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The Potential Role of Leptin in Tumor Invasion and Metastasis

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Abstract

The adipocyte-released hormone-like cytokine/adipokine leptin behaves differently in obesity compared to its functions in the normal healthy state. In obese individuals, elevated leptin levels act as a pro-inflammatory adipokine and are associated with certain types of cancers. Further, a growing body of evidence suggests that higher circulating leptin concentrations and/or elevated expression of leptin receptors (Ob-R) in tumors may be poor prognostic factors. Although the underlying pathological mechanisms of leptin's association with poor prognosis are not clear, leptin can impact the tumor microenvironment in several ways. For example, leptin is associated with a number of biological components that could lead to tumor cell invasion and distant metastasis. This includes interactions with carcinoma-associated fibroblasts, tumor promoting effects of infiltrating macrophages, activation of matrix metalloproteinases, transforming growth factor-β signaling, etc. Recent studies also have shown that leptin plays a role in the epithelial-mesenchymal transition, an important phenomenon for cancer cell migration and/or metastasis. Furthermore, leptin's potentiating effects on insulin-like growth factor-I, epidermal growth factor receptor and HER2/neu have been reported. Regarding unfavorable prognosis, leptin has been shown to influence both adenocarcinomas and squamous cell carcinomas. Features of poor prognosis such as tumor invasion, lymph node involvement and distant metastasis have been recorded in several cancer types with higher levels of leptin and/or Ob-R. This review will describe the current scenario in a precise manner. In general, obesity indicates poor prognosis in cancer patients.

Graphical Abstract

Leptin-linked Pathological Mechanisms in Tumor Proliferation and Migration

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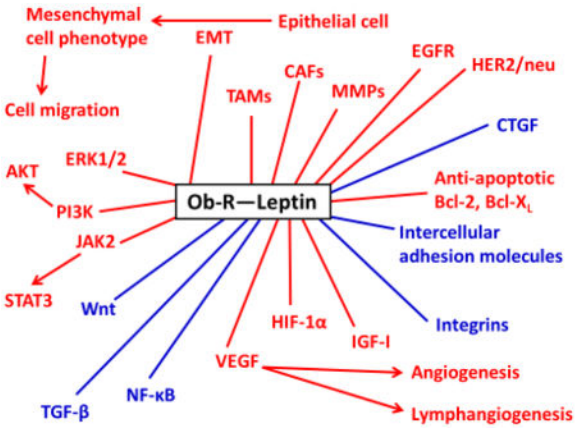
Conflict of Interest

The authors have no conflict of interest to declare.



Red: Tumor-promoting effect

Blue: Dual role (tumor–suppressor or –promoting effect depending on the situation)

CAFs: Cancer-associated fibroblasts, CTGF: Connective tissue growth factor, EGFR: Epidermal growth factor receptor, EMT: Epithelial–mesenchymal transition, ERK1/2: Extracellular signal-regulated kinases 1 and 2, HER2: Human epidermal growth factor receptor 2, HIF-1α: Hypoxia-inducible factor-1 alpha, IGF-I: Insulin-like growth factor-I, JAK2: Janus kinase 2, MMPs: Matrix metalloproteinases, NF-κB: Nuclear factor-kappa B, Ob-R: Leptin receptor, PI3K: Phosphatidylinositol 3-kinase, STAT3: Signal transducer and activator of transcription 3, TAMs: Tumor-associated macrophages, TGF-β: Transforming growth factor-beta, VEGF: Vascular endothelial growth factor

Keywords

Invasive cancer; Leptin; Metastasis; Obesity; Prognosis

1. Introduction

Obesity is associated with poor prognosis among cancer patients, apart from its link to other disease processes such as type-2 diabetes, cardiovascular disorders and increased risk of certain malignancies. Hormone-like cytokines or adipokines released from the expanded adipose tissue mass in obesity are thought to be responsible for these pathophysiological changes. One of the important adipokines is leptin, which may influence several biological processes in both normal and disease conditions. Leptin is a 16 kDa protein mainly synthesized by adipocytes that maintains energy homeostasis by influencing the central anorexigenic pathway [1]. However, in obesity, leptin displays diverse abnormal functions. Leptin acts via transmembrane receptors (Ob-R), which include at least 6 alternatively spliced isoforms. The long form Ob-Rb appears to be most important for leptin's weight regulating effects.

The tumor microenvironment, which consists of extracellular matrix (ECM) and various populations of stromal cells such as fibroblasts, adipocytes and macrophages, may influence the behavior of cancer cells through expression of cytokines, growth factors, and proteases

[2]. For instance, both cancer-associated fibroblasts (CAFs) and matrix metalloproteinases (MMPs) are involved in different phases of cancer progression including invasiveness, and metastasis [3,4]. In fact, cancer cells and CAFs crosstalk enhances the production of growth factors, cytokines, chemokines, MMPs, and inflammatory mediators, which eventually facilitates tumor growth [3]. Interestingly, leptin has been demonstrated to be closely associated with activities of CAFs and expression levels of MMPs [5–7]. On the other hand, many reports have revealed leptin's link with a number of ECM components such as fibronectin, transforming growth factor-beta (TGF-β), and connective tissue growth factor (CTGF) as well as several intracellular signaling molecules including AKT, phosphatidylinositol 3-kinase (PI3K), and signal transducer and activator of transcription (STAT) [8–10]. Therefore, leptin is capable of inducing the local autocrine/paracrine cascades within the tumor microenvironment and likely of stimulating neoplastic growth and progression.

The epithelial-mesenchymal transition (EMT) is an intricate biological process by which epithelial cells acquire mesenchymal morphology. This phenomenon is observed during tumor invasion and metastasis, apart from normal embryological development and wound healing. Interestingly, blocking leptin's availability to estrogen receptor positive (ER+) MCF-7 breast cancer cells significantly decreased the number of metastatic lesions in SCID/beige mice [11]. A growing body of evidence shows that leptin may promote EMT, and a number of mechanisms have been proposed. For example, Choi et al. found that leptin activated hedgehog signaling in hepatic stellate cells, which in turn promoted EMT [12]. Further, a potential cross-talk between leptin and Wnt signaling in EMT has been reported in breast cancer cell-lines [13]. This was demonstrated by an increased accumulation and nuclear translocation of β-catenin by leptin due to a decrease in the formation of glycogen synthase kinase 3β (GSK3β)-liver kinase B1 (LKB1)-axin complex in MCF-7, MDA-MB-468 and MDA-MB-231 breast cancer cells. In addition, using A549 human lung cancer cells, Feng and colleagues reported that leptin significantly upregulated TGF-β that may play an important role in inducing EMT [14]. Leptin-mediated activation of intracellular signaling molecules such as STAT3, AKT, and PI3K could contribute to EMT-linked mechanisms [15,16].

Invasion and distant metastases by cancer cells are the final event that leads to the majority of cancer deaths. Leptin potentially has a potent impact on the spread of cancer to distinct organs. Apart from the pathological effects that have been mentioned above, leptin may influence disease processes through a number of mechanisms such as enhanced cellular proliferation, angiogenesis, and interaction with various cytokines (Fig. 1). In this review, we analyze leptin's role in tumor invasion and metastasis.

2. Obesity-related microenvironment and its influence on tumor progression

A growing body of evidence suggests that adipocytes in the tumor microenvironment play a crucial role in disease progression by providing fatty acids, pro-inflammatory cytokines and proteases [17,18]. Interestingly, cancer cells utilize adipocyte-released fatty acids for energy

production through β-oxidation. Therefore, adequate supply of fatty acids/lipids from cancer-associated adipocytes favors uncontrolled growth and progression of malignancy [17]. A recent study showed that after co-culture with isolated omental adipocytes, MKN-45 and AGS gastric cancer cells exhibited a significant increase in lipid uptake and enhanced invasiveness [19]. Similarly, co-culture of adipocytes with SW480 and DLD1 human colon cancer cells led to a transfer of free fatty acids from the adipocytes to the cancer cells [20]. Furthermore, this study found an accumulation of adipocytes in close association with invasive tumor cells in colon cancer patients. In an *in vitro* study, murine 3T3L1 adipocytes were co-cultured with pancreatic intraepithelial neoplasia and ductal adenocarcinoma cells derived from PKCY mice. Adipocytes promoted proliferation of both cell types [21].

Prostate cancer progression may also be impacted by adipose tissue microenvironment. For example, androgen independent RM1 mouse prostate carcinoma cells were co-cultured with pre-adipocytes or adipocytes as well as with their respective conditioned medium [22]. Both adipocytes and its conditioned media significantly increased RM1 cell proliferation and greater cell migration was observed with pre-adipocyte and adipocyte conditioned media. Moreover, RM1 cell invasion was significantly increased after co-culture with pre-adipocytes and adipocytes. Another study using human prostate cancer cell-lines (DU145, LNCaP, and PC-3) found that conditioned medium from pre-adipocytes increased cell proliferation and invasive activity [23]. Interestingly, Ribeiro et al. reported higher MMP-2 activity in periprostatic adipose tissue compared to pre-peritoneal visceral adipose tissue [24]. In addition, increased proliferation and migration of hormone-refractory PC-3 cells were detected after stimulation with conditioned medium from periprostatic adipose tissue explants.

A number of studies have been conducted on the interactions between breast tumors and adipocytes in the tumor microenvironment. For example, Zhu et al. analyzed 294 cases and concluded that invasive portions of breast cancer were generally situated adjacent to breast adipose tissue [25]. Intriguingly, Fletcher et al. noted that adipocytes associated with the invasive front are reduced in size compared to adipocytes that are farther away [26]. Many investigators have recorded considerable morphological and functional changes of adipocytes which are adjacent to tumors and referred to as cancer-associated adipocytes [27,28].

Additional evidence for an interaction of adipocytes with breast cancer development comes from studies with co-cultures of human breast cancer lines with adipocytes. When MDA-MB-231, MCF-7 and ZR-75-1 human breast cancer cell lines were co-cultured in the presence of adipocytes significant increases in proliferation rate were determined [29]. Furthermore, an enhanced MDA-MB-231 cancer cell invasiveness has been documented when co-cultured with adipocytes [30]. Similarly, Lee et al. observed that adipocytes induced migration and invasion in MCF-7, MDA-MB-435S, and MDA-MB-231 breast cancer cells after co-culture with adipocytes [31]. In this study, increased cell migration and invasion were accompanied by the upregulation of MMP-9 and TWIST1. In an interesting study, MCF-7 cells were co-cultured with SGBS adipocytes (which originated from a patient with Simpson-Golabi-Behmel syndrome, an overgrowth disorder). Gene expression levels of EMT-inducing transcription factors FOXC2 and TWIST1 were increased in the MCF-7 cells

while in the SGBS cells hypoxia-inducible factor-1α (HIF-1α), TGF-β, and lectin-type oxidized LDL receptor 1 (LOX1) mRNA levels were increased [32].

In a somewhat different approach to evaluate the potential impact of adipose tissue in the microenvironment on breast cancer development experiments have been conducted using conditioned medium from isolated normal breast adipocytes as well as cancer associated adipocytes. MCF-7 and MDA-MB-231 cells were used for the purpose of detecting phenotypic changes resulting from culture with the different media. Both breast cancer cell-lines had higher migration in conditioned medium obtained from the cancer-associated adipocytes in comparison to the media from normal breast adipocytes. The conditioned medium from the cancer associated adipocytes had higher levels of monocyte chemoattractant protein-1 (MCP-1) and interleukin-6 (IL-6) than that from the normal breast adipocytes [33].

In the breast tumor microenvironment, adipocytes have been reported to produce a number of biologically active compounds including different cytokines, e.g., MMP-11, plasminogen activator inhibitor-1 (PAI-1), collagen VI, IL-1β, IL-6, tumor necrosis factor – α (TNF-α), insulin-like growth factor-I (IGF-I), and leptin; many of which are metastasis-associated proteins [34–36]. In particular, Wolfson et al. suggested that leptin has a key effect on breast cancer; and in adipocyte-adjacent breast cancer cells, due to stimulation of various regulatory pathways such as Notch, Wnt and Nanog signaling axis [37]. Clearly, the significance of leptin at the adipocyte-breast cancer cell interface, and its effects in the neighboring breast cancer cells are complex (Fig. 2). A potential role of leptin acting to increase aromatase activity in adipose tissue associated with breast cancer is supported by interesting *in vivo* studies. Liu et al. co-injected pre-adipocytes (F442A) with MCF-7 breast cancer cells subcutaneously into SCID mice [38]. The pre-adipocytes developed into fat pads and interestingly tumors also developed in these mice while those with only the MCF-7 cells injected did not. Further investigation indicated that leptin signaling in these adipocytes increased aromatase expression and further that direct injection of leptin into these fat pads increased mRNA for aromatase 6-fold [38]. Their findings suggest that leptin in the tumor microenvironment could play a crucial role in the progress of hormone-dependent breast cancer.

3. Leptin biology and molecules of relevant signal transduction pathways

The leptin gene (*ob*) is located on the long arm of chromosome 7. Initially a 167 amino acid peptide is translated, which forms the mature 146 amino acid peptide after processing through the endoplasmic reticulum [39]. Leptin protein sequences are highly conserved among mammals; however, it exhibits considerable sequence variation among other vertebrates [40]. Due to poor solubility of natural leptin, a mutant leptin has been constructed with a single amino acid substitution of glutamic acid for tryptophan at position 100 [41]. For improved solubility, it has been possible to crystallize this leptin analog W100E. The crystallographic structure has displayed the secondary and tertiary structure of the leptin molecule, which consists of four antiparallel α-helices (A, B, C, and D) and two relatively long interconnected loops [39,41,42]. Two conserved cysteine residues (C96 in the

CD loop and C146 as the C-terminal residue) form a disulfide bridge that is essential for structural stability and thus biological activity [43].

Leptin belongs to the family of class-I cytokines, which also includes biomolecules such as IL-6, leukemia inhibitory factor (LIF), and ciliary neurotrophic factor (CNTF) [44,45]. For this reason, like other members, Ob-R mainly functions via the activation of Janus kinases (JAK) and STAT, particularly through JAK2-STAT3 signal transduction pathway (Fig. 3 and Fig. 4a–c). Finally, activated/phosphorylated STAT3 (p-STAT3) translocate to the nucleus and stimulates transcription. Other pathways that support neoplastic growth include PI3K and mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) signaling pathways [46,47]. Evidence to support the role of these proteins in cancer progression will be presented next.

3.1. STAT3 activation in neoplastic process

STAT3 activation has been documented in both solid and hematological malignancies [48,49]. It has been suggested that aberrant signaling of STAT3 is associated with different biological processes of tumor progression such as angiogenesis, cellular proliferation and survival of cells or inhibition of apoptosis leading to invasion and metastasis. Furthermore, STAT3 is involved in EMT by regulating various EMT-related proteins, e.g., Twist, Snail, and vimentin [50–52]. A recent study on intrahepatic cholangiocarcinoma found that patients with high STAT3 expression levels had a poor overall and disease-free survival [53]. After analyzing a large number of gastric cancer cases, both STAT3 and p-STAT3 were shown to be associated with an increased mortality risk [54,55]. In another study, Deng et al. reported that 62.6% (67/107) and 38% (41/107) of gastric cancer tissues exhibited positive immunostaining for STAT3 and p-STAT3, respectively [56]. In a study on gastric cancer conducted by Song et al., immunostaining for STAT3 and p-STAT3 was 81.7% (49/60) and 58% (35/60), respectively [57]. Moreover, their study showed that p-STAT3 played a significant role in prognosis. Similarly, in colon cancer, positive immunostaining rates for STAT3 and p-STAT3 were 72% (36/50) and 76% (38/50), respectively [58]. After reviewing 17 studies and a total of 2,346 colon cancer patients, the authors revealed a positive association between p-STAT3 expression and lymph node metastasis [59].

Likewise, results of another meta-analysis on non-small-cell lung cancer (NSCLC), which included 17 retrospective trials and 1,793 patients, suggested that high STAT3 or p-STAT3 expression was a strong predictor of poor prognosis [60]. Of note, the majority of lung cancers are NSCLC, which includes predominant squamous cell carcinomas and adenocarcinomas. Nevertheless, in a study conducted by Zhao et al., STAT3, and p-STAT3 expressions were observed in 72.1% (49/68) and 58.8% (40/68) of NSCLC cases, respectively [61]. Furthermore, immunohistochemical analyses on 82 surgically resected NSCLC tissues revealed p-STAT3 expression in 59.7% of tumors [62]. The investigators also recorded that p-STAT3 expression was correlated with tumor’s degree of differentiation, lymph node metastasis, clinical staging and prognosis after surgical resection.

Liu et al. analyzed 208 breast cancer tissues, and they noticed high level of STAT3 immunoreactivity in 72% (n=150) of cancer samples; whereas high p-STAT3 expression was present in 43.8% (n=91) cases [63]. The expression levels of both STAT3 and p-STAT3 were

increased with higher TNM stage and lymph node metastasis. In another study on 76 breast cancer cases, 66% (n=50) of the cancer specimens showed positive immuno-expression of STAT3, which was significantly associated with the clinical stage, tumor differentiation and lymph node metastasis [64]. Additionally, a key role of STAT3 in the regulation of invasion and metastasis has been revealed by a recent study [65].

3.2. PI3K involvement in cancers

An important intermediate, which functions in diverse hormone signaling pathways including insulin and leptin, is PI3K (Fig. 4d). Possibly, the PI3K signaling pathway is a significant link between obesity, leptin and an increased risk of cancer [66]. In a nutshell, activation of PI3K causes phosphorylation of AKT and stimulation of the mammalian target of rapamycin (mTOR); while the phosphatase and tensin homolog (PTEN) is a negative regulator. Remarkably, PI3K pathway regulates various biological processes such as cellular metabolism, proliferation (Fig. 4e), and apoptosis (Fig. 4f); aberrant responses of these phenomena trigger the malignant pathology (Fig. 3). It is noteworthy that PI3K is composed of a regulatory subunit and a 110 kDa catalytic subunit. The gene encoding PI3K catalytic subunit α (PIK3CA/p110α) is frequently activated by mutation in cancers [67].

PIK3CA mutations represent one of the most common genetic aberrations in breast cancer [68]. In a study on 241 patients with breast cancer, PIK3CA mutations were found in 15.8% cases [69]. On the other hand, Firoozinia et al. analyzed 50 breast cancer samples; 72% samples showed significant amplification of PIK3CA gene, while 24% exhibited protein overexpression [70]. In another study that examined 95 breast cancer tissue samples, PIK3CA gene mutations were present in 25.3% cases [71]; and in a recent study, mutations of PIK3CA were found in 28% (21/75) cases [72].

A number of studies have shown the involvement of PI3K pathway in carcinomas of the gastrointestinal tract. In a recent study on Japanese patients with gastric cancer, PIK3CA mutations were detected in 12% (25/208) [73]. In another study on gastric cancer patients, both PIK3CA gene mutations and amplification were assessed in connection with clinicopathological characteristics and disease outcome [74]. PIK3CA mutations and amplification were found in 7.1% (8/113) and 67% (88/131), respectively. Furthermore, patients with PIK3CA amplification had significantly shorter survival times. A study, which analyzed PIK3CA mRNA levels in both gastric cancer tissues and metastases, observed 2 fold higher mRNA in lymph node metastasis compared to primary tumors [75]. On the other hand, an immunohistochemical analysis of tissue samples from 70 patients with gastric cancer revealed that expression level of PI3K protein was significantly correlated with TNM stage, differentiation grade, lymph node metastasis and distant metastases [76]. In the same way, after analyzing 60 colon cancer specimens immunohistochemically, the investigators concluded that high PI3K expression was associated with cancer metastasis [77]. They also noticed a significant difference in the proportion of PI3K in primary lesions (30%, 18/60) vs metastatic lesions (46.7%, 28/60). Overall, PI3K pathway alteration is common in human cancers, and overexpression of this pathway is associated with the uncontrollable cellular proliferation and growth.

3.3. Overexpression of AKT and ERK in cancers

Different investigators have reported an interaction between PI3K-AKT and ERK signaling pathways [78–80]. A number of studies, which evaluated both AKT and ERK in tumors, recorded similar frequencies of expression [81–83]. In various cancers, high expression rates of both AKT and ERK have been documented [84–88]. Regarding overexpression of AKT and its association with patients' prognosis, conflicting reports have been reported. For example, David et al. noticed that lung cancer patients with strong phosphorylated AKT (p-AKT) expression were more likely to die early [89]. In a study that analyzed endometrial adenocarcinomas, p-AKT expression was significantly higher in women with positive lymph nodes, and higher expression was significantly associated with poor survival [81]. Jia et al. recorded that p-AKT expression correlated with higher histological grade and resistance to chemotherapy in ovarian cancer [90]. On the other hand, a study in colorectal cancer patients showed that p-AKT expression was associated with lower stage and good prognosis [91]. Interestingly, in renal cell carcinoma, Pantuck et al. found that high nuclear p-AKT expression was associated with a favorable prognosis, while high cytoplasmic p-AKT expression was associated with a poor prognosis [92]. Conversely, a study in patients with non-small cell lung cancer observed that higher levels of cytoplasmic p-AKT had a trend toward longer overall survival; whereas higher levels of nuclear p-AKT had a poorer prognosis [93]. In general, investigators have identified an association between ERK activation and poor prognosis such as aggressive neoplastic features, metastasis, and reduced survival [94,95]. In a study among breast carcinoma patients, p-ERK was shown to be associated with higher TNM stage and lymph node metastasis [96]. Similar pathologic characteristics with regard to ERK expression/activation have also been detected in digestive tract cancers. In esophageal carcinoma, increasing p-ERK nuclear and cytoplasmic expression was found to be significantly correlated with tumor grade [97]. Likewise, high nuclear p-ERK expression in colorectal cancer was associated with highly invasive disease [98]. In addition, the investigators showed that nuclear but not cytoplasmic p-ERK expression correlated with the patients' overall survival rate. Perhaps, activation of ERK may increase the metastatic potentiality of cancer cells.

4. Leptin and tumor behavior of the gastrointestinal tract

4.1. Upper gastrointestinal tract

An association between high levels of leptin and risk of esophageal adenocarcinoma in obese individuals has been reported [99]. Adenocarcinoma in the lower part of esophagus most often arises from the epithelium of intestinal metaplasia (Barrett's); while squamous cell carcinoma occurs mainly in the middle and lower regions and originates from non-keratinized stratified epithelium [100]. In a study of esophageal adenocarcinoma increased peri-tumoral adipocyte size was associated with higher leptin expression, angiogenesis, lymphangiogenesis and nodal metastasis [101]. These findings suggest that leptin secreted from peri-tumoral adipocytes may play a key role in the progression of cancer. In esophageal squamous cell carcinoma, leptin immunoexpression also has been demonstrated to be upregulated. Moreover, the expression of leptin was significantly correlated with lymph node involvement and advanced tumor stage in esophageal squamous cell carcinoma [102].

With respect to gastric carcinoma Ob-R expression was correlated with poor survival in 47% of patients with lymph node metastasis, 60% of the patients with advanced gastric cancer, and 41% of the cases defined as Lauren diffuse pathology [103] (Table 1). Of note, gastric cancer has been classified in many different ways such as Lauren, World Health Organization (WHO), and Borrmann classifications [104]. Poorly cohesive carcinomas belong to the diffuse type according to the Lauren classification. By analyzing 61 gastric cancer cases, Zhao et al. observed a significant association between leptin expression and vascular endothelial growth factor (VEGF) expression [105]. Further, leptin expression in these gastric cancer tissues was significantly associated with histology, Borrmann classification, lymph node metastasis and stage. Moreover, in patients with poorly differentiated gastric cancer, a poor prognosis was found for those with a strong expression of leptin. Similarly, Geng et al. recorded that the survival of gastric cancer patients with leptin positive expression was significantly poorer than those with negative expression [106]. They also noted a correlation between the expression of leptin and HER2, both of which were significantly associated with invasion depth, lymph node metastasis, tumor stage and expression of VEGF. Interestingly, Ishikawa et al. found that the immunohistochemical expression levels of both leptin and Ob-R tended to increase as the depth of either gastric tumor invasion or TNM stage increased [107]. Among stages I and II, 31% and 27% of the cases exhibited significant immunostaining for leptin and Ob-R, respectively. Whereas, in stages III and IV, 48% of the cases were strongly positive for leptin and 47% expressed Ob-R. In this study, lymph node metastasis was detected in approximately half of the leptin strong positive and Ob-R positive cases. Both venous and lymphatic invasion was more frequently observed in tumors with high leptin and positive Ob-R expression.

To explain the mechanisms of leptin-mediated invasion and metastasis in gastric cancer, Dong and colleagues reported that leptin promoted gastric cancer cell invasion by upregulating the expression of membrane type-1 matrix metalloproteinase (MT1-MMP or MMP14), which is an ECM degrading protease which plays crucial roles in tumor invasion [108]. Furthermore, they observed that leptin and intercellular adhesion molecule-1 (ICAM-1) were both overexpressed in gastric cancer tissue, and there was a strong positive correlation between them [109]. Both were related with clinical stage and lymph node metastasis. In addition, they demonstrated that leptin induced migration of human gastric carcinoma cells (AGS and MKN-45 cell-lines) by upregulating ICAM-1. It is known that ICAM-1 or CD54 is a transmembrane glycoprotein of the immunoglobulin superfamily and it can facilitate the metastasis of cancer cells by escaping the recognition and attack of immunocytes [110]. Interestingly, leptin expression in gastric adenocarcinoma cell-lines was reported to be correlated with resistance to the chemotherapeutic agent cisplatin [111]. Furthermore, including a leptin antagonist increased the sensitivity of both the cisplatin-resistant gastric cancer AGS Cis5 and esophageal adenocarcinoma OE33 cell-lines to cisplatin, which greatly illustrated the impact of leptin in the disease course (Table 2 [101,111,112]).

Leptin may also be involved with the severity of gall bladder cancer. For example, in a recent *in vitro* study using gallbladder cancer cells the addition of leptin increased the levels of MMPs (−3 and −9) and VEGF (-C and -D, responsible for lymphangiogenesis) [113].

Since these proteins increase proliferation, migration and invasion of cancer cells, leptin could act through them to eventually promote metastasis.

4.2. Lower gastrointestinal tract

At another gastrointestinal cancer site, i.e., colorectal cancer, a relationship between higher serum leptin levels and lymph node involvement has been documented [114,115]. Moreover, after analyzing the data of 130 colorectal cancer patients, Healy et al. found that serum leptin level was associated with microvascular invasion and advanced tumor stage [115]. Similarly, leptin and Ob-R protein expressions in tumor tissue have been found to be correlated with aggressive characteristics of cancer, e.g., tumor grade and stage, depth of bowel wall invasion, lymph node metastasis and distant metastasis [116,117]. Pro-angiogenic and pro-migratory effects of leptin are possibly important in progression of colon cancer. In a study conducted by Ratke and colleagues using SW480, SW620 and HCT116 human colon carcinoma cell-lines, leptin significantly enhanced the migratory activity of these cell-lines through the activation of STAT3 and JAK [118]. The investigators performed three-dimensional collagen-based migration assays, and carcinoma cells exhibited a significant increase of their migratory activity in response to 100 ng/ml of human leptin. It is noteworthy that a positive correlation was observed among leptin, Ob-R, VEGF, and microvessel density (MVD) in colorectal carcinoma [117]. In accordance with the protein expression (i.e., Ob-R) in tumor tissue that has been discussed above, Erkasap et al. documented higher mRNA expression of Ob-R in metastatic growth compared to primary colorectal cancer [119].

Cancers of the gastrointestinal tract are common causes of death worldwide. High levels of leptin released from excessive adipose tissue in obesity may play a key role in different stages of cancer. It is known that leptin can affect several aspects of the gastrointestinal tract such as interactions with local hormones, food absorption and inflammatory condition. Therefore, it is important to understand the precise role of leptin in gastrointestinal tumors for better management and patient outcome.

5. Tumors of endocrine and associated organs

5.1. Pancreas

Adipocyte-derived leptin and insulin which is secreted from the pancreas have fascinating similarities and complex interactions in normal health and conditions such as obesity, leptin resistance and insulin resistance. Overall, an intricate relationship has been observed in energy homeostasis including glucose and lipid metabolism. Leptin resistance in obesity markedly influences the status of insulin sensitivity, and vice versa. Therefore, it is imperative to understand the role of leptin in various disorders of the pancreas, which is a storehouse of several exocrine and endocrine factors.

Fan et al. found an increased expression of leptin's functional receptor Ob-Rb in pancreatic tumor tissue which was significantly associated with the level of MMP-13, lymph node metastasis and TNM stage [120]. Further, in *in vitro* experiments these investigators showed that addition of leptin stimulated the expression of MMP-13 as well as increased the

migration and invasion of PANC-1 human pancreatic cancer cells. Likewise, in a xenograft model with the PANC-1 cell-line, they observed that the overexpression of leptin in tumor tissue significantly increased tumor growth and lymph node metastasis. In this experiment, the investigators used the PANC-1 cells that were transfected with the recombinant lentivirus carrying the human leptin gene; the cell-line stably overexpressed leptin. In another study, Ren et al. found that HIF-1α expression was correlated with Ob-R in pancreatic cancer tissue in xenograft mouse model that was inoculated with PANC-1 cells [121]. This study included human clinical data indicating that overexpression of HIF-1 was associated with lymph node metastasis and overall survival. These findings are consistent with the fact that HIF-1α appears to be involved in tumorigenesis and angiogenesis [122].

5.2. Thyroid gland

Recently there has been an increasing incidence of thyroid cancer. One of the suspected risk factors is the increasing prevalence of obesity [123,124]. Since the level of leptin is elevated in obesity, this adipokine could be involved in the development and progression of this malignancy [124]. The thyroid gland contains two types of endocrine cells – follicular cells that synthesize thyroid hormone, and parafollicular or C cells that produce calcitonin. Papillary carcinoma, the most common type of thyroid cancer, originates from follicular cells; whereas medullary carcinoma develops from C cells. A study on different subtypes of thyroid carcinoma revealed that immunohistochemical expression of Ob-R was significantly correlated with nodal metastasis and advanced stage in medullary thyroid carcinoma. On the other hand, both leptin and Ob-R levels were strongly associated with larger tumor size, nodal metastasis and advanced stage in papillary thyroid carcinoma [125]. Several studies on patients with papillary thyroid cancer documented that expression of leptin and/or Ob-R was directly linked with disease aggressiveness including increased tumor size and lymph node metastasis [126–128]. In addition, it has been found that Ob-R overexpression was significantly associated with the overexpression of anti-apoptotic XIAP and Bcl-X_L proteins as well as with poor disease-free survival in cases with papillary thyroid cancer [128]. In a study on K1 and BCPAP human papillary thyroid cancer cell-lines, tumor cell migration was significantly enhanced by leptin in a dose-dependent manner [129].

6. Female reproductive system cancers

6.1. Gynecologic cancers

Malignancies of the female reproductive system are responsible for significant morbidity and mortality throughout the world. Among all gynecological malignancies, ovarian cancer is the most deadly disease. Due to unique anatomical position of the ovary, dissemination of cancer cells within the peritoneal cavity is common in ovarian cancer. The disease is generally diagnosed at a late stage and prognosis is poor.

Similar to colon cancer [119], metastases from ovarian cancers were found to have higher Ob-Rb expression than what was associated with primary tumors [16]. *In vitro* studies with various ovarian cancer cell-lines further demonstrated that leptin stimulated cell migration and invasion. In a study on endometrial cancer, elevated levels of patients' serum leptin were associated with invasiveness [114]. Similar findings were observed with regard to

immunohistochemical expression of both leptin and Ob-R in endometrial cancer tissue. The expressions of leptin and Ob-R were positively correlated with the invasiveness of cancer, lymph node metastasis, and poorer prognosis [130].

6.2. Breast cancer

Higher breast cancer incidence has been shown to be attributable to increased body mass index (BMI) [131,132]. Leptin is considered as an important adipokine that may modulate this disease process. A number of studies on breast cancer patients have noted an association of higher serum leptin levels with aggressive malignant features. For instance, in Italian women, serum leptin concentrations significantly correlated with tumor size, lymph node involvement and metastasis among ER+ breast cancer cases [133]. Similarly in another study, serum leptin levels were positively correlated with TNM staging, tumor size, lymph node metastasis, and histological grading among postmenopausal women with breast cancer [134]. A number of investigators also included measurement of adiponectin and expressed results in relation to leptin [134–136]. Adiponectin is an adipokine with anti-inflammatory effects unlike the pathophysiological functions of leptin [137]. Nonetheless, Hou et al. observed that reduced serum levels of adiponectin and elevated leptin were associated with lymph node metastasis in breast cancer patients [135]. Chen et al. also reported that an increased serum ratio of leptin/adiponectin (L/A ratio) was positively correlated with tumor size [136].

Similar to serum levels, tumor tissue expressions of leptin and/or Ob-R also indicate the presence of aggressive behavior. For example, Ishikawa and colleagues reported that distant metastasis was detected in Ob-R-positive tumors with leptin overexpression compared to tumors that lacked Ob-R expression or leptin overexpression [138]. Similarly in another study, overexpression of leptin and Ob-R in breast cancer was positively correlated to lymph node metastasis [139]. In this study, analyses of 60 cases of breast cancer and adjacent normal tissue along with benign breast disease tissues revealed that immunostaining intensity for both parameters (co-expression) in cancer tissue was directly proportional to the degree of cancer cell dissemination. Interestingly, Alshaker et al. detected that Ob-R genes were elevated in metastases of ER-negative breast cancer patients [140]. Results from a mouse model have also provided evidence for a role of leptin in the metastatic process in breast cancer. Park et al. observed an absence of Ob-R attenuated tumor progression and lung metastasis through a reduction of ERK1/2 and JAK2/STAT3 pathways in the MMTV-PyMT mammary tumor model of breast cancer [141]. For this purpose, the brain-specific long form of leptin receptor (NseLEPR-B) transgenic mouse was crossed with the MMTV-PyMT mouse strain. Normally, MMTV-PyMT female mice develop mammary adenocarcinomas that metastasize to the lung. Additional support comes from *in vitro* studies. For example, in mouse (4T1, EMT6 and MMT) mammary cancer cell-lines, the addition of leptin induced cell proliferation and migration, as well as upregulated VEGF and its receptor VEGFR-2 [142]. McMurtry et al. reported that leptin increased the invasiveness and MMP-2 activity of the ER+ MCF-7 human breast cancer cell-line [143]. Additionally, in an experiment using breast cancer cells and tumor-associated macrophages (TAMs), leptin was shown to stimulate the expression of IL-18, which is closely linked with inflammatory reactions [144]. In this study, THP-1 human monocytic cell-line and MCF-7, SK-BR-3 and

MDA-MB-231 human breast cancer cell-lines were used. Furthermore, the study documented that leptin could induce IL-8 expression in both breast cancer cells and TAMs.

It is interesting to note leptin's influence on TAMs, which are a crucial component in the tumor microenvironment and the principal source of inflammatory cytokines [144]. Moreover, clinical evidence has revealed a strong correlation between a high density of TAMs and poor prognosis in breast cancer [144]. As such, TAMs could play key roles in cancer progression including EMT. It is noteworthy that macrophages can be classified into two major categories – classically activated M1 macrophages are linked with tumoricidal functions, and alternatively activated M2 macrophages are involved in tumor promotion [145]. Fascinatingly, TAMs have been considered as M2 macrophages, which could promote the EMT [145–147]. A recent study has shown that leptin may promote breast cancer progression by stimulating M2 macrophages [148]. Using a different approach Strong et al. [11] co-cultured adipose stromal/stem cells isolated from obese women with either ER+ MCF-7, ZR75, or T47D human breast cancer cells, which resulted in enhanced expression of EMT and metastasis related genes, SERPINE1, MMP-2, and IL-6. Of note, adipose stromal/stem cells have the capability to differentiate along the adipocyte and other lineage pathways. Nevertheless, these investigators concluded that leptin from adipose stromal/stem cells could contribute to the aggressiveness of breast cancer in obese women [11]. In a similar way, migration and colonization patterns of MDA-MB-231 and MCF-7 breast cancer cells were studied in co-culture with cancellous or spongy bone tissue fragments. The results showed that breast cancer cells migrated to human bone tissue-conditioned medium in association with increasing levels of leptin and IL-1β, and colonized the bone marrow adipose tissue compartment of cultured fragments [149].

Leptin has been demonstrated to influence numerous biological elements such as signal transduction proteins like JAK and PI3K, cellular receptors such as epidermal growth factor receptor (EGFR) and androgen receptor, and the enzymes aromatase and cytochrome P450 1B1 (CYP1B1): all of these biomolecules could be associated with neoplastic process [10,137]. Perera et al. identified a number of leptin-regulated genes in MCF-7 cells by using a microarray system; and observed that leptin up-regulated the expression of CTGF and the anti-apoptotic genes Bcl-2 and survivin, and also reduced the expression of apoptotic genes [150]. Furthermore, experiments demonstrated a bidirectional crosstalk between leptin and IGF-I signalling that transactivated EGFR and promoted the migration of breast cancer cells [151]. Overall, the results of different clinical, *in vivo* and *in vitro* studies have suggested that leptin could contribute to the aggressiveness of breast cancer.

7. Tumors of other organs

Leptin has been shown to stimulate the migration of human prostate cancer and chondrosarcoma cells [152,153]. In both human prostate cancer and chondrosarcoma cells, one of the underlying mechanisms of leptin-influenced migration was transcriptional up-regulation of αvβ3 integrin expression through Ob-R and activation of associated signaling pathways such as PI3K, AKT, and nuclear factor-κB (NF-κB). Of note, integrins are cell adhesion molecules that connect the cytoskeleton with ECM/other cells. The integrin αvβ3 or vitronectin receptor is linked to many biological processes such as angiogenesis and

tumor metastasis [154]. It may be worth mentioning that obesity has not been shown to be involved in prostate cancer development per se. However, obesity is associated with more aggressive prostate cancer, and this could be mediated by leptin [155].

In a study on patients with renal cell carcinoma, it was observed that serum leptin levels were significantly higher in those with venous invasion. There were significant associations between high Ob-R expression in tumor tissue and the presence of venous invasion, tumor histology, and lymph node metastasis. Patients with higher serum leptin had significantly shorter progression-free survival than patients with lower levels [156].

Investigators have observed that leptin levels were significantly higher in melanoma patients with positive sentinel nodes [157]. Their follow-up analysis revealed more aggressive disease in diabetic patients. It may be worth mentioning that melanoma is a highly aggressive form of skin cancer and the condition is frequently fatal if the tumor is not diagnosed early. An excess of adipose tissue may influence the development of melanoma and disease progression by different obesity-linked phenomena such as type 2 diabetes and enhanced release of leptin [158–161]. In an interesting study on melanoma, RNA was extracted from nodal tissue of 13 tumor-negative sentinel nodes, 10 tumor-positive sentinel nodes (micro-metastases<2 mm), and 11 tumor-negative non-sentinel nodes [162]. Expression levels of leptin were significantly higher in tumor-positive as compared with tumor-negative sentinel nodes.

In a study on lung cancer patients that included both primary and metastatic bone lesions, leptin was present at higher levels in samples associated with diagnosis of lung cancer bone metastastic tissue than lung cancer tissue [14]. In another study, the authors determined the association between the expression level of leptin and the survival of patients suffering from NSCLC [163]. They observed that leptin expression in NSCLC tissue affected the survival time of patients. Therefore it has been suggested that the expression of leptin may be an independent prognostic factor for NSCLC. On the other hand, in a syngeneic murine model study of Lewis lung carcinoma, consumption of a high-fat diet significantly increased plasma leptin in male C57BL/6 mice and resulted in a two-fold increase in the number of lung metastases along with a 50% increase in tumor volume compared to mice fed a low fat diet [164]. These studies have clearly shown that higher concentrations of leptin were associated with tumor progression and this adipokine could serve as an additional parameter for prognosis.

8. Leptin in tumor progression

Deregulated cell proliferation is the key feature in all phases of cancer development. Uncontrolled cell proliferation associated with suppression of apoptosis, proper supply of oxygen and nutrients, and removal of metabolic wastes provide an appropriate condition to support disease progression from in situ neoplastic growth to tissue invasion and metastasis. Remarkably in normal and cancer cells, leptin has been shown to stimulate proliferation. In a study of normal uterine smooth muscle cells collected from myometrial biopsies, leptin induced cell proliferation at both low and high doses (6.25 and 50 ng/ml) [165]. In the same way, leptin was documented to stimulate the proliferation of normal intestinal epithelial cells

(IEC-6 cells) [166]. With regard to the proliferation of cancer cells, the pertinent information has been provided in Table 3 along with a brief etiologic account [116,167–180].

As mentioned above, both inhibition of apoptosis and formation of new blood vessels for adequate supply of oxygen with nutrients and removal of cellular wastes are the essential requirements for the progression of neoplastic disease. Anti-apoptotic effect of leptin in cancer is a frequent observation of many investigators [181–185]. Similarly, several reports have documented that leptin can facilitate the formation of new blood vessels or angiogenesis, along with biosynthesis of pro-angiogenic factors like VEGF [185–187]. It is worth mentioning that without angiogenesis usually a tumor cannot grow beyond 2 mm in diameter [188]. Nonetheless, the presence of vascular channels in the tumor microenvironment definitely promotes the process of metastasis.

Metastasis is a highly complex process, which leads to a number of hypotheses such as progression, transient compartment, and fusion models [189]. The progression model proposes the development of metastatic subpopulations or clone of mutated cells with metastatic potential within the primary tumor. It is known that tumor aneuploidy (chromosome number variation) and genetic heterogeneity/diversity can influence the disease behavior including metastatic capability [190–192]. Apart from genetic changes, epigenetic alterations such as DNA methylation and histone modifications could play a significant role in metastasis [193,194]. Accumulating evidence suggests that both genetic and epigenetic changes can occur in obesity [195–199]. Interestingly, epigenetic regulation of the leptin signaling pathway may cause leptin resistance in obesity [200]. It is noteworthy that an epigenetic phenomenon is the notion of the transient metastatic compartment model [189]. On the other hand, an important aspect of the genetic events is the role of metastasis suppressor genes in secondary tumors. Metastasis suppressors are molecules that inhibit metastasis formation without affecting primary tumor growth [201]. Functionally, leptin is associated with a number of metastasis suppressor molecules, e.g., caspase 8, CD44, kisspeptin, and thioredoxin interacting protein (TXNIP) [172,202–204].

For the development of secondary tumors, cancer cells undergo various steps from local invasion to distant metastasis. This process is involved with numerous intracellular signaling pathways and associated transduction molecules. Some of the well-studied signaling pathways/molecules include: MAPK/ERK, PI3K/AKT, STAT, TGF-β, and Wnt/β-Catenin [205]. Fascinatingly, many of these molecules are linked with the signal transduction pathways of leptin (Table 4 [14,108,109,113,118,120,129,142,144,148,152,153,206–222]).

A highly complex role is played by the tumor infiltrating immune cells, particularly macrophages. In general, studies have indicated that the presence of macrophages within the tumor microenvironment (i.e., TAMs) was associated with poor prognosis in cancer patients [223,224]. Often TAMs display M2 phenotype [225–227]. On the other hand, evidence suggests that obesity can favor M2 polarization [228,229]. Furthermore, leptin has been shown to influence M2 macrophages [148,230]. Nonetheless, macrophages are highly specialized cells having character of plasticity/adaptability in diverse microenvironments. Interestingly, many features of macrophages are also seen in metastatic cancer cells [231]. For instance, carcinomas of the lining epithelial cells can form a solid mass like

mesenchymal tissue. Of note, macrophages arise from the myeloid lineage that originates from mesenchymal cells (derivative of mesoderm). Moreover, many metastatic cancers express aerobic glycolysis (Warburg effect), which may occur in activated macrophages in chronic inflammatory lesions. Additionally, acquiring mesenchymal characteristics in the process of EMT happens during metastasis. Other interesting macrophage-like features of metastatic cells are: they can migrate and extravasate/infiltrate into the surrounding tissues, show phagocytic behavior, and release various pro-angiogenic molecules. All of these features have led to the proposition for the fusion model, which suggests the formation of fusion hybrids between cancer cells and macrophages [189,231]. So, there could be generation of fusion subclones with varying dissemination potentials.

In a recent report, Clawson and colleagues described macrophage-tumor cell fusions that were isolated from the blood of pancreatic ductal adenocarcinoma patients [232]. They observed that fusion cells expressed markers characteristic of pancreatic ductal adenocarcinoma and stem cells, as well as M2-polarized macrophages. In another study, M2 macrophages (derived from U937 cells) were fused with MCF-7 and MDA-MB-231 breast cancer cells [233]. The investigators found that the fusion hybrids had a more aggressive phenotype. It is worth noting that macrophages have the capacity to form fusion cells in physiologic and different pathologic circumstances. Multinucleated cells such as osteoclasts and foreign-body giant cells are thought to be generated by fusion of macrophages. Although the dichotomy of M1 and M2 macrophages is not based on very clear ideas, it has been suggested that M2 macrophages appear at the later phase of a lesion, they stimulate angiogenesis, synthesize ECM components and work towards resolution of inflammation [234]. Obviously, these properties are beneficial for the development of secondary tumors. Overall, excess adipose tissue is an attractive place for macrophages – because, obesity is considered as a state of low grade chronic inflammation. Like other inflammatory conditions, macrophages may influence the disease process or contribute to disease progression in obesity.

9. Conclusions

Leptin could influence cancer cells through numerous phenomena, e.g., inflammation and oxidative stress, cell proliferation, inhibition of apoptosis, angiogenesis, immune modulation, etc. In obesity, cancer cells constantly receive growth promoting stimuli in an environment where pro-inflammatory cytokines or adipokines in particular leptin dominate. Obviously, manipulation of this environment surrounding the neoplastic growth or tumor microenvironment is a challenging task. However, already some potential directions have been proposed such as increased biosynthesis of anti-inflammatory substances like adiponectin, leptin antagonists, and other pharmaceutical agents like metformin.

Leptin antagonists can be divided into 2 major groups: leptin analogs and antibodies against leptin or Ob-R. Leptin interacts with Ob-R through three different binding sites: I-III. Different analogs or muteins are formed by changing the amino acid sequence such as LDFI (in binding site I), LPrA2 (II), and Allo-aca (III). In general, administration of antagonists has been reported to cause weight gain. It is anticipated that orexigenic neuropeptide Y (NPY) will dominate in the hypothalamus in the absence of appropriate leptin signaling.

However, some adverse effects are serious and could be dangerous, e.g., insulin resistance, increased bone mass, disrupted locomotor activity, depressive behavior, and peripheral inflammation [235,236]. All these studies were conducted in experimental animals and for a relatively short duration. In real clinical situations, the treatment is expected to continue for a considerable time period and thus the adverse effects could be substantial. Furthermore, malignancies are often associated with comorbidities, which are common in patients with colon, breast, and lung cancers [237]. Consequently complications in such patients may aggravate in additional disorders such as obesity, insulin resistance or any inflammatory conditions. Development of methods for the local delivery of leptin antagonists at the tumor site is definitely a superior alternative.

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Abbreviations

AKT	protein kinase B (a serine/threonine kinase)
CAF	cancer-associated fibroblast
CTGF	connective tissue growth factor
ECM	extracellular matrix
EGFR	epidermal growth factor receptor
EMT	epithelial-mesenchymal transition
ER	estrogen receptor
ERK	extracellular signal-regulated kinase
HIF	hypoxia-inducible factor
ICAM	intercellular adhesion molecule
IGF	insulin-like growth factor
IL	interleukin
JAK	Janus kinase
MCP-1	monocyte chemoattractant protein-1
MMP	matrix metalloproteinase
NSCLC	non-small-cell lung cancer
Ob-R	leptin receptor
PI3K	phosphatidylinositol 3-kinase

STAT	signal transducer and activator of transcription
TAM	tumor-associated macrophage
TGF	transforming growth factor
TNF	tumor necrosis factor
TNM	tumor-lymph node-distant metastasis
VEGF	vascular endothelial growth factor

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Biographies

Amitabha Ray

Amitabha Ray completed his graduation in medicine (M.B.B.S.) from University of Calcutta, postgraduation (M.D.) from B.H.U., Varanasi, and Ph.D. from J.M.I., New Delhi, India. He pursued his postdoctoral research in Dr. Cleary's laboratory at the Hormel Institute, University of Minnesota. Currently, he is working with LECOM at Seton Hill University, Greensburg, PA, as an Associate Professor.

Margot P. Cleary

Dr. Margot P. Cleary is a professor at the Hormel Institute, University of Minnesota. She received an undergraduate degree in Chemistry from Regis College in Weston, MA and then did graduate work at Columbia University at the Institute of Human Nutrition earning MS, MPhil and Ph.D. degrees. Dr. Cleary's laboratory was one of the first to study the interactions of body weight with the development of breast and prostate cancers using preclinical models. In addition, she carried out extensive studies on the potential role of leptin as a growth factor linking obesity with breast cancer and proposed that the leptin to adiponectin ratio may be an important determinate in the development of some cancers.

In an interesting study, a leptin antagonist was demonstrated to stimulate appetite and weight gain in chronic kidney disease-associated cachexia in experimental animals [238]. The investigators also observed that the leptin antagonist normalized the expression of pro-inflammatory cytokines such as IL-6 and TNF-α. Cachexia in cancer is associated progressive wasting of body tissues, systemic inflammation, and poor prognosis. Leptin antagonists could be a promising inclusion in the therapy.

Highlights

1. The multifaceted action of leptin involves multiple cell types in the tumor microenvironment and intracellular signaling molecules; cooperation/interactions between these biomolecules in obesity could accelerate the process of cancer cell migration and metastasis.
2. Approximately half of the cancer burden and two-fifths of cancer deaths are attributable to cancers of the digestive and reproductive systems: usually leptin's role is important in these cancers.
3. Leptin has been demonstrated to affect the prognosis of both adenocarcinomas and squamous cell carcinomas, although obesity is not a risk factor for squamous cell carcinomas.
4. Several cancer dissemination mechanisms such as the involvement of matrix metalloproteinases (MMPs), M2 macrophages, and epithelial-mesenchymal transition (EMT) have been documented to be linked with leptin.

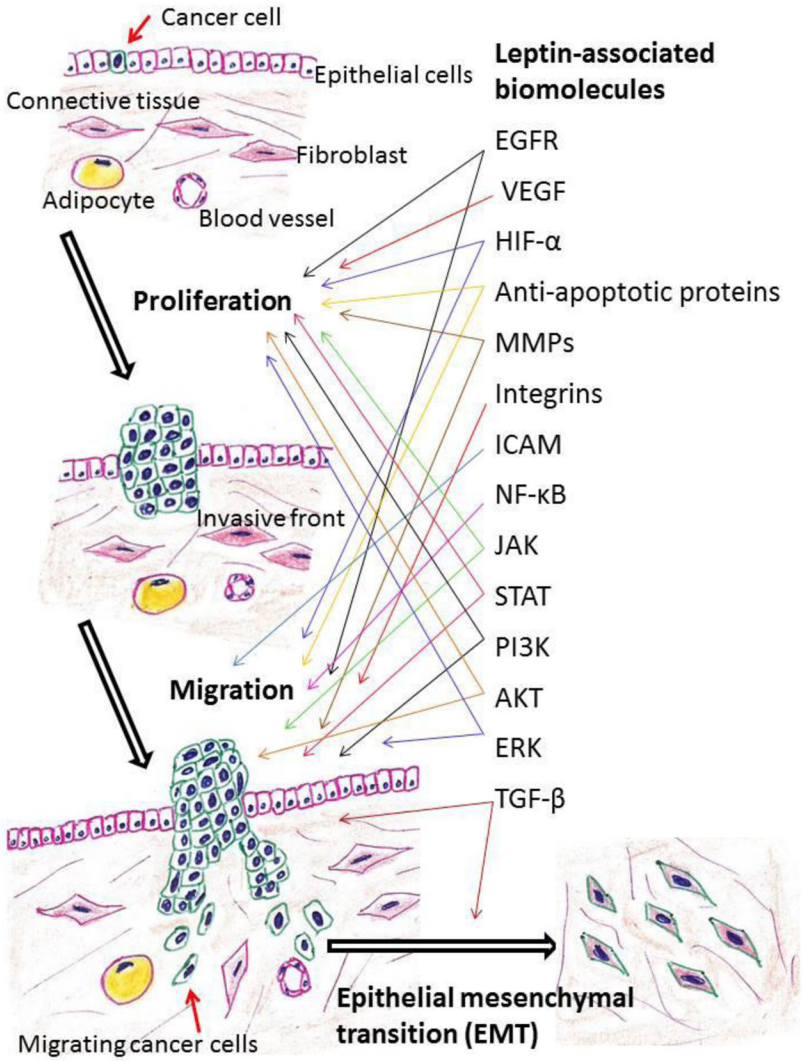

Fig. 1. Leptin-associated biomolecules that may promote cancer cell proliferation and migration

EGFR: Epidermal growth factor receptor, ERK: Extracellular signal-regulated kinases, HIF-1α: Hypoxia-inducible factor-1 alpha, ICAM: Intercellular adhesion molecule, JAK: Janus kinase, MMPs: Matrix metalloproteinases, NF-κB: Nuclear factor-kappa B, PI3K: Phosphatidylinositol 3-kinase, STAT: Signal transducer and activator of transcription, TGF-β: Transforming growth factor-beta, VEGF: Vascular endothelial growth factor

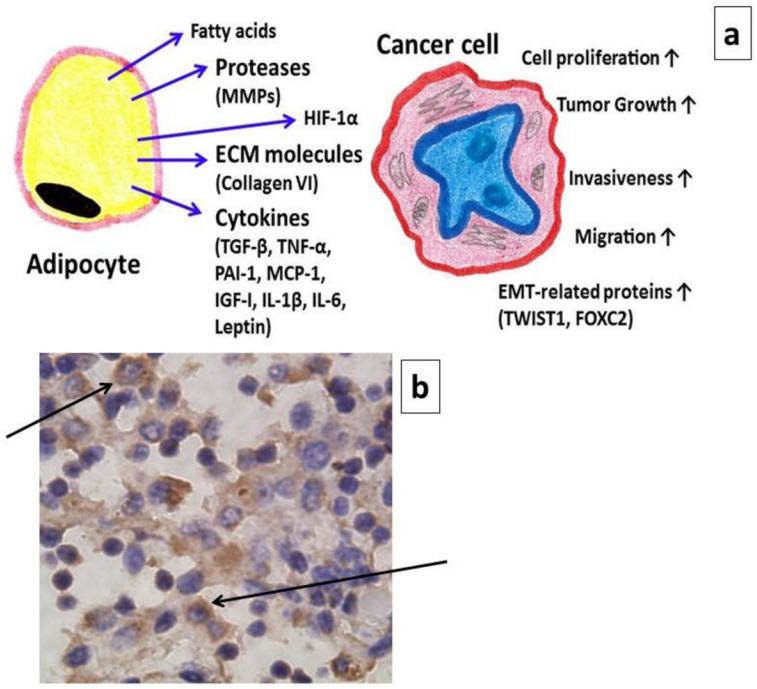

Fig. 2. Adipocytes in tumor microenvironment

(a) A schematic diagram to show the influence of adipocytes on the progression of cancer. (b) Immunohistochemical expression of leptin in tumor-adjacent adipose tissue, which was collected from the mammary fat pad of a CD-1 female mouse. In this xenograft model, T47D breast cancer cells were inoculated. The arrows indicate the presence of leptin in the cytoplasm of cells. (↑ - Increase)

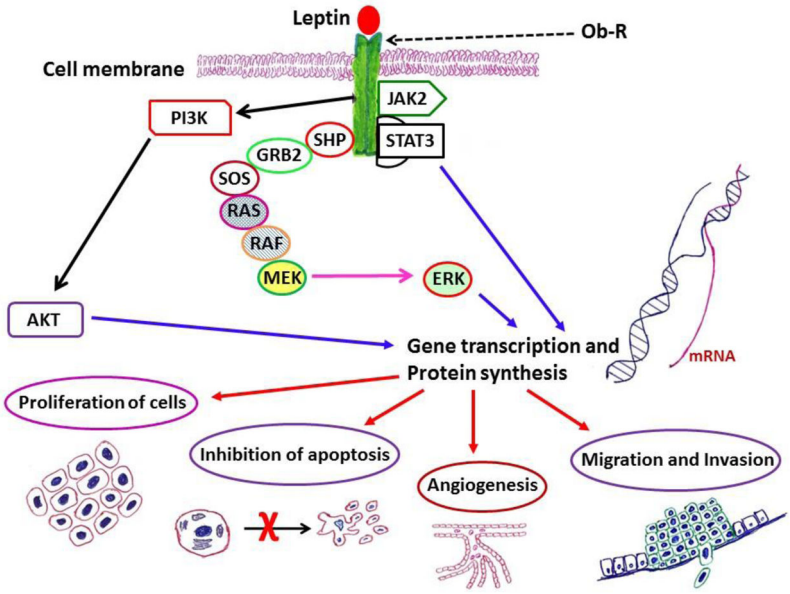

Fig. 3.

Leptin-related intracellular signaling molecules and their association with survival/anti-apoptotic and proliferative potential of cells.

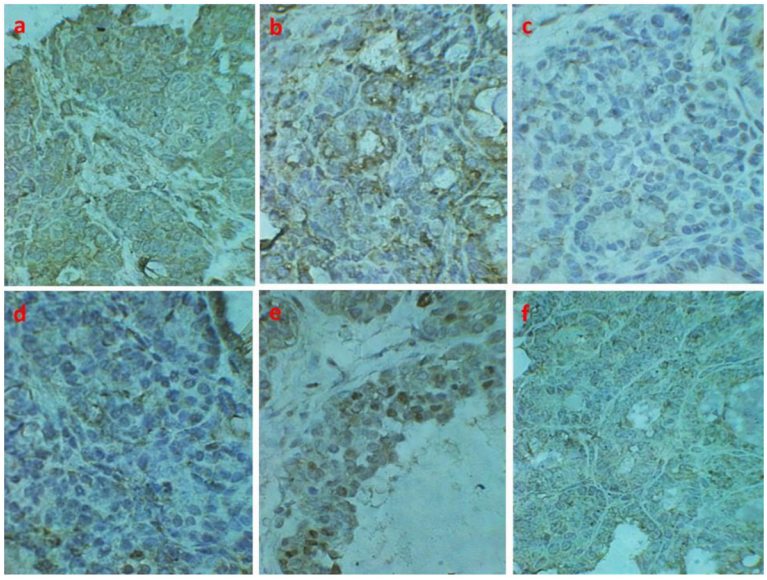

Fig. 4. Immunohistochemical expression of leptin receptor and relevant effector molecules in tissue sections from mammary tumors of TGFα mice

Expression of (a) Ob-Rb, (b) STAT3, (c) p-STAT3, (d) PI3K, (e) cell proliferation marker proliferating cell nuclear antigen (PCNA), and (f) anti-apoptotic protein Bcl-2. Presence of yellowish- to dark-brown color indicates positive staining.

Table 1

Summary of selected clinical studies that recorded a positive association between overexpression of leptin and/or its receptor proteins in tumor tissue and cancer cell invasion or metastasis

Site	Subjects	Findings	Investigators
Breast	76 invasive ductal carcinomas and 32 normal control samples.	Distant metastasis was detected in 21 (34%) of 61 Ob-R-positive tumors with leptin overexpression, but in none of the 15 tumors that lacked Ob-R expression or leptin overexpression.	Ishikawa et al. 2004 [104]
Colon	68 cases of colorectal carcinoma tissue, tumor adjacent tissue and normal colorectal tissue.	Expression of leptin and Ob-R in tumor was correlated with depth of bowel wall invasion, lymph node metastasis, and distant metastasis.	Liu et al. 2011 [117]
Colon	108 patients with colorectal carcinoma.	Leptin/Ob-R expression was significantly associated with TNM stage, lymph node metastasis, and distant metastasis.	Wang et al. 2012 [116]
Esophagus	122 cases of esophageal squamous cell carcinoma and 40 normal esophageal mucosa tissue.	Expression of leptin was significantly correlated with lymph node involvement and advanced tumor stage.	Duan et al. 2014 [102]
Lung	50 patients with lung cancer along with another 50 lung cancer patients with metastatic bone lesions.	Leptin was present at higher levels in bone metastasis cases.	Feng et al. 2013 [14]
Ovary	70 ovarian cancer cases.	Ob-Rb was highly overexpressed in metastases compared to primary tumors.	Kato et al. 2015 [16]
Pancreas	60 pancreatic cancer tissue specimens.	Expression of Ob-Rb was significantly stronger in patients with lymph node metastasis than in those without lymph node metastasis.	Fan et al. 2015 [90]
Stomach	343 cases of gastric carcinoma.	Ob-R expression was correlated with poor survival in 207 patients with advanced gastric cancer (muscle or deeper invasion), 139 of the Lauren diffuse group (poorly differentiated tumor cells scatter throughout the organ), and in 160 patients with lymph node metastasis.	Choi et al. 2015 [103]
Stomach	84 cases from primary gastric carcinoma.	Moderate to strong immnoreactivity for leptin was identified in 57% (n=48) cases. Leptin was positively correlated with lymph node metastasis and clinical stage.	Dong et al. 2014 [109]
Stomach	110 gastric cancer specimens and 96 normal gastric mucosa were analyzed.	Expression of leptin was associated with tumor invasion depth, lymph node metastasis, and expression of HER2 and VEGF.	Geng et al. 2012 [106]
Stomach	207 gastric carcinomas (100 early and 107 advanced carcinomas)	Expression levels of both leptin and Ob-R tended to increase as the depth of tumor invasion or TNM stage increased.	Ishikawa et al. 2006 [107]
Stomach	61 gastric cancer specimens.	Leptin expression was significantly associated with lymph node metastasis and higher stage.	Zhao et al. 2007 [105]
Thyroid	49 papillary thyroid carcinomas.	Coexpression of leptin and Ob-R in primary neoplasms had greater incidence of lymph node metastasis.	Cheng et al. 2010 [95]
Thyroid	93 cases with papillary thyroid carcinoma and 25 cases with medullary thyroid carcinoma.	For papillary thyroid carcinoma, expression of leptin and Ob-R was significantly correlated with lymph node metastasis and advanced stage. For medullary thyroid carcinoma, Ob-R was significantly correlated with lymph node metastasis and advanced stage.	Fan and Li 2015 [93]

Table 2

Relevant leptin antagonists that have been exhibited to prevent aggressive tumor behavior

Authors/Tissue	Antagonist used	Experimental design	Major observations
Bain et al. 2014 [111] Gastroesophageal adenocarcinomas (clinical samples) as well as in vitro experiments on gastric cancer cells and esophageal cancer cells.	Recombinant super human leptin antagonist (SHLA), which is a polypeptide chain containing 146 amino acids.	Cisplatin-resistant gastric cancer cell-line AGS Cis5 and esophageal adenocarcinoma cell-line OE33 were used. Originally, AGS cell-line was derived from a gastric adenocarcinoma of a Caucasian female. In a 96-well plate, cells were grown. Cisplatin and/or SHLA were added. Following incubation, MTT cell proliferation assay was performed.	Leptin antagonist SHLA increased the sensitivity of AGS Cis5 and OE33 cell-lines to cisplatin. SHLA inhibited the growth of OE33 cells when given alone. In both AGS Cis5 and OE33, SHLA inhibited leptin-induced cell proliferation.
Trevellin et al. 2015 [101] Esophageal adenocarcinoma patients, and in vitro study on esophageal adenocarcinoma cells.	Super human leptin antagonist (SHLA).	Treatment of OE33 cells with conditioned media (CM) collected from cultured biopsies of adipose tissue and peritumoral adipose tissue of patients with lymph node metastasis.	After treatment with CM, mRNA levels of two key EMT regulator genes, alpha-smooth muscle actin (α-SMA) and E-cadherin, were increased. However, SHLA diminished the mRNA expression of α-SMA and E-cadherin.
Catalano et al. 2015 [112] In vitro and in vivo experimental models using ERα-positive (MCF-7) and -negative (SK-BR-3) breast cancer cells.	Pegylated LDFI (LDFI-PEG): polyethylene glycol (PEG)-attached to tetrapeptide that mimics the sequence of leptin binding site I. Leptin interacts with Ob-R through 3 different binding sites: I–III. Site I is crucial for the formation of an active leptin–Ob-R complex and in its subsequent activation. Amino acids 39–42 (Leu-Asp-Phe-Ile-LDFI) were shown to contribute to leptin binding site I.	Injected SK-BR-3 breast cancer cells into the intrascapular region of female nude mice (nu/nu Swiss) and followed tumor growth after administration of LDFI-PEG.	After LDFI-PEG treatment, tumor volumes continued to reduce over control for the duration of experiment. At the end of treatment (28 days) it was observed that PEG-LDFI induced a significant tumor growth inhibition compared to vehicle-treated mice. Sections of tumors from PEG-LDFI-treated mice exhibited a reduction in the expression of proliferation marker Ki-67, and phosphorylated levels of STAT3, MAPK and AKT than controls.

AKT: Protein kinase B/a serine/threonine kinase, EMT: Epithelial-mesenchymal transition, MAPK: Mitogen-activated protein kinase, STAT3: Signal transducer and activator of transcription 3

Table 3

Possible mechanisms of leptin-induced cancer cell proliferation

Investigators and Cancer types	Involved intracellular signaling pathways/Mechanisms	Principal mediating factors/phenomena
Blanquer-Rosselló Mdel et al. 2016 [167] MCF-7 breast cancer cells	Lipid metabolism pathways, AMPK	Leptin favored the use of glucose for biosynthesis and lipids for energy production
Chen et al. 2013 [168] OVCAR-3 ovarian cancer cells	PI3K/AKT and MEK/ERK1/2 pathways	Leptin enhanced the expression of regulators of cell proliferation and apoptosis inhibition, cyclin D1 and Mcl-1
Chin et al. 2017 [169] SKOV-3 and OVCAR-3 ovarian cancer cells	PI3K, STAT3, ERK1/2	Leptin increased FSH, stimulated ERα and the expression of ERα-responsive genes
Dubois et al. 2014 [170] MCF-7 and T47D breast cancer cells	Estrogenic pathway, leptin autocrine/paracrine signaling loop	Leptin stimulated proliferation, overexpression of leptin, Ob-R, ER, and aromatase
Habib et al. 2015 [171] PC-3 prostate cancer cells	Estrogenic pathway	Leptin increased the expression of ERα, aromatase and CYP1B1, and decreased the expression of ERβ and COMT
Harbuzariu et al. 2017 [172] BxPC-3, MiaPaCa-2, Panc-1, AsPC-1 pancreatic cancer cells	Notch signaling pathway, leptin autocrine/paracrine signaling loop	Leptin increased cell cycle progression and proliferation
Kim et al. 2017 [173] MCF-7 breast cancer cells	STAT3, AKT, ERK, JNK, PKA, cAMP	Leptin increased COX-2 expression and PGE2 production, increased aromatase expression
Liu et al. 2013 [174] Ishikawa endometrial cancer cells	Estrogenic pathway	Leptin stimulated cell proliferation via enhancing aromatase expression and estradiol synthesis
Nepal et al. 2015 [175] HepG2 hepatoma and MCF-7 breast cancer cells	p53/FoxO3A axis	Leptin augmented the expression of autophagy-related genes, including beclin-1, Atg5 and LC3 II, which caused increase in cell number and suppression of apoptosis
Ptak et al. 2013 [176] OVCAR-3 ovarian cancer cells	Increase in cyclin D1, cyclin A2, and a decrease in p21WAF1/CIP1, Bad, TNFR1, and caspase 6	Leptin promoted cell proliferation and downregulated apoptotic pathway
Qian et al. 2015 [177] MCF-7 and tamoxifen-resistant cells	ERK1/2, STAT3	Leptin enhanced proliferation, and CCND1 (cyclin D1) gene transcription by inducing the binding of ERα to the promoter of CCND1 gene
Shouman et al. 2016 [178] MCF-7 breast cancer cells	Estrogenic pathway	Leptin increased CYP1B1 expression and DNA adducts, and diminished COMT protein expression
Wang et al. 2012 [116] HCT-116 colon cancer cells	PI3K/AKT/mTOR pathway	Leptin stimulated proliferation and inhibited apoptosis
Xu et al. 2013 [179] SKOV3 and A2780 ovarian cancer cells	STAT5	Leptin promoted cell proliferation in cooperation with microRNA-182 and microRNA-96 (targeting FoxO3)
Yoon et al. 2014 [180] LS174T, HCT-116 and CaCo-2 colon cancer cells	JAK and ERK signaling pathways	Leptin increased the number of cells

AKT: v-Akt murine thymoma viral oncogene or protein kinase B (a serine/threonine kinase), AMPK: AMP-activated protein kinase, Atg5: autophagy protein 5, cAMP: cyclic AMP, COMT: catechol-o-methyltransferase, COX-2: cyclooxygenase-2, CYP1B1: cytochrome P450 1B1, ER: estrogen receptor, ERK: extracellular signal-regulated kinase, FoxO3A: forkhead box O3, FSH: follicle-stimulating hormone, JAK: Janus kinase, JNK: c-Jun N-terminal kinase, LC3: light chain 3 (microtubule-associated), Mcl-1: myeloid cell leukemia 1, MEK: mitogen-activated protein kinase kinase, mTOR: mechanistic/mammalian target of rapamycin, Ob-R: leptin receptor, p21WAF1/CIP1: cyclin-dependent kinase inhibitor, PGE2: prostaglandin E2, PI3K: phosphatidylinositol-3-kinase, PKA: protein kinase A, STAT3: signal transducer and activator of transcription 3, TNFR1: tumor necrosis factor receptor 1

Table 4

Proposed mechanisms of cancer cell migration and metastasis under the influence of leptin

Investigators and Cancer types	Nature of neoplastic progression	Mechanisms or involved intracellular signaling pathways	Observed phenomena
Cao et al. 2016 [148] MCF-7 and MDA-MB-231 breast cancer cells, and xenograft model	Migration and metastasis	p38 MAPK and ERK1/2	Leptin-induced tumor-associated M2 macrophage-derived IL-8 promoted the migration and invasion/ metastasis of cancer cells in both in vitro and xenograft model
Cheng et al. 2011 [129] K1 and B-CPAP papillary thyroid cancer cells	Migration	AKT and ERK pathways	Leptin enhanced the migratory activity
Ding et al. 2016 [206] HepG2 hepatocellular carcinoma and MCF-7 breast cancer cells	Migration	STAT3, ERK1/2, and AKT	APPL1 positively mediated leptin signaling and promoted leptin-induced proliferation and migration of cancer cells
Dong et al. 2013 [108] AGS, MKN-28 and MKN-45 gastric cancer cells	Migration	AKT and ERK1/2	Leptin upregulated MT1-MMP expression and enhanced the interaction of MT1-MMP with KIF1B, which contributed to cancer cell invasion
Dong et al. 2014 [109] AGS and MKN-45 gastric cancer cells	Migration	Rho/ROCK pathway	Leptin enhanced cancer cell migration by upregulating ICAM-1
Fan et al. 2015 [120] PANC-1 and AsPC-1 pancreatic cancer cells, and clinical samples	Migration and metastasis	JAK2/STAT3 pathway	Leptin upregulated the expression of MMP-13
Fava et al. 2008 [207] Intrahepatic cholangiocarcinoma HuH-28 cells	Migration	STAT3 and ERK1/2	Leptin increased the growth and migration of cancer cells
Feng et al. 2013 [14] A549 lung cancer cells	Metastasis	TGF-β	Leptin promoted the metastasis by inducing EMT in a TGF-β-dependent manner
Frankenberry et al. 2004 [208] androgen-independent DU145 and PC-3 prostate cancer cells	Migration	MAPK and PI3K (mainly)	Leptin enhanced cancer cell migration, and induced expression of VEGF, TGF-β1, and bFGF
Ghasemi et al. 2017 [209] OVCAR3, SKOV3 and CaoV-3 ovarian cancer cells	Migration	RhoA/ROCK, PI3K/AKT, JAK/ STAT pathways and NF-κB activation	Leptin induced cancer cell invasion via upregulating uPA
Ghasemi et al. 2017 [210] SKOV3 and OVCAR3 ovarian cancer cells	Migration	ERK and JNK pathways	Leptin regulated cancer cell invasion by promoting MMP-7 expression
Guo and Gonzalez-Perez 2011 [142] Mouse 4T1, EMT6 and MMT breast cancer cells	Migration	JAK2/STAT3, MAPK, PI3K/ mTOR, p38 MAPK and JNK signaling pathways	Leptin upregulated cell proliferation/ migration and pro-angiogenic factors Notch, IL-1 and VEGF/VEGFR-2
Huang et al. 2011 [152] PC-3, DU145, and LnCaP prostate cancer cells	Migration	IRS-1, PI3K, AKT, and NF-κB	Leptin increased the migration of cancer cells. Moreover, leptin upregulated αvβ3 integrin expression
Huang et al. 2017 [211] MCF-7 and T47D breast cancer cells	Migration	PI3K/AKT/SREBP2 pathway	Leptin enhanced the proliferation, migration and invasion of cancer cells via ACAT2 upregulation
Knight et al. 2011 [212] MCF-7 and MDA-MB-231 breast cancer cells	Migration	EGFR, Notch1, and survivin	Leptin increased the migration potential of cancer cells

Investigators and Cancer types	Nature of neoplastic progression	Mechanisms or involved intracellular signaling pathways	Observed phenomena
Li et al. 2016 [144] THP1 human monocytes, and MCF-7, SK-BR-3 and MDA-MB-231 breast cancer cells, and xenograft model	Migration and metastasis	NF-κB signaling in TAMs, and PI3K-AKT/ATF-2 signaling in cancer cells	Leptin stimulated IL-18 expression in both TAMs and breast cancer cells
Martín et al. 2017 [213] Human astrocytoma 1321N1 cells	Migration	EGFR, and ERK, AKT/mTOR pathways	Leptin and sPLA2-IIA increased growth and migration in these cells
Mendonsa et al. 2015 [214] Murine Panc02 and human Panc1 pancreatic cancer cells	Migration	PI3K/AKT pathway	Leptin increased migration of cancer cells
Mishra et al. 2017 [215] Breast epithelial and cancer cells: MCF10A, MCF10AT1, MCF-7 and MDA-MB-231	Metastasis	TGF-β1 pathway	Leptin-mediated changes represented EMT and a cancer stem cell-like phenotype
Noda et al. 2015 [216] LNCaP, DU145 and PC-3 prostate cancer cells	Migration	PI3K/AKT	Leptin increased the cell proliferation, migration, and invasion. Leptin also increased the phosphorylation of FOXO1, the expression of cyclin D1, and decreased the expression of p21 protein
Ratke et al. 2010 [118] SW480, SW620, and HCT-116 colon cancer cells	Migration	JAK, STAT3, Src kinase, FAK, PI3K, PKCδ	Leptin enhanced the migratory activity of cancer cells
Saxena et al. 2007 [217] HepG2 and Huh7 hepatocellular carcinoma cells	Migration	JAK/STAT3, ERK, and PI3K	Leptin promoted cancer cell growth, invasiveness, and migration
Sobrinho Santos et al. 2017 [218] SCC-9 and SCC-4 oral squamous cell cancer cells, and clinical samples	Migration	E-cadherin, Col1A1, MMP-2 and -9, mir-210 and HIF-1α	Leptin favored higher cell proliferation, migration, and reduced apoptosis. Furthermore, leptin decreased caspase-3 mRNA expression
Wang et al. 2013 [219] MCF-7 breast cancer cells	Migration	JAK/STAT and PI3K/AKT signaling pathways	Leptin promoted migration and invasion. Leptin also increased the expression of MMP-9 and TGF-β
Wei et al. 2016 [220] MCF-7, SK-BR-3, and MDA-MB-468 breast cancer cells	Migration and metastasis	PI3K/AKT signaling pathway	Leptin promoted EMT in breast cancer cells via upregulation of PKM2
Yang et al. 2009 [153] JJ012 and SW1353 chondrosarcoma cells	Migration	IRS-1, PI3K, AKT and NF-κB	Leptin increased migration and expression of αvβ3 integrin in human chondrosarcoma cells
Yeh et al. 2009 [221] C6 glioma cells	Migration	p38 MAPK and NF-κB pathways	Leptin enhanced the migration and invasion of glioma cells. Leptin also upregulated the expression of MMP-13
Yuan et al. 2014 [222] MCF-7 breast cancer cells	Migration	ERK pathway	Leptin increased the proliferation and migration of cancer cells
Zou et al. 2016 [113] Gallbladder cancer cell subline GBC-SD, xenograft model, and clinical samples	Migration	JAK2/STAT3/SOCS3	Leptin promoted the proliferation, migration and invasion of cancer cells. Leptin upregulated VEGF-C and VEGF-D levels and activated MMP-3 and MMP-9

ACAT2: acetyl-CoA acetyltransferase 2, AKT: v-Akt murine thymoma viral oncogene or protein kinase B (a serine/threonine kinase), APPL1: adaptor protein, phosphotyrosine interacting with PH domain and leucine zipper 1 (involved in the regulation of cell proliferation), ATF-2: activating transcription factor 2, bFGF: basic fibroblast growth factor, Col1A1: collagen type I alpha 1, EGFR: epidermal growth factor receptor, EMT: epithelial-mesenchymal transition, ERK: extracellular signal-regulated kinase, FAK: focal adhesion kinase, FOXO1: forkhead box O1, HIF-1α: hypoxia-inducible factor 1α, ICAM-1: Intercellular adhesion molecule-1, IL: interleukin, IRS: insulin receptor substrate, JAK: Janus kinase, JNK: c-Jun N-terminal kinase, KIF1B: kinesin family member 1B (involved in the transport of materials), MAPK: mitogen-activated protein kinase, mir-210: mir-210 microRNA (predominant hypoxia-responsive miRNA), MMP: matrix metalloproteinase, MT1-MMP: membrane

type 1-matrix metalloproteinase, mTOR: mechanistic/mammalian target of rapamycin, NF-κB: nuclear factor-kappa B, PI3K: phosphatidylinositol-3-kinase, PKC: protein kinase C, PKM2: pyruvate kinase M2 isoform, Rho: G protein under the Ras homologue gene family, RhoA: Rho family small GTPase-member A, ROCK: Rho-associated coiled-coil-forming protein kinase, SOCS3: suppressor of cytokine signaling-3, sPLA2-IIA: secreted phospholipase A2-IIA, Src: avian sarcoma viral oncogene homolog, SREBP2: sterol regulatory element-binding protein 2, STAT3: signal transducer and activator of transcription 3, TAM: tumor-associated macrophage, TGF-β: transforming growth factor beta, uPA: urokinase plasminogen activator, VEGF: vascular endothelial growth factor, VEGFR-2: vascular endothelial growth factor receptor 2.

Oncology Evidence-Based Nutrition Practice Guideline for Adults

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Editor's note: Figure 4 and Tables 1, 2, 3, and 4 that accompany this article are available online at www.andjrnl.org.

CANCER IS A TERM USED TO describe a group of more than 100 multifactorial diseases in which abnormal cells reproduce in an uncontrolled manner and are able to spread to other parts of the body and invade healthy tissues.[1] Numbers of cancer-related deaths have fallen steadily since the 1990s, and the number of cancer survivors has increased.[2] The National Cancer Institute has estimated that 1,685,210 new cases will be diagnosed and 595,690 deaths will occur in 2016.[2] Cancers develop from complex interactions between genes and the environment.[3] Although many of the specific pathways by which nutritional status can impact cancer remain poorly understood,[4] it is well recognized that nutrition plays important roles in cancer prevention and treatment.[4-8]

In 2007, the Academy of Nutrition and Dietetics (Academy) published guideline recommendations on the Evidence Analysis Library (EAL) related to nutrition interventions for specific types of cancer and cancer treatments. In 2010, a new evidence analysis workgroup was formed to supplement the original guideline, which was subsequently published on the EAL during November 2013. The current guideline focuses on comprehensive oncology nutrition practice for the care of adult patients with cancer. Although the recommendations are written for registered dietitian nutritionists (RDNs), others may find them helpful.

The guideline developed by the workgroup will be reviewed, beginning with the recommendations that are based on the related EAL systematic review, followed by a brief review of recommendations based on organization guidelines outside of the Academy.[9-11] The latter were included to further expand the scope of the evidence-based recommendations. Finally, a brief review of the consensus-based recommendations will be provided to further guide the RDN, where there is less nutrition research or the research is difficult to elucidate.

DEVELOPMENT OF CONCLUSION STATEMENTS AND RECOMMENDATIONS

The Academy's 5-step systematic review process[12] was followed throughout the project. The Oncology Workgroup chose to principally target four areas of oncology nutrition in adults where there was an adequate pool of evidence related to nutritional status and nutrition interventions:

- validity of malnutrition screening and nutrition assessment tools;
- the association among nutritional status and morbidity and mortality outcomes;
- the effect of medical nutrition therapy (MNT)[13] on patients undergoing chemotherapy (CT) and radiation treatment (RT); and
- cancer cachexia and the effect of dietary supplements and medical food supplements (MFS) containing fish oil (specifically eicosapentaenoic acid [EPA]), on body weight and lean body mass (LBM).

A comprehensive literature search was conducted using PubMed and Cumulative Index to Nursing and Allied Health Literature databases, with search inclusion dates 1993 to 2011. For the final questions on fish oil, search inclusion dates were 1990 to 2013 to adequately evaluate the body of literature on this topic. Additional articles were identified by hand searching reference lists from pertinent review articles. Figure 1 shows the criteria applied to the inclusion and exclusion of studies for each question. Figure 2 illustrates the search strategy and study selection process.[14] A total of 102 primary research articles were included in the final analysis.

Following the research analysis, conclusion statements were written and the strength of the evidence was graded by the workgroup based on quality, consistency, sample size, clinical impact, and generalizability of the studies. Full conclusion statements are found on the EAL (www.andeal.org). Conclusion statements were graded as I (Good/strong), II (Fair), III (Limited/weak), IV (Expert

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Criteria	Inclusion	Exclusion
Age	≥18 y	<18 y
Setting	Ambulatory care, acute care	Intensive situations such as intensive care unit and critical care
Health status	Cancer patients undergoing treatment or not undergoing treatment	Cancer prevention; cancer survivorship
Study design preferences	Randomized controlled trials; controlled clinical studies; cohort studies; case-control studies; systematic reviews; meta-analyses	Review articles; hand search pertinent review articles
Size of study groups	≥10 subjects in each study group	<10 subjects in each group
Subject dropout rate	<20% <35% (advanced stage cancer patients)	>20% >35% (advanced stage cancer patients)
Year range	1990 through March 2013[a] 1993 through October 2011[b] 1993 through May 2011	Before 1990[a] Before 1993
Language	English	Not in English
Subjects	Human	Animal
Other	Article must be published in a peer-reviewed journal	Not peer-reviewed journal Studies by same author similar in content Abstracts or presentations
Subtopic-specific criteria applied in addition to the above criteria		
Anthropometric data[a]	Focus of study is on weight, lean body mass, or both; change from baseline must be reported	Focus of study is not on weight, lean body mass, or both; change from baseline not reported
Fish oil components[a]	At least 1 arm includes medical food supplement or dietary supplement containing EPA[c] and DHA[d] (with no confounding factors)	No medical food supplement or dietary supplement containing EPA and DHA; confounding factors
Medical nutrition therapy[ef]	Dietary intervention provided by registered dietitian nutritionist (credentialed in the United States; has a reciprocity agreement with the Commission on Dietetic Registration or reasonably equivalent); >1 medical nutrition therapy visit; individualized approach	Dietary intervention not provided by a registered dietitian nutritionist (or equivalent); only 1 medical nutrition therapy visit; approach not individualized
Anticancer Treatment[e]	Undergoing chemotherapy or radiation therapy during nutrition intervention	Not undergoing chemotherapy or radiation therapy during nutrition intervention
Validated tools[g]	Must include reference standard used for malnutrition screening or nutrition assessment tool validation	No reference standard used for tool validation

[a]Criteria applicable only to the following subtopic: fish oil.

[b]Criteria applicable only to the following subtopics: nutritional status and outcomes, malnutrition screening tools.

[c]EPA=eicosapentaenoic acid.

[d]DHA=docosahexaenoic acid.

[e]Criteria applicable only to the following subtopics: medical nutrition therapy and chemotherapy; medical nutrition therapy and radiation treatment.

[f]Medical nutrition therapy is provided by a registered dietitian nutritionist.

[g]Criteria applicable only to the following subtopics: malnutrition screening tools; nutrition assessment tools.

Figure 1. Search strategy and inclusion and exclusion criteria for articles for the Oncology Guideline 2013.

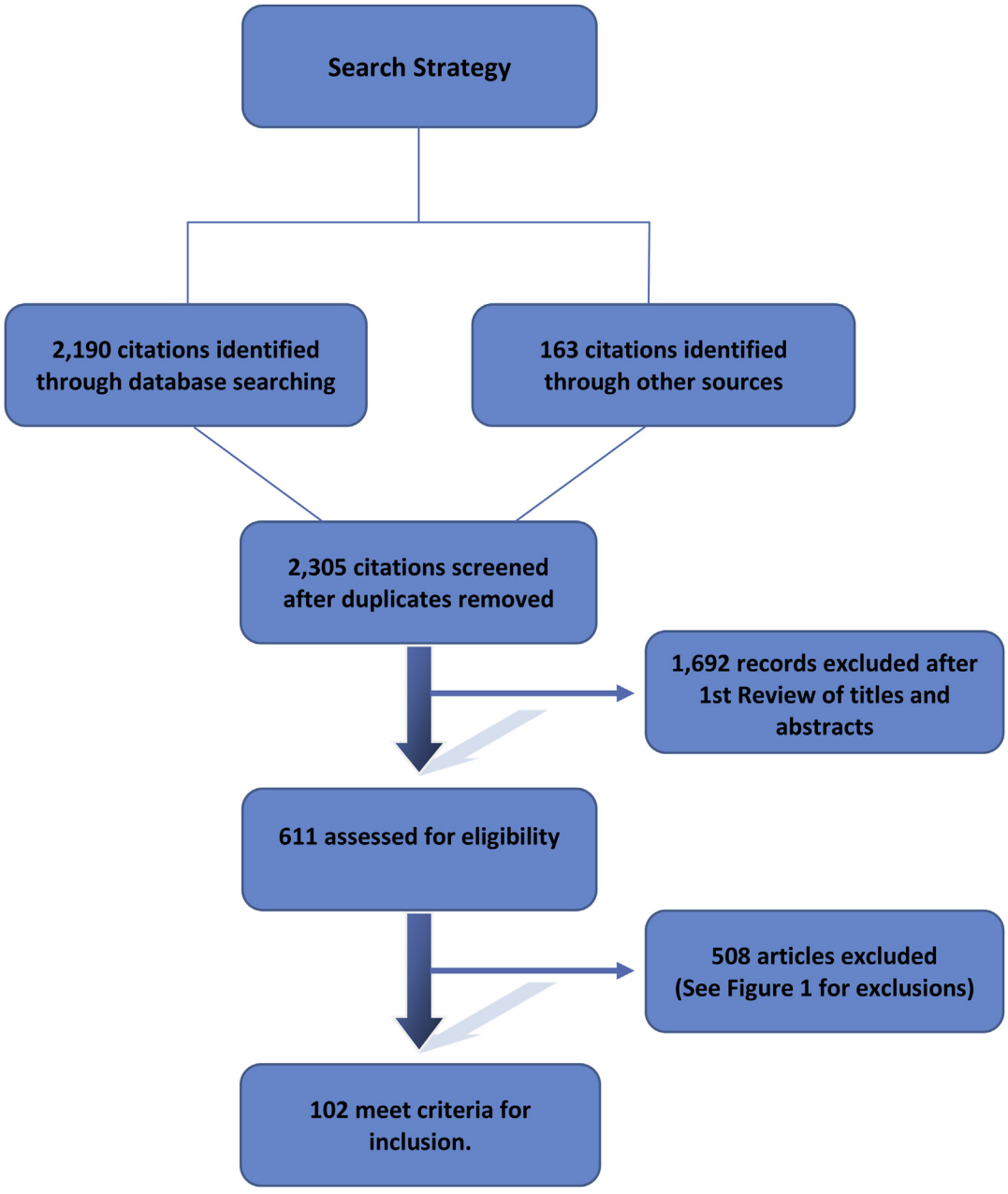

Figure 2. Flow diagram of the search strategy and selection process used in the Evidence Analysis Library systematic review for the Oncology Guideline 2013. Flow diagram template adapted from Moher and colleagues.[14]

opinion only), or V (Not assignable). A complete description of the grading definitions is described on the EAL and elsewhere.[12]

The 16 conclusion statements for the four EAL oncology subtopic areas were integrated into the formulation of seven recommendations. In addition to the seven evidence-based recommendations, the workgroup incorporated five nutrition-related recommendations based on external organization guidelines and 11 consensus recommendations to broaden the comprehensiveness of the guideline. In all, 23 nutrition practice recommendations were created.

The workgroup used expert consensus to write conditional (clearly defines a specific situation) or imperative (broadly applicable to a target population, with restraints on their pertinence) recommendation statements. The recommendations were rated as strong, fair, weak, consensus, or insufficient, based on standardized rating rubrics developed by the Academy. A complete description of the criteria for recommendation rating is available on the EAL. For the inclusion of specific oncology nutrition recommendations from sources outside of the Academy, the Evidence-Based

External Guideline[a] Recommendation rating scale	EAL rating[b] equivalent
American Society of Parenteral and Enteral Nutrition Clinical Guidelines: Nutrition Support Therapy During Adult Anticancer Treatment and in Hematopoietic Cell Transplantation, 2009[9]	
A: Supported by at least 2 level I investigations	Strong
B: Supported by 1 level I investigation	Fair
C: Supported by level II investigations only	Fair
D: Supported by at least 2 level III investigations	Weak
E: Supported by level IV or level V evidence	Consensus
Clinical Oncological Society of Australia: Evidence-Based Practice Guidelines for the Nutritional Management of Adult Patients with Head and Neck Cancer, 2011[10]	
A: Body of evidence can be trusted to guide practice	Strong
B: Body of evidence can be trusted to guide practice in most situations	Fair
C: Body of evidence provides some support for recommendation(s) but care should be taken in its application	Fair
D: Body of evidence is weak and recommendation(s) must be applied with caution	Weak
Oncology Nursing Society: Putting Evidence into Practice, 2009[11]	
Recommended for practice Effectiveness is demonstrated by strong evidence from rigorously designed studies, meta-analyses, or systematic reviews. Expected benefit exceeds expected harms.	Strong
Likely to be effective Evidence is less well established than for those listed under recommended for practice	Fair, or Consensus (if based on consensus documents)
Benefits balanced with harms Clinicians and patients should weigh the beneficial and harmful effects according to individual circumstances and priorities	Weak
Effectiveness not established Data currently are insufficient or are of inadequate quality	Weak
Effectiveness unlikely Lack of effectiveness is less well established than those listed under not recommended for practice	Weak
Not recommended for practice Ineffectiveness or harm clearly is demonstrated, or cost or burden exceeds potential benefit	Strong, Fair, or Weak

[a]Guidelines published outside of the Academy of Nutrition and Dietetics.

[b]EAL Guideline Recommendation Rating System (www.andeal.org/recommendation-ratings).

Figure 3. Academy of Nutrition and Dietetics Evidence Analysis Library (EAL) external guideline rating equivalencies.

Practice Committee approved the use of guidelines from three external organizations[9-11] that had similar methodology. Because the grading systems were not correlated clearly to the Academy's EAL rating scale, the Evidence-Based Practice Committee approved a rating equivalency (Figure 3), which allowed the Academy's ratings to be applied consistently.

The guideline underwent both an internal and external review, the latter of which consisted of an interdisciplinary group of health professionals. The guideline was adjusted by consensus of the expert work group and approved by the Academy's Evidence-Based Practice Committee before publication on the EAL. The final recommendations and the conclusion statements (or external guidelines) supporting them are listed in order of the Nutrition Care Process in Figure 4 (available online at www.andjrnl.org) and are on the EAL website (www.andeal.org).[15] For each of the recommendations below, the rationale summarizes the evidence, followed by the rationale based on external guidelines, and finally the rationale for recommendations based on consensus publications.

GUIDELINE APPLICATION

This guideline was developed for RDNs caring for adult oncology patients in

ambulatory and acute care settings; therefore, clinical judgment is crucial in the application of these guidelines to adult oncology patients in other settings or to children and adolescents with cancer. Careful consideration should be given to the application of these guidelines for patients receiving hospice, palliative care, or those with significant medical comorbidities. Advance directives may also indicate whether treatment is desired or not.

EAL RECOMMENDATIONS

Validated Tools for Malnutrition Screening and Nutrition Assessment

Recommendation

- Adult oncology patients should be screened using a malnutrition screening tool validated in the setting in which the tool is intended for use. The following tools have been shown to be valid and reliable in identifying malnutrition risk in adult oncology patients:
  - Inpatient settings: Malnutrition Screening Tool (MST), Malnutrition Screening Tool for Cancer Patients, and Malnutrition Universal Screening Tool.
  - Ambulatory/outpatient settings: MST.

Rating: Strong; Imperative

Recommendation

- RDNs should use an assessment tool validated in the setting in which the tool is intended for use as part of the complete nutrition assessment. The Patient Generated–Subjective Global Assessment (PG-SGA) and Subjective Global Assessment tools have been shown to elicit valid and reliable data as part of a comprehensive nutrition assessment of adult oncology patients in ambulatory and acute care settings.

Rating: Strong; Imperative

Rationale: Malnutrition screening and rescreening identifies patients who would benefit from nutrition assessment and intervention by an RDN. The importance of using a tool validated in the population and setting in which it is intended has been described elsewhere.[16] Seven studies,[17-23] shown in Table 1 (available online at www.andjrnl.org), evaluated the validity and reliability of one malnutrition screening tool (ie, MST) in the ambulatory setting and five tools in the acute care setting (ie, the 2-item nutrition screen from the Zung Self-Rating Depression Scale, Malnutrition Advisory Group Malnutrition Screening tool, MST, Malnutrition Screening Tool for Cancer Patients, and Malnutrition Universal Screening Tool). In the ambulatory setting, the MST[19,21] was found to be valid and reliable for identifying malnutrition risk in adult oncology patients. In acute care settings, three tools were found to be valid and reliable for identifying malnutrition risk in adult oncology patients: the MST,[17,20] the Malnutrition Screening Tool for Cancer Patients,[20] and Malnutrition Universal Screening Tool,[17] whereas the Malnutrition Advisory Group Malnutrition Screening tool[18] and the 2-item nutrition screen from the Zung Self-Rating Depression Scale[23] were not found to be valid and reliable in this setting. The PG-SGA tool is often used as both a screening tool (to determine risk for malnutrition) and an assessment tool (to determine presence of malnutrition) in adult oncology patients. At the time this review was completed, there were no validation studies for the PG-SGA short form, which consists of four history-related questions completed by the patient. Thus, the tool was included in the nutrition assessment evidence below, rather than as a separate malnutrition screening tool.

Seven studies,[24-30] shown in Table 1 (available online at www.andjrnl.org), evaluated the validity and reliability of nutrition assessment tools. The PG-SGA[24-29] and the Subjective Global Assessment[28] were found to be valid and reliable in identifying malnutrition as part of a comprehensive nutrition assessment in adult oncology patients in both ambulatory and acute care settings. The Malnutrition Assessment[30] was evaluated in patients in ambulatory care settings, and was found to have the sensitivity to diagnose oncology patients with malnutrition in the ambulatory setting, but was only moderately specific in identifying malnutrition when compared with the PG-SGA. The Malnutrition Assessment was not evaluated in an acute care setting.

Of the 14 studies included in the malnutrition screening and nutrition assessment tool questions, six studies[18-20,24,25,27] used the Subjective Global Assessment as the reference standard, whereas four[21-23,30] used the PG-SGA as the reference standard. Other reference standards included anthropometric[26] and biochemical measures,[28] food and nutrition practitioner assessment,[20,29] and the Nutritional Risk Screening 2002 (NRS-2002).[17]

Evaluation of Nutritional Status as Key Component in Patient Care Process

Recommendation

- RDNs should collaborate with other health care professionals, administrators, and public policy decision makers to ensure that the evaluation of nutritional status is a key component of the adult oncology patient care process.

Rating: Strong; Imperative

Rationale: The workgroup selected six outcomes where the impact of nutritional status could be measured. These outcomes included mortality because it relates to the cancer diagnosis, and five morbidity outcomes (hospital admissions and readmissions, hospital length of stay [LOS], quality of life [QoL], and RT and CT treatment tolerance). The studies included in this topic may have included other outcomes that were not reviewed in this analysis.

Forty-five studies,[17,25,29,31-72] shown in Table 2 (available online at www.andjrnl.org), examined the associations among nutritional status and the six outcomes. Several studies reported on more than one outcome. Nutritional status was measured in a number of ways, including body composition (eg, LBM,[62,67] loss of subcutaneous fat,[38] and sarcopenia[60,61]), weight status (eg, body mass index[44,71]), weight loss,[38,41,44,47,52,56,67] functional status (eg, handgrip strength[41,49]), biochemical indicators (eg, albumin[40,44,46,49,50]), and food and nutrition intake.[37] In addition, a number of studies measured nutritional status using

nutrition assessment tools that use multiple indicators to score nutritional status (eg, Subjective Global Assessment[34,37] and PG-SGA[25,29,32,38,45,46,53,54]).

The studies provide strong evidence that poor nutritional status in adult cancer patients is associated with higher rates of hospital admissions or readmissions (six studies[33,36,45,50,55,58]), increased LOS (11 studies[17,32,33,35,46-49,51,63,70]), lower QoL (14 studies[25,34,37,38,41,44,47,49,54,56,64-66,69]), and mortality (17 studies[29,36,39,41-44,47,52,53,63,67,70,71]), and with decreased tolerance to CT (11 studies[31,36,40,45,55,57,59,61,62,67,68]) and RT (six studies[36,45,55,64,66]).

Only four studies did not find associations between poor nutritional status and a negative outcome. These outcomes included QoL,[44] hospital LOS,[47] and mortality.[47] One study found decreased nutritional status associated with greater numbers of hospital admissions, but the magnitude of the effect was not statistically significant.[33] Another study[55] did not find a significant difference between groups in dose of CT received in esophageal cancer patients, although the trend was toward fewer dose reductions in nutrition pathway patients.

Eight studies specifically recommended nutrition intervention by an RDN or other food and nutrition practitioner for adult oncology patients.[34,47,54,56,64-66,69] Two studies found that dietary counseling using regular foods was superior in maintaining QoL to interventions providing only oral supplements.[65,66] Because of the strong connection between nutritional status and QoL, one study suggested that all adult oncology patients be provided with a plan for nutrition care upon diagnosis, and that nutrition therapy should be an integral part of the overall care provided to patients.[54]

MNT in Patients Undergoing CT and RT

Recommendation

- If an adult oncology patient is undergoing CT or RT, RDNs should provide MNT.

Rating: Strong; Conditional

- RDNs should be members of interdisciplinary teams providing multimodal therapy to adult oncology patients undergoing CT or RT.

Rating: Fair; Conditional

Rationale: Nineteen studies,[55,56,64-66,73-86] mostly international, examined the effect of MNT intervention on patients undergoing anticancer treatments (eg, CT, RT, or combined therapy) in ambulatory and inpatient oncology centers. Accepted studies were those in which an RDN (or international equivalent food and nutrition practitioner) provided the dietary intervention. That is, the RDN practiced in a country holding a reciprocity agreement with the Commission on Dietetic Registration or the description of the practitioner was reasonably equivalent. Studies are shown in Table 3 (available online at www.andjrnl.org).

Early and intensive MNT intervention was effective in improving multiple treatment outcomes in patients with a variety of cancers (eg, breast, ovary, lung, leukemias, head and neck, colorectal, upper gastrointestinal [GI]) undergoing CT (five studies) and RT (11 studies). Improvement in treatment outcomes related to MNT included weight gain and preservation of desirable weight status[56,66,77,78] and LBM,[80,81] enhanced QoL,[55,64,66,78,81] perceived health benefits and patient satisfaction,[79] reduction in hospital admissions,[55] reduced hospital LOS,[55] better appetite, better treatment tolerance,[55,65,66] and increased energy and protein intake.[64-66,78,79,83,84] One small study showed that nutrition intervention to manage symptoms resulted in improved nutrition impact symptoms, including patient weight status, function score, endurance, grip strength, and C-reactive protein value.[77]

Four studies[73-75,85] examined the effectiveness of RDNs as members of multidisciplinary teams providing multimodal therapy, a treatment approach combining multiple elements or modalities that work together and support each other to optimize a patient's care. All studies found that MNT provided by an RDN as part of multimodal therapy was effective in improving one or more outcomes, including weight preservation,[75] general well-being,[85] and disease-free survival[73] in patients receiving CT[73] and RT.[74,75,85] One study found that timely and multidisciplinary care, including MNT, is feasible in clinical oncology settings[74] and that nutrition care recommendations result in an a decrease in symptom distress.[85]

DIETARY SUPPLEMENTS OR MFS CONTAINING FISH OIL

Recommendation

- If suboptimal symptom control or inadequate dietary intake has been addressed and the adult oncology patient is still experiencing loss of weight and LBM, an RDN may consider use of dietary supplements containing EPA as a component of nutrition intervention.

Rating: Strong; Imperative

- If suboptimal symptom control or inadequate dietary intake has been addressed and the adult oncology patient is still experiencing loss of weight and LBM, an RDN may consider use of MFS containing EPA as a component of nutrition intervention.

Rating: Strong; Imperative

Rationale: A dietary supplement is a single nutrient supplement in the form of a pill, capsule, liquid, chew, or other form. Twelve dietary supplement studies,[87-98] shown in Table 4 (available online at www.andjrnl.org), examined the effect of EPA on weight status and five studies[89,92,96-98] reported LBM outcomes. Actual consumption of EPA in the studies ranged from approximately 0.77 to 6 g/day. Dietary supplements containing fish oil resulted in statistically significant preservation of weight or increase in weight in eight of 12 studies, which included 10 solid tumor types.[87,90,92,94-98] Three studies, including one in patients with leukemia,[88] showed similar results, and the improvements in weight may have been clinically relevant, although they were not statistically significant.[88,91,93] One study showed a positive effect for a subgroup of the population (GI cancer patients), but not for the total population.[89] Four studies in weight-losing patients with mainly lung, pancreatic, and GI cancers showed statistically significant increase or preservation in LBM with use of dietary supplements containing fish oil.[92,96-98] Another study showed a non-statistically significant gain of 0.9 kg in LBM, although this was accompanied

by a significant improvement in functional status compared with placebo.[89] The change in functional status is likely due to improvement in LBM.

An MFS is a commercial or prepared food or beverage that supplements energy, protein, carbohydrate, fiber, or fat intake. Eleven studies[99-110] of MFS containing EPA reported on weight[99,104,110] and nine studies reported LBM outcomes.[99,102,104,107,110] Actual consumption of EPA in the studies ranged from 1.2 to 2.2 g/day. All but one study,[102] used the same commercially available MFS product containing EPA. Slight variations in international regulation account for the differences in nutrient labeling.

MFSs containing fish oil showed statistically significant increase or preservation in weight status in nine of 11 studies in patients with lung,[105,109] pancreatic,[97-99] head and neck,[102,103,109,110] or GI cancers.[107] Seven studies in weight-losing patients with solid tumors of the lung,[109] pancreas,[99,100,104] GI tract,[107,108] and head and neck[110] showed a statistically significant increase or preservation in LBM. Whereas four other studies did not find a statistically significant improvement in weight[104,106] or LBM,[101,102] the studies showed similar trends. These small gains may have been clinically relevant, although they were not statistically significant.

RECOMMENDATIONS BASED ON EXTERNAL ORGANIZATION SYSTEMATIC REVIEW

Glutamine (GLN) and Oral Mucositis in Patients with Solid Tumors and Hematologic Malignancies

Recommendation

- If use of parenteral GLN is proposed to prevent or treat oral mucositis in oncology patients with solid tumors, RDNs should advise that its use may or may not be beneficial.

Rating: Weak; Conditional

Rationale: Research was evaluated by the Oncological Nursing Society in head and neck and stem cell transplantation patients receiving parenteral L-alanyl-L-glutamine to treat or preventing oral mucositis. Because of the limited research available on its effectiveness, the Oncological Nursing Society gave parenteral GLN a grade of *Effectiveness Not Established*.[111]

Parenteral GLN and Hematopoietic Cell Transplantation

Recommendation

- When parenteral nutrition is required for patients undergoing hematopoietic cell transplant, RDNs may or may not recommend parenteral GLN in doses ranging from 0.2 to 0.5 g/kg/day.

Rating: Fair; Conditional

Rationale: Research evaluated by the American Society for Enteral and Parenteral Nutrition (A.S.P.E.N.) showed that for patients undergoing hematopoietic cell transplant, parenteral GLN in doses ranging from 0.2 to 0.5 g/kg/day should be initiated early in the treatment course.[112,113] Parenteral GLN was associated with improved nitrogen balance and decreased morbidity. However, decreased hospital LOS was found only when data from allogeneic and autologous transplants were combined. Enteral or oral provision of glutamine was not evaluated. A.S.P.E.N. concluded that parenteral GLN in pharmacologic doses may be beneficial in patients undergoing hematopoietic cell transplant and the evidence was given a *Grade C*.

Nutritional Substances and Chemotherapy-Induced Peripheral Neuropathy (CIPN)

Recommendation

- If an adult oncology patient is at risk for or has CIPN, RDNs should advise the patient that the use of nutritional substances (eg, vitamin E, calcium and magnesium infusions, acetyl-L-carnitine, GLN, and glutathione) may or may not be beneficial as a means of preventing or improving CIPN.

Rating: Weak; Conditional

Rationale: CIPN is a significant debilitating symptom directly related to the administration of neurotoxic CT for the treatment of cancer. Oncological Nursing Society found that the following nutritional substances (vitamin E, calcium and magnesium infusions, acetyl-L-carnitine, GLN, and glutathione) had only limited success in preventing or improving CIPN in oncology patients receiving specific chemotherapeutic agents.[114] Their conclusion received a Weight of the Evidence Category grade of *Effectiveness was not established.*

Neutropenic Dietary Precautions

Recommendation

- If an adult oncology patient has neutropenia, RDNs should provide dietary counseling on safe food handling and foods that may pose infectious risks during the period of neutropenia. A neutropenic diet is not necessary, but safe food counseling is recommended as a prudent precaution.

Rating: Fair; Conditional

- If an adult oncology patient is undergoing bone marrow transplant (BMT), RDNs should provide dietary counseling on safe food handling and foods that may pose infectious risks during the period of neutropenia. A neutropenic diet is not necessary, but safe food counseling is recommended as a prudent precaution.
- **Rating:** Weak; Conditional

Rationale: Research on the effectiveness of low-microbial diets was evaluated by Oncological Nursing Society, in patients undergoing BMT,[115] and by A.S.P.E.N. in non-BMT patients with neutropenia.[112,113] In non-BMT patients, the Oncological Nursing Society grade for Low Microbial Diet for Neutropenic Patients was *Effectiveness Unlikely*. In BMT patients, A.S.P.E.N. recommended safe food counseling regarding which foods may pose infectious risks during the period of neutropenia as a prudent precaution, but graded the evidence for neutropenic diets as *Grade C*. Based on these findings, the workgroup suggested safe food counseling for both BMT and non-BMT patients.

RECOMMENDATIONS BASED ON CONSENSUS PUBLICATIONS

Screening for Malnutrition Risk and Referral to RDNs

Recommendation

- All adult patients should be screened for malnutrition risk on

entry into oncology services. Rescreening should be repeated routinely throughout treatment to facilitate referral as needed.

Rating: Consensus: Imperative

- If an adult oncology patient has been identified at screening to be at risk for malnutrition, the patient should be referred to an RDN for evaluation. In cases where it is indicated, an RDN conducts a nutrition assessment and provides MNT, including the Nutrition Care Process.

Rating: Consensus; Conditional

Rationale: Nutrition screening triggers the entry of a patient into the Academy's Nutrition Care Process.[16] Timely screening and rescreening and prompt identification of malnutrition facilitates referral to an RDN for nutrition management and leads to improved outcomes.[116]

Malnutrition has been defined as "a state of nutrition in which a deficiency or excess (or imbalance) of energy, protein, and other nutrients causes measurable adverse effects on tissue/body form (body shape, size and composition) and function and clinical outcome."[117] The work group limited the definition of malnutrition in the oncology population to undernutrition. Patients may have a cachexia syndrome in addition to malnutrition.

Nutrition Assessment Criteria

Recommendation

- RDNs should assess the following:
  - Food, beverage, and nutrient intake and related history, including but not limited to energy and protein intake; changes in food and fluid/beverage intake; adequacy and appropriateness of nutrient intake or nutrient administration; actual daily intake from enteral nutrition and parenteral nutrition and other nutrient sources; changes in type, texture, or temperature of food and liquids; use of MFS; food avoidance and intolerances; meal or snack pattern changes; prescription medications, over-the-counter medications, herbal preparations, and complementary or alternative medicine products; and factors affecting access to food.

Rating: Consensus; Imperative

- Anthropometric measurements in adult oncology patients: height and weight, weight change, and body mass index.

Rating: Consensus; Imperative

- RDNs should evaluate available data regarding:
  - Biochemical data, medical tests, and procedures of adult oncology patients. Examples include glucose; white blood cell count; nutritional anemia profile (ie, hemoglobin, hematocrit, folate, vitamin B-12, and iron values); electrolyte and renal profile; liver function; inflammatory profile, including C-reactive protein value; and GI function tests (ie, swallowing study, abdominal films, gastric emptying, and transit time). In cases where biochemical data are not available, RDNs should recommend, as indicated.

Rating: Consensus; Imperative

- Nutrition-focused physical findings and client history of adult oncology patients, including but not limited to age older than 65 years, loss of muscle mass, loss of subcutaneous fat, presence of pressure ulcers or wounds, nutrition impact symptoms, changes in appetite or vital signs, change in functional indicators (ie, Karnofsky performance scale[118] and grip strength), and localized or generalized fluid accumulation. Client history—patient/family/client medical/health history, including but not limited to dysphagia, depression, and pain fatigue; medical treatment or therapy; other diseases, conditions, and illnesses, including cancer cachexia. Social history should include psychological/socioeconomic factors (eg, social support).

Rating: Consensus; Imperative

Rationale: Assessment is needed to effectively determine nutrition diagnoses and plan nutrition interventions. An adult oncology nutrition assessment should characterize and document the presence of, or expected potential for altered nutritional status[119] and nutrition impact symptoms, shown in Figure 5. These symptoms that impede intake, digestion, or absorption can be caused by the cancer itself or the oncology treatment.[120-122]

Any unintended weight loss (UWL) in adult oncology patients has potential significance because oncology patients often experience weight loss.[119] Accurate determination of a baseline weight and documentation of any weight loss before diagnosis or during treatment is vital to intervene and impact outcomes. In elderly patients, studies have shown an association between increased mortality and underweight with UWL of 5% in 30 days or a body mass index <20 rather than the usual <18.5.[71,123,124] Low muscle mass is an independent predictor of mortality,[60] is

- Adverse effects on weight or body composition
- Impaired immune response
- Decreased muscle strength
- Increased fatigue
- Impaired wound healing
- Impaired glucose function
- Impaired psychosocial function, including depression
- Reduced quality of life
- Reduced response to treatment
- Increased treatment toxicities
- Treatment delays
- Increased hospitalizations or length of stay

Figure 5. Common nutrition impact symptoms in adult oncology patients. Adapted from references 120, 121, and 122.

The presence of two or more of the following criteria or characteristics supports a nutrition diagnosis of malnutrition in adult oncology patients:

- Insufficient energy intake[119]
- Unintended weight loss[119]
- Loss of subcutaneous fat[71,119,129]
- Loss of muscle mass[61,119]
- Localized or generalized fluid accumulation (that may mask weight loss)[119]
- Reduced grip strength[119,128]

Figure 6. Criteria for nutrition diagnosis of malnutrition in adult oncology patients. Adapted from references 61, 71, 119, and 128.

a particularly adverse prognostic indicator in obese patients, and is associated with greater toxicities of CT leading to treatment interruptions, dose reductions, and delays or terminations of treatment.[59,61,124-126]

In addition to the five domains of the Nutrition Care Process (Food/Nutrition Related History; Anthropometric, Measurements; Biochemical Data, Medical Tests, and Procedures; Nutrition-Focused Physical Findings; and Client History), RDNs should consider the six identifiers of malnutrition, shown in Figure 6. The identifiers of malnutrition in the Academy/A.S.P.E.N. consensus statement[119] (pending validation) were adapted by the Oncology Workgroup for the Adult Oncology Population. The characteristics of malnutrition should be considered within the context of systemic inflammation and/or the presence of cachexia.[119,127,128]

Nutrition Assessment for the Stages of Cancer Cachexia

Recommendation

- As part of a nutrition assessment in patients with lung, pancreatic, or head and neck and GI cancers or those who are at high risk for weight loss or have experienced UWL, RDNs should assess for nutrition impact symptoms, markers of inflammation (eg, elevated C-reactive protein value), and other signs of wasting that may indicate precachexia or cancer cachexia.

Rating: Consensus; Conditional

Rationale: Further nutrition assessment is needed for patients with lung, pancreatic, or head and neck and GI cancers or those who are at high risk for weight loss or have experienced UWL. Patients with these diagnoses are more at risk for cachexia and therefore have more to gain from timely identification and nutrition intervention.

Nutrition assessment and intervention by an RDN is most effective when provided in the stages of precachexia and cachexia.[129] The stages of cancer cachexia are shown in Figure 7.

The metabolic response to cancer is heterogeneous, so it is important to intervene and manipulate the factors that are behavior-related, to address the direct causes of decreased intake (eg, obstruction or dysphagia), and address the secondary causes (eg, depression, fatigue, pain, or GI function). Symptom management in patients with advanced cancer can improve survival.[130]

Cancer cachexia A multifactorial syndrome characterized by an ongoing loss of skeletal muscle mass (with or without loss of fat mass) that cannot be fully reversed by conventional nutritional support and leads to progressive functional impairment. The pathophysiology is characterized by a negative protein and energy balance, driven by a variable combination of reduced food intake and abnormal metabolism.[129]

Precachexia (in general) Defined by the presence of all of the following criteria: underlying chronic disease, unintended weight loss of up to 5% usual body weight during the past 6 months, chronic or recurrent systemic inflammatory response, and anorexia or anorexia-related symptoms.[130]

Precachexia (in cancer) Characterized by early clinical and metabolic signs such as loss of appetite and impaired glucose tolerance. Can precede substantial involuntary weight loss (ie, up to 5%). The risk of progression is variable and depends on cancer type, stage, presence of systemic inflammation, low food intake, and lack of response to anticancer therapy.[129]

Refractory cachexia May be a result of very advanced cancer (preterminal) or the presence of rapidly progressive cancer unresponsive to anticancer therapy. This stage is associated with active catabolism or the presence of factors that make active management of weight loss no longer possible or appropriate. Refractory cachexia is characterized by a low performance score (eg, World Health Organization grade 3 or 4) and a life expectancy <3 months.[129]

Figure 7. Definitions of cachexia. There are several stages of cancer cachexia: precachexia, cachexia, and refractory cachexia. Adapted from references 129 and 130.

Nutrition Diagnosis of Malnutrition

Recommendation

- RDNs should use clinical judgment in interpreting nutrition assessment data to diagnose malnutrition in adult oncology patients. The presence of two or more of the following criteria or characteristics supports a nutrition diagnosis of malnutrition in an adult oncology patient: insufficient energy intake, UWL, loss of subcutaneous fat, loss of muscle mass, localized or generalized fluid accumulation (that may mask weight loss), and reduced grip strength.

Rating: Consensus; Imperative

Rationale: Although there is no universally accepted approach to the diagnosis and documentation of adult malnutrition, the workgroup developed guidance for adult oncology patients, based on the Academy/A.S.P.E.N. consensus document guidance,[119] shown in Figure 6. RDNs should use clinical judgment in interpreting nutrition assessment data to make a

nutrition diagnosis of malnutrition in adult oncology patients.

Nutrition Intervention

Recommendation

- Cachexia In adult oncology patients who have been identified to have precachexia or cancer cachexia, prompt and aggressive intervention to address nutrition impact symptoms and preserve or prevent loss of LBM and weight should be initiated by an RDN.

Rating: Consensus; Conditional

Rationale: Early rather than later intervention to prevent weight loss in patients with precachexia or cancer cachexia (Figure 7) is more likely to be effective. The metabolic derangements in cancer cachexia that promote wasting can lead to loss of weight and LBM and poor outcomes.

Monitoring and Evaluation

Recommendation

- To check progress, an RDN should monitor and evaluate the following components of adult oncology patients at each visit and compare with desired individual outcomes. This may include, but is not limited to anthropometric measurements; food- and nutrition-related history; biochemical data, medical tests, and procedures; nutrition-focused physical findings; client history; patient/family/client medical/health history; social history; and psychological/socioeconomic issues.

Rating: Consensus; Imperative

- In patients with lung, pancreatic, or head and neck and GI cancers, or those who are at high risk for weight loss or have experienced UWL, RDNs should monitor and evaluate nutrition impact symptoms, markers of inflammation (eg, elevated C-reactive protein values), and other signs of wasting, which may indicate precachexia or cancer cachexia.

Rating: Consensus; Conditional

Rationale: Frequent monitoring and evaluation should be performed to document the presence of (or expected potential for) altered nutritional status, nutrition impact symptoms or measureable adverse effects on body composition, function, QoL, or clinical outcome and includes the six indicators of malnutrition, as well as laboratory values and planned oncology treatments. Monitoring and evaluation of these factors is needed to correctly/effectively diagnose nutrition-related problems that should be the focus of further nutrition interventions. Inability to achieve optimal nutrient intake may contribute to poor outcomes.

SUGGESTIONS FOR FUTURE RESEARCH

During the literature review process several points regarding future research directions became clear. We suggest that research methods and consistency in outcomes reporting by investigators be addressed as follows:

1. The qualifications of clinicians (eg, RDN; nutrition and dietetics technician, registered; or nurse) providing the nutrition intervention can be described for studies to be compared or repeated in other settings.
2. Validated malnutrition screening and nutrition assessment tools can be used and clearly stated. Nutritional status (reported as PG-SGA or Subjective Global Assessment score)[24,27,131] may improve, although weight does not.
3. Research is needed in US patients, under the US health care system, with RDNs providing or leading the intervention as MNT.
4. Body weight (reported as kilograms and pounds) and LBM can be reported as lost, gained, or maintained.
5. Use of validated tools for measuring QoL can include oncology-specific instruments such as the Functional Assessment of Anorexia Cachexia Therapy[132] and European Organization for the Research and Treatment of Cancer Quality of Life Questionnaire-Core 30 (QLQ-C30).[133]
6. Performance status as a means of quantifying patients' general well-being can be reported using Karnofsky Performance Score or Eastern Cooperative Oncology Group score,[134,135] (also called World Health Organization [WHO] or Zubrod score). A translation of Zubrod and Karnofsky scales has been validated in lung cancer[135]; performance is sometimes used as a QoL surrogate.
7. Research is needed on oncology treatment outcomes anticipated to change by nutrition intervention, such as dose reductions, treatment delays, treatment completion, or treatment toxicities (reported as Common Terminology Criteria for Adverse Events current version).[136]
8. Efforts can be made to blind investigators who report and evaluate outcomes.
9. Because trials are in the design phase, investigators can have inclusion of the study in meta-analyses or systematic reviews as a goal. Inclusion of studies in meta-analyses or systematic reviews is a means to the creation of strong guidelines.

SUMMARY

The Academy of Nutrition and Dietetics Oncology Evidence-Based Nutrition Practice Guideline for Adults is a valuable resource for RDNs as well as other clinicians involved in the care of adult oncology patients. The Academy has published the Oncology Guideline 2013[15] on the EAL. MNT provided by an RDN is effective and essential to securing the best possible clinical outcomes for patients undergoing cancer treatments. These evidence-based guidelines highlight the importance of malnutrition screening and rescreening, timely referral to an RDN for patients identified as being at nutritional risk, and nutrition assessment and periodic reassessment using tools validated in the appropriate setting and with an oncology population. Early identification and diagnosis of malnutrition leading to intervention can positively impact body composition, functional status, QoL, treatment tolerance, and other clinical outcomes. Use of dietary supplements or an MFS containing EPA may be considered as an intervention because both have a significant effect on preserving weight and LBM in adult oncology patients. Finally, nutrition monitoring and evaluation of anthropometric measurements; food- and nutrition-related

history; biochemical data; medical tests and procedures; and nutrition-focused physical findings, client history, and social history help determine whether the nutrition-related goals and expected outcomes are met.

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STATEMENT OF POTENTIAL CONFLICT OF INTEREST

No potential conflict of interest was reported by the authors.

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Conclusion statements[a] and recommendations Green shading = recommendations supported by EAL systematic review No shading = recommendations supported by consensus publications Gray shading = recommendations supported by external guidelines	Number of studies (quality rating),[b] conclusion statement grade,[c] and EAL recommendation rating
Nutrition Screening and Referral	
1. Malnutrition screening tools for adult oncology patients	
Which malnutrition screening tools have been found to be valid and reliable for identifying malnutrition risk in adult oncology patients in ambulatory and acute care settings? *Conclusion:* These tools were found to be valid and reliable for identifying malnutrition risk in adult oncology patients as follows: MST[d] *(Ambulatory and acute care settings)* and MSTC;[e] MUST[f] *(Acute care setting only).* The MAG-MST[g] and the 2-item nutrition screen from the ZSDS[h] were not found to be valid and reliable for identifying malnutrition risk in adult oncology patients in acute care settings. Validity and reliability of the MSTC, MUST, MAG-MST, and 2-item nutrition screen from the ZSDS tools were not evaluated in adult oncology patients in the ambulatory setting.	7 studies (5+; 2Ø); Grade I
Recommendation: Adult oncology patients should be screened using a malnutrition screening tool validated in the setting in which the tool is intended for use. The following tools have been shown to be valid and reliable in inpatient settings: MST, MSTC, and MUST and in ambulatory/outpatient settings: MST.	Strong; Imperative
2. Screening for malnutrition risk and referral of adult oncology patients	
2.a. Screening for malnutrition risk and rescreening of adult oncology patients	
Conclusion: None *Recommendation:* All adult patients should be screened for malnutrition risk on entry into oncology services. Early identification and management of malnutrition risk improves and protects nutrition status and QoL,[i] which leads to improved outcomes. Rescreening should be repeated routinely throughout treatment to facilitate referral as needed.	Not applicable Consensus: Imperative
2.b. Referral of adult oncology patients identified at malnutrition risk to RDN[j]	
Conclusion: None *Recommendation:* If an adult oncology patient has been identified at screening to be at risk for malnutrition, the patient should be referred to an RDN for evaluation. If indicated, an RDN conducts a nutrition assessment and provides MNT,[k] including the nutrition care process: Nutrition assessment, nutrition diagnosis, nutrition intervention, and nutrition monitoring and evaluation. Management of malnutrition risk improves and protects nutrition status and QoL, which leads to improved outcomes.	Not applicable Consensus; Conditional
MNT	
3. MNT in adult oncology patients	
3.a. MNT in adult oncology patients undergoing CT[l] and RT[m]	
Is MNT provided by an RDN effective in adult oncology patients receiving CT? *Conclusion:* MNT provided by an RDN (or equivalent food and nutrition practitioner) was effective in improving multiple treatment outcomes[n] in adult oncology patients with a variety of cancers (breast, ovary, lung, leukemias, colorectal, and upper GI[o]) receiving CT in ambulatory and inpatient oncology centers.	5 studies (1+; 4Ø); Grade: II
Is MNT provided by an RDN effective in adult oncology patients receiving RT? *Conclusion:* MNT provided an RDN (or equivalent food and nutrition practitioner) was effective in improving multiple treatment outcomes[n] in adult oncology patients with a variety of high-risk cancers (head and neck or GI) receiving RT or combined RT in ambulatory and inpatient oncology centers.	11 studies (6+; 5Ø); Grade: I

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Figure 4. Oncology Guideline 2013 Evidence Analysis Library (EAL) conclusion statements and recommendations.

Conclusion statements[a] and recommendations Green shading = recommendations supported by EAL systematic review No shading = recommendations supported by consensus publications Gray shading = recommendations supported by external guidelines	Number of studies (quality rating),[b] conclusion statement grade,[c] and EAL recommendation rating
Recommendation: If an adult oncology patient is undergoing CT or RT, the RDN should provide MNT. MNT has been shown to be effective in improving multiple treatment outcomes[n] in patients undergoing CT, RT, or chemoradiotherapy in ambulatory or outpatient and inpatient oncology settings.	Strong; Conditional
3.b. MNT as part of multimodal therapy in adult oncology patients undergoing CT and RT	
Is MNT provided by an RDN as part of multimodal therapy effective in adult oncology patients receiving RT? *Conclusion:* MNT provided by an RDN (or equivalent food and nutrition practitioner), as part of multimodal therapy, was effective in improving outcomes[p] in adult oncology patients receiving RT.	3 studies (3Ø); Grade: II
Is MNT provided by an RDN as part of multimodal therapy effective in adult oncology patients receiving CT? *Conclusion:* MNT provided by an RDN, as part of multimodal therapy, was found to be effective in improving outcomes[q] in adult breast cancer patients receiving CT.	1 study (1+); Grade: III
Recommendation: RDNs should be members of interdisciplinary teams providing multimodal therapy to adult oncology patients undergoing CT or RT.	Fair; Conditional
NUTRITION ASSESSMENT	
4. Nutrition assessment tools for adult oncology patients	
Which nutrition assessment tools have been found to be valid and reliable to assess nutritional status of adult oncology patients in ambulatory and acute care settings? *Conclusion:* The PG-SGA[r] and SGA[s] tools have been found to be valid and reliable in assessing the nutritional status of adult oncology patients in ambulatory and acute care settings. The Mini Nutrition Assessment tool was found to have the sensitivity to diagnose oncology patients with malnutrition in ambulatory settings, but was only moderately specific in identifying malnutrition when compared with the PG-SGA. The Mini Nutrition Assessment tool was not evaluated in an acute care setting.	7 studies (5+; 2Ø); Grade: I
Recommendation: RDNs should use an assessment tool validated in the setting in which the tool is intended for use as part of a complete nutrition assessment. Research indicates that the PG-SGA and SGA tools elicit valid and reliable data as part of a comprehensive nutrition assessment of adult oncology patients in ambulatory and acute care settings.	Strong; Imperative
5.a. Assessment of food/nutrition-related history of adult oncology patients	
Conclusion: None. *Recommendation:* RDNs should assess the food, beverage, and nutrient intake and related history of adult oncology patients including, but not limited to the following: • Energy and protein intake • Changes in food and fluid/beverage intake • Adequacy and appropriateness of nutrient intake or nutrient administration • Actual daily intake from enteral and parenteral nutrition and other nutrient sources • Changes in type, texture, or temperature of food and liquids • Use of MFS[t] • Food avoidance and intolerances • Meal or snack pattern changes • Prescription medications, over-the-counter medications, herbal preparations, and complementary or alternative medicine products • Factors affecting access to food.	Not applicable Consensus; Imperative

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Figure 4. *(continued)* Oncology Guideline 2013 Evidence Analysis Library (EAL) conclusion statements and recommendations.

Conclusion statements[a] and recommendations Green shading = recommendations supported by EAL systematic review No shading = recommendations supported by consensus publications Gray shading = recommendations supported by external guidelines	Number of studies (quality rating),[b] conclusion statement grade,[c] and EAL recommendation rating
Assessment of the above factors is needed to effectively determine nutrition diagnoses and plan the nutrition interventions. Inability to achieve optimal nutrient intake may contribute to poor outcomes.	
5.b. Assessment of anthropometric measurement in adult oncology patients	
Conclusion: None *Recommendation:* RDNs should assess the following anthropometric measurements in adult oncology patients: • Height and weight • Weight change • BMI[u] Any weight loss that is unintended in adult oncology patients has potential significance, because oncology patients often experience weight loss before admission to oncology services. Low muscle mass is a common and independent predictor of immobility and mortality; is a particularly adverse prognostic indicator in obese patients; and is associated with greater toxicities of CT leading to treatment interruptions, including dose reductions, treatment delays, and treatment termination. Assessment of the above factors is needed to effectively determine nutrition diagnoses and plan the nutrition interventions.	Not applicable Consensus; Imperative
5.c. Assessment of biochemical data, medical tests, and procedures on adult oncology patients	
Conclusion: None *Recommendation:* RDNs should evaluate available data and recommend as indicated: biochemical data, medical tests, and procedures of adult oncology patients. Examples include: • Glucose; • White blood cell count; • Nutrition-related anemia profile (hemoglobin, hematocrit, folate, B-12, and iron); • Electrolyte and renal profile; • Liver function; • Inflammatory profile, including C-reactive protein; and • GI function tests (ie, swallowing study, abdominal films, gastric emptying, and transit time). Assessment of these factors is needed to effectively determine nutrition diagnoses and plan the nutrition interventions.	Not applicable Consensus; Imperative
5.d. Assessment of nutrition-focused physical findings and client history of adult oncology patients	
Conclusion: None *Recommendation:* RDNs should evaluate available data regarding the nutrition-focused physical findings and client history of adult oncology patients, including but not limited to: Nutrition-focused physical findings: • Older than age 65 y; • Loss of muscle mass; • Loss of subcutaneous fat; • Presence of pressure ulcers or wounds;	Not applicable Consensus; Imperative

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Figure 4. *(continued)* Oncology Guideline 2013 Evidence Analysis Library (EAL) conclusion statements and recommendations.

Conclusion statements[a] and recommendations Green shading = recommendations supported by EAL systematic review No shading = recommendations supported by consensus publications Gray shading = recommendations supported by external guidelines	Number of studies (quality rating),[b] conclusion statement grade,[c] and EAL recommendation rating
• Nutrition impact symptoms, including but not limited to nausea, vomiting, diarrhea, constipation, stomatitis, mucositis, alterations in taste, and smell and anxiety; • Changes in appetite; • Vital signs; • Functional indicators (ie, Karnofsky performance scale[118] score and grip strength); and • Localized or generalized fluid accumulation. Client history: • Patient/family/client medical/health history: • Nutrition impact symptoms, including but not limited to dysphagia, depression, and pain fatigue; • Medical treatment or therapy; • Other diseases, conditions, and illnesses, including cancer cachexia. Social history: Psychological/socioeconomic factors (eg, social support). Assessment of the above factors is needed to effectively determine nutrition diagnoses and plan the nutrition interventions	
6. Nutrition assessment for the stages of cancer cachexia in adult oncology patients	
Conclusion: None *Recommendation:* As part of the nutrition assessment, in patients with lung, pancreatic, or head and neck and GI cancers or those who are at high risk for weight loss or have experienced unintended weight loss, RDNs should assess for nutrition impact symptoms, markers of inflammation (eg, elevated C-reactive protein level) and other signs of wasting that may indicate precachexia or cancer cachexia. The presence of cachexia does not always indicate end of life or need for hospice. Therefore, the identification of cachexia leading to intervention can positively impact clinical outcomes.	Not applicable Consensus; Conditional
NUTRITION DIAGNOSIS	
7. Nutrition diagnosis of malnutrition in adult oncology patients	
Conclusion: None *Recommendation:* RDNs should use clinical judgment in interpreting nutrition assessment data to diagnose malnutrition in adult oncology patients. Early identification and diagnosis of malnutrition leading to intervention can positively impact body composition, function, QoL, treatment tolerance, and clinical outcomes. The presence of two or more of the following criteria or characteristics supports a nutrition diagnosis of malnutrition in adult oncology patients. • Insufficient energy intake, • Unintended weight loss, • Loss of subcutaneous fat, • Loss of muscle mass, • Localized or generalized fluid accumulation (that may mask weight loss), and • Reduced grip strength.	Not applicable Consensus; Imperative
NUTRITION INTERVENTION	
8. Nutrition intervention of adult oncology patients with cancer cachexia	
Conclusion: None *Recommendation:* In adult oncology patients who have been identified to have precachexia or cancer cachexia, prompt and aggressive intervention to address nutrition impact symptoms	Not applicable Consensus; Conditional

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Figure 4. *(continued)* Oncology Guideline 2013 Evidence Analysis Library (EAL) conclusion statements and recommendations.

Conclusion statements[a] and recommendations Green shading = recommendations supported by EAL systematic review No shading = recommendations supported by consensus publications Gray shading = recommendations supported by external guidelines	Number of studies (quality rating),[b] conclusion statement grade,[c] and EAL recommendation rating
and preserve or prevent loss of LBM[v] and weight should be initiated by an RDN. Early rather than later intervention to prevent weight loss in this population is more likely to be effective. The metabolic derangements in cancer cachexia that promote wasting can lead to loss of weight and LBM and poor outcomes.	
9. Fish oil, weight, and lean body mass in adult oncology patients	
9.a. Dietary supplements containing fish oil for adult oncology patients	
What is the effect of a dietary supplement containing fish oil on weight in adult oncology patients? *Conclusion:* Eight studies found that dietary supplements containing fish oil (actual consumption 0.77-6.0 g EPA[w]/d), resulted in weight gain or weight stabilization in adult oncology patients with weight loss. Three studies showed the same effect, but were not statistically significant. One study showed a positive effect for a subgroup of the population (GI cancer patients), but not for the total population. More research is needed to determine the optimal dose.	12 studies (7+; 3Ø; 2-); Grade: I
What is the effect of a dietary supplement containing fish oil on lean body mass in adult oncology patients? *Conclusion:* Four studies found that dietary supplements containing fish oil (actual consumption approximately 0.77-6.0 g EPA/d) resulted in improvement or preservation of LBM in adult oncology patients with weight loss. The fifth study showed the same effect, but was not statistically significant. More research is needed to determine the optimal dose.	5 studies (2+; 2Ø; 1-); Grade: II
Recommendation: If suboptimal symptom control or inadequate dietary intake has been addressed and the adult oncology patient is still experiencing loss of weight and LBM, an RDN may consider use of dietary supplements containing EPA as a component of a nutrition intervention.	Strong; Imperative
9.b. Medical food supplements containing fish oil for adult oncology patients	
What is the effect of MFS containing fish oil on weight in adult oncology patients? *Conclusion:* Nine studies found that MFS containing fish oil (actual consumption 1.2-2.2 g EPA/d) resulted in weight gain or weight stabilization in adult oncology patients with weight loss. Two studies showed the same effect, but were not statistically significant. More research is needed to determine the optimal dose.	11 studies (6+; 5Ø); Grade: I
What is the effect of MFS containing fish oil on LBM in adult oncology patients? *Conclusion:* Seven studies found that MFS containing fish oil (actual consumption 1.2-2.2 g EPA/d) resulted in improvement or preservation of LBM in adult oncology patients with weight loss. Two studies showed the same effect, but were not statistically significant. More research is needed to determine the optimal dose.	9 studies (4+; 5Ø); Grade: I
Recommendation: If suboptimal symptom control or inadequate dietary intake has been addressed and the adult oncology patient is still experiencing loss of weight and LBM, an RDN may consider use of an MFS containing EPA as a component of a nutrition intervention.	Strong; Imperative
10. Glutamine and oral mucositis in adult oncology patients with solid tumors and hematologic malignancies	
Guideline: The effectiveness of treating of oral mucositis with glutamine has not been established.[111] *Recommendation:* If use of parenteral glutamine is proposed to prevent or treat oral mucositis in oncology patients with solid tumors, an RDN should advise that its use may or may not	Weight of Evidence Category: Effectiveness Not Established. Weak; Conditional

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Figure 4. *(continued)* Oncology Guideline 2013 Evidence Analysis Library (EAL) conclusion statements and recommendations.

Conclusion statements[a] and recommendations Green shading = recommendations supported by EAL systematic review No shading = recommendations supported by consensus publications Gray shading = recommendations supported by external guidelines	Number of studies (quality rating),[b] conclusion statement grade,[c] and EAL recommendation rating
be beneficial. Limited research in head and neck and stem cell transplantation patients receiving parenteral glutamine has not established the effectiveness of L-alanyl-L-glutamine in treating or preventing oral mucositis. Enteral or oral provision of glutamine was not evaluated.	
11. Parenteral glutamine and hematopoietic cell transplantation in adult oncology patients	
Guideline: Parenteral glutamine in pharmacologic doses may be beneficial in patients undergoing hematopoietic cell transplantation.[112,113] *Recommendation:* When parenteral nutrition is required for patients undergoing hematopoietic cell transplantation, an RDN may or may not recommend parenteral glutamine in doses ranging from 0.2-0.5 g/kg/d. Research indicates parenteral glutamine should be initiated early in the treatment course. Parenteral glutamine is associated with improved nitrogen balance and decreased morbidity. However, decreased hospital LOS was found only when data from allogeneic and autologous transplants were combined.	Grade: C Fair; Conditional
12. Nutrition substances and CT-induced peripheral neuropathy	
Guideline: No nursing interventions (vitamin E, calcium, and magnesium infusions; acetyl-L-carnitine; glutamine; and glutathione) for the prevention or treatment of CIPN[x] can be categorized as recommended for practice or likely to be effective.[114] *Recommendation:* If an adult oncology patient is at risk for or has CIPN, an RDN should advise the patient that the use of nutritional substances (vitamin E, calcium, and magnesium infusions; acetyl-L-carnitine; glutamine; and glutathione) may or may not be beneficial as a means of preventing or improving CIPN. Research indicates that these substances have had only limited success in preventing or improving CIPN in oncology patients receiving specific chemotherapeutic agents.	Weight of the Evidence Category: Effectiveness Not Established Weak; Conditional
13. Neutropenic dietary precautions for adult oncology patients	
13.a. Neutropenic dietary precautions for adult oncology patients with neutropenia (nonbone marrow transplant)	
Guideline: Patients should receive dietary counseling regarding foods that may pose infectious risks and safe food handling during the period of neutropenia.[112,113] *Recommendation:* In a case where a an adult oncology patient has neutropenia, an RDN should provide dietary counseling on safe food handling and foods that may pose infectious risks during the period of neutropenia. A neutropenic diet is not necessary, but safe food counseling is recommended as a prudent precaution. Research has not demonstrated the effectiveness of low-microbial diets.	Grade: C Fair; Conditional
13.b. Neutropenic dietary precautions for adult oncology patients undergoing bone marrow transplant	
Guideline: Studies have linked dietary restrictions with a lower risk of infection for neutropenic patients with cancer; however, basic principles, such as avoiding uncooked meats, seafood, eggs, and unwashed fruits and vegetables, may be prudent.[115] *Recommendation:* If an adult oncology patient is undergoing bone marrow transplant, an RDN should provide dietary counseling on safe food handling and foods that may pose infectious risks during the period of neutropenia. A neutropenic diet is not necessary, but safe food counseling is recommended as a prudent precaution. There is conflicting research regarding the effectiveness of neutropenic diets in the bone marrow transplant population.	Grade: Effectiveness Unlikely Weak; Conditional

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Figure 4. *(continued)* Oncology Guideline 2013 Evidence Analysis Library (EAL) conclusion statements and recommendations.

Conclusion statements[a] and recommendations Green shading = recommendations supported by EAL systematic review No shading = recommendations supported by consensus publications Gray shading = recommendations supported by external guidelines	Number of studies (quality rating),[b] conclusion statement grade,[c] and EAL recommendation rating
Nutrition monitoring and evaluation	
14. Nutrition monitoring and evaluation in adult oncology patients	
14.a. ONC: Nutrition monitoring and evaluation of adult oncology patients	
Conclusion: None *Recommendation:* Following the nutrition intervention, to check progress, an RDN should monitor and evaluate the following components of adult oncology patients at each visit and compare with desired individual outcomes relevant to the nutrition diagnosis and intervention. This may include, but is not limited to: Anthropometric measurements: • Weight change, and • BMI. Food- and nutrition-related history: • Energy and protein intake; • Changes in food and fluid/beverage intake; • Adequacy and appropriateness of nutrient intake or nutrient administration; • Actual daily intake from enteral and parenteral nutrition and other nutrient sources; • Changes in type, texture, or temperature of food and liquids; • Use of MFS; • Food avoidance and intolerances; • Meal or snack pattern changes; • Prescription medications, over-the-counter medications, herbal preparations, and complementary alternative medicine products; • Factors affecting access to food; and • Feeding method or need for placement (eg, oral, enteral, or parenteral). Biochemical data, medical tests, and procedures: • Biochemical indexes, and • Implications of diagnostic tests and therapeutic procedures. Nutrition-focused physical findings: • Vital signs; • Loss of muscle mass; • Loss of subcutaneous fat; • Nutrition impact symptoms, including but not limited to nausea, vomiting, diarrhea, constipation, stomatitis, mucositis, alterations in taste and smell, and anxiety; • Presence of pressure ulcers or wounds; • Functional indicators (ie, Karnofsky performance scale score and grip strength); and • Localized or generalized fluid accumulation. Client history: • Patient/family/client medical/health history: ○ Nutrition impact symptoms, including but not limited to dysphagia, depression, and pain fatigue; ○ Medical treatment or therapy; and ○ Other diseases; conditions; and illnesses, including cancer cachexia.	Not applicable Consensus; Imperative

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Figure 4. *(continued)* Oncology Guideline 2013 Evidence Analysis Library (EAL) conclusion statements and recommendations.

Conclusion statements[a] and recommendations Green shading = recommendations supported by EAL systematic review No shading = recommendations supported by consensus publications Gray shading = recommendations supported by external guidelines	Number of studies (quality rating),[b] conclusion statement grade,[c] and EAL recommendation rating
Social history: • Psychological/socioeconomic issues (eg, social support). Monitoring and evaluation of the above factors is needed to correctly and effectively diagnose nutrition problems that should be the focus of further nutrition interventions. Inability to achieve optimal nutrient intake may contribute to poor outcomes.	
14.b. Nutrition monitoring and evaluating adult oncology patients with cancer cachexia	
Conclusion: None *Recommendation:* As part of monitoring and evaluation, in patients with lung, pancreatic, or head and neck and GI cancers, or those who are at high risk for weight loss or have experienced unintended weight loss, an RDN should monitor and evaluate nutrition impact symptoms, markers of inflammation (eg, elevated C-reactive protein value), and other signs of wasting that may indicate precachexia or cancer cachexia.	Not applicable Consensus; Conditional
Outcomes management	
15. Nutritional status and outcomes in adult oncology patients	
What is the relationship between nutritional status and hospital admissions or readmissions in adult oncology patients? *Conclusion:* Poor nutritional status is associated with higher rates of hospital admissions or readmissions in adult oncology patients. Five studies found that a decreased nutritional status is associated with greater numbers of hospital admissions. A sixth study showed the same effect, but was not statistically significant.	6 studies (2+; 4Ø); Grade: II
What is the relationship between nutritional status and hospital LOS[y] in oncology patients? *Conclusion:* Poor nutritional status is associated with increased LOS in adult oncology patients. Ten studies found that a decreased nutritional status is associated with longer LOS, whereas one study found no statistical difference between groups.	11 studies (9+; 2Ø); Grade: I
What is the relationship between nutritional status and QoL in oncology patients? *Conclusion:* Poor nutritional status is associated with lower QoL in adult oncology patients. Thirteen studies found that a decreased nutritional status is associated with a lower QoL. All 8 studies using the PG-SGA found that a higher score (higher nutritional risk) was associated with a lower QoL in oncology patients.	14 studies (9+; 5Ø); Grade: I
What is the relationship between nutritional status and RT tolerance in oncology patients? *Conclusion:* Poor nutritional status is associated with decreased tolerance to RT in adult oncology patients undergoing RT. All studies found positive associations between nutritional status and 2 or more of the following: reduced treatment interruptions, unplanned hospital admissions, treatment toxicity, PG-SGA score over time, and QoL.	6 studies (4+; 2Ø); Grade: I
What is the relationship between nutritional status and CT tolerance in oncology patients? *Conclusion:* Poor nutritional status is associated with decreased tolerance to CT treatment in adult oncology patients undergoing CT. Ten studies found positive associations in 1 or more of the following: treatment interruptions, infections, unplanned hospital admissions, treatment toxicity (including dose-limiting treatment toxicity), neutropenic fever, fatigue, and severe thrombocytopenia. One additional study showed a similar trend toward fewer dose reductions, but the difference was not significant.	11 studies (4+; 7Ø); Grade: I

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Figure 4. *(continued)* Oncology Guideline 2013 Evidence Analysis Library (EAL) conclusion statements and recommendations.

Conclusion statements[a] and recommendations Green shading = recommendations supported by EAL systematic review No shading = recommendations supported by consensus publications Gray shading = recommendations supported by external guidelines	Number of studies (quality rating),[b] conclusion statement grade,[c] and EAL recommendation rating
What is the relationship between nutritional status and mortality in oncology patients? *Conclusion:* Poor nutritional status is associated with mortality in adult oncology patients. Sixteen studies found positive associations among one or more of the following and mortality: weight loss, malnutrition, poor scores on validated malnutrition and QoL screening tools, sarcopenia, cachexia, and fatigue.	17 studies (9+; 8Ø); Grade: I
Recommendation: RDNs should collaborate with other health care professionals, administrators, and public policy decision-makers to ensure that the evaluation of nutritional status is a key component of the adult oncology patient care process.	Strong; Imperative

[a]Conclusion statement for EAL systematic review questions or guideline statement for external guidelines.

[b]Number of studies and quality rating [positive (+), neutral (Ø) or negative (-)] included in the EAL systematic review conclusion statement.

[c]Grade for external guideline statements. See Figure 3 for EAL rating equivalents.

[d]MST=Malnutrition Screening Tool.

[e]MSTC=Malnutrition Screening Tool for Cancer Patients.

[f]MUST=Malnutrition Universal Screening Tool.

[g]MAG-MST=Malnutrition Advisory Group Malnutrition Screening Tool.

[h]ZSDS=Zung self-rating depression scale.

[i]QoL=quality of life.

[j]RDN=registered dietitian nutritionist.

[k]MNT=medical nutrition therapy.

[l]CT=chemotherapy treatment.

[m]RT=radiation therapy or treatment.

[n]Improvement in treatment outcomes included weight gain and preservation of weight, QoL, increased energy and protein intake, and management of nutrition impact symptoms resulting in improved weight status, function score, endurance, grip strength, and C-reactive protein status.

[o]GI=gastrointestinal.

[p]Improvement in treatment outcomes included weight gain and preservation of weight and LBM, QoL, increased energy and protein intake, appetite, perceived health benefits and patient satisfaction, reduction in hospital admissions, and LOS, better treatment tolerance, and management of nutrition impact symptoms, as above.

[q]Improvement in treatment outcomes included weight status, patient satisfaction, decreased symptom distress, improved function, and provision of care in patient's preferred outpatient setting.

[r]PG-SGA=Patient Generated Subjective Global Assessment.

[s]SGA=Subjective Global Assessment.

[t]MFS=medical food supplement.

[u]BMI=body mass index.

[v]LBM=lean body mass.

[w]EPA=eicosapentaenoic acid.

[x]CIPN=chemotherapy-induced peripheral neuropathy.

[y]LOS=length of stay.

Figure 4. *(continued)* Oncology Guideline 2013 Evidence Analysis Library (EAL) conclusion statements and recommendations.

Table 1. Summary of studies included in the Academy of Nutrition and Dietetics Evidence Analysis Library systematic review of the validity and reliability of malnutrition screening tools and nutrition assessment tools used with adult oncology patients in ambulatory and acute care settings

Author(s), year Study design Location Quality rating	Population Cancer site	Tool Reference standard	Results Sensitivity/specificity (%)	Interrater reliability	Positive predictive value/negative predictive value (%)	Other	Recommended for Use[a]
Malnutrition screening tools							
Amaral and colleagues, 2008[17] DVR[b] Portugal Positive	N=130 inpatients Various	MST[c] MUST[d] NRS-2002[e]	48.7/94.6 97.3/77.4	NR[f] NR	78.3/82.2 63.2/98.6	Agreement: 81.50% (k[g]=0.49) Agreement: 83.10% (k=0.64) Malnutrition risk identification: MUST, n=57 (43.8%); NRS-2002, n=37 (28.5%); MST, n=23 (17.7%)	Yes, MUST had higher concurrence with NRS-2002 than MST
Bauer and colleagues, 2003[18] Cross-sectional Australia Neutral	N=65 inpatients Lymphoma, breast	Malnutrition Advisory Group Malnutrition Screening tool SGA[h]	59/75	NR	88/38	Significant linear trends toward agreement with SGA for % BW[i] ↓ over past 6 mo, $F_{(1, 64)}$=26.5; P<0.0001 and for BMI,[j] $F_{(1, 58)}$=7.9; P<0.007	No
Ferguson and colleagues, 1999[19] Cross-sectional Australia Positive	N=106 outpatients undergoing radiation therapy Various	MST SGA	100/81	NR	40/100	NA[k]	Yes
Ferguson and colleagues, 1999[20] DVR Australia Positive	N=408 inpatients Various	MST SGA; dietitian	93/93	93-97% k=0.84-0.93 (P<0.01)	98.4/72.7	NA	Yes

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Table 1. Summary of studies included in the Academy of Nutrition and Dietetics Evidence Analysis Library systematic review of the validity and reliability of malnutrition screening tools and nutrition assessment tools used with adult oncology patients in ambulatory and acute care settings (*continued*)

Author(s), year Study design Location Quality rating	Population Cancer site	Tool Reference standard	Results: Sensitivity/ specificity (%)	Interrater reliability	Positive predictive value/negative predictive value (%)	Other	Recommended for Use[a]
Isenring and colleagues, 2006[21] Cross-sectional Australia Positive	N=50 outpatients undergoing chemotherapy Head and neck, rectum, abdomen	MST PG-SGA[l]	100/92	k=0.83-0.88; n=20	80/100	NA	Yes
Kim and colleagues, 2011[22] DVR Republic of Korea Positive	N=257 inpatients Various	MST for Cancer Patients PG-SGA	Low risk: 94/84.2 High risk: 85.4-98.3/ 78.2-89.1	NR	Low risk: 67.8/97.6 (95% CI) High risk: 57.3-77.1/ 93.9-99.3 (95% CI)	k=0.7 for low risk; k=NR for high risk	Yes, recommend further research
Kirsh and colleagues, 2003[23] DVR United States Neutral	N=50 inpatients Various	2-item ZSDS[m] (Item #5 and #7) PG-SGA	50/88 For 2-item screen	NR	NR/NR	Significant relationship between PG-SGA and ZSDS (R=0.63; P<0.01); and items #5/#7 of ZSDS (F=13.99; P<0.001)	No, recommend further research
Nutrition assessment tools							
Bauer and colleagues, 2002[24] Cross-sectional Australia Positive	N=71 inpatients Lymphoma, breast, prostate, esophagus, lung, sarcoma, myeloma	PG-SGA[n] SGA	98/82	NR	95/93	NA	Yes

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Table 1. Summary of studies included in the Academy of Nutrition and Dietetics Evidence Analysis Library systematic review of the validity and reliability of malnutrition screening tools and nutrition assessment tools used with adult oncology patients in ambulatory and acute care settings (*continued*)

Author(s), year Study design Location Quality rating	Population Cancer site	Tool Reference standard	Results				Recommended for Use[a]
			Sensitivity/ specificity (%)	Interrater reliability	Positive predictive value/negative predictive value (%)	Other	
Isenring and colleagues, 2003[25] DVR Australia Positive	N=57 outpatients undergoing radiation therapy Head and neck, abdominal, rectal	PG-SGA SGA and global quality of life (EORTC QLQ-C30)[o]	NR/NR	NR	NR/NR	Significant linear trend between PG-SGA scores for each SGA classification ($P<0.001$) Change in PG-SGA score was significantly different between subjects who improved, maintained, or deteriorated in status according to SGA ($F_{(3,53)}=23.48$; $P<0.001$) PG-SGA score was correlated with baseline BMI ($P<0.008$) and 6 mo BW ↓ prior to baseline ($P<0.001$)	Yes
Kwang and Kandiah, 2010[26] Cross-Sectional Malaysia Positive	N=58 inpatients and outpatients Advanced cancers	PG-SGA Anthropometric measures[p]	NR	NR	NR	Significant difference ($P<0.05$) in anthropometric measures for the 3 PG-SGA stages Low readings of anthropometric measures were associated with higher PG-SGA scores ($R=$ -0.32; $P<0.05$)	Yes, recommend further research
Laky and colleagues, 2008[27] DVR Australia Positive	N=194 outpatients Gynecologic	PG-SGA SGA	NR/NR	NR	NR/NR	Area under the curve: For scored PG-SGA: 0.92 (95% CI, 0.83-1.01; $P<0.001$) For pretreatment albumin: 0.92 (95% CI 0.84-1.01; $P<0.001$) For total body potassium: 0.77 (95% CI 0.61-0.94; $P<0.005$) For triceps skinfold: 0.70 (95% CI 0.53-0.88; $P<0.041$)	Yes

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Table 1. Summary of studies included in the Academy of Nutrition and Dietetics Evidence Analysis Library systematic review of the validity and reliability of malnutrition screening tools and nutrition assessment tools used with adult oncology patients in ambulatory and acute care settings (*continued*)

Author(s), year Study design Location Quality rating	Population Cancer site	Tool Reference standard	Results Sensitivity/specificity (%)	Interrater reliability	Positive predictive value/negative predictive value (%)	Other	Recommended for Use[a]
Li and colleagues, 2010[28] Descriptive China Neutral	N=148 inpatients Lung; benign lung cancer	PG-SGA SGA BMI and biochemical parameters	NR/NR	NR	NR/NR	Weight/BW ↓ predicted SGA and PG-SGA ratings in both groups Albumin, total lymphocyte count, hemoglobin, transferring, and prealbumin predicted SGA rating for lung cancer Transferrin and hemoglobin predicted SGA rating for benign lung cancer; transferrin predicted PG-SGA rating for benign lung cancer Albumin, total lymphocyte count, transferring, and prealbumin predicted PG-SGA for lung cancer Prealbumin was the only predictor of SGA (severe malnutrition) for both groups. Prealbumin accurately predicted PG-SGA (severe malnutrition for lung cancer only) Highest receiver-operating characteristic area under the curve was for PG-SGA score, BMI, and BW	Yes
Persson and colleagues, 1999[29] DVR Sweden Neutral	N=87 outpatients GI, urologic	PG-SGA Physician, dietitian	NR/NR	NR	NR/NR	PG-SGA classification agreement: 90% Muscle wastage: 53% Subcutaneous fat ↓: 61% Multivariate analysis for PG-SGA predictors of nutritional status classification: Level of food intake (odds ratio 35.87; $P<0.02$), BW ↓ past 6 mo (odds ratio 7.54; $P<0.02$), muscle wastage (odds ratio 20.09; $P<0.001$)	Yes

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Table 1. Summary of studies included in the Academy of Nutrition and Dietetics Evidence Analysis Library systematic review of the validity and reliability of malnutrition screening tools and nutrition assessment tools used with adult oncology patients in ambulatory and acute care settings (*continued*)

Author(s), year Study design Location Quality rating	Population Cancer site	Tool Reference standard	Results				Recommended for Use[a]
			Sensitivity/ specificity (%)	Interrater reliability	Positive predictive value/negative predictive value (%)	Other	
Read and colleagues, 2005[30] DVR Australia Positive	N=126 outpatients reassessed at Weeks 4-6 N=104 outpatients reassessed at Weeks 8-12 Colorectal, lung, esophageal, gastric, or pancreatic	Mini Nutrition Assessment PG-SGA	97/54 (Baseline) 79/69 (at 4-6 wk) 93/82 (At 8-12 wk)	NR	59/NR 54/NR 66/NR	χ^2 goodness-of-fit test confirmed nonsignificant (P=0.631) difference between the 2 methods	No

[a]Tool recommended by author for use in the studied population.
[b]DVR=diagnostic, validity, or reliability study.
[c]MST=Malnutrition Screening Tool.
[d]MUST=Malnutrition Universal Screening Tool.
[e]NRS-2002=Nutrition Risk Screening 2002 tool.
[f]NR=not reported.
[g]k=kappa.
[h]SGA=Subjective Global Assessment.
[i]BW=body weight.
[j]BMI=body mass index.
[k]NA=not applicable.
[l]PG-SGA=Patient Generated Subjective Global Assessment.
[m]ZSDS=Zung Self-Rating Depression Scale.
[n]The PG-SGA was evaluated as a nutrition assessment tool in this systematic review, because no validation studies for the PG-SGA short form were available within the search inclusion dates.
[o]EORTC QLQ=European Organization for Research and Treatment of Cancer Quality of Life Questionnaire.
[p]Height, weight, BMI, midarm muscle circumference, triceps skinfold, and % BW change within 1 and 6 mo.

Table 2. Summary of studies included in the Academy of Nutrition and Dietetics Evidence Analysis Library systematic review of the relationships among nutritional status and hospital admissions/readmissions, hospital length of stay (LOS), quality of life (QoL), radiation treatment (RT) tolerance, chemotherapy treatment (CT) tolerance, and mortality outcomes in adult oncology patients

Author(s), year Study design Location Quality rating	Population Cancer site	Nutritional status indicator(s)	Results
Alexandre and colleagues, 2003[31] Case-control France Positive	N=107 Advanced solid tumors (primarily breast, gynecologic, and GI[a]; also lung and other)	Nutritional and Inflammatory status score	Nutritional and inflammatory status score and performance status were determinants of severe hematologic toxicity associated with CT
Amaral and colleagues, 2008[17] DVR[b] Portugal Positive	N=130 Various (head and neck, GI, gynecologic, and other)	MST[c] score MUST[d] score NRS-2002[e] score (reference standard)	Mean LOS: 12.5 d for undernourished vs 7.5 d for well nourished (P=0.016) Undernourished or at nutritionally at risk patients (classified by NRS-2002 or MUST), respectively, had an independent higher risk of ↑ LOS (≥7 d)
Antoun and colleagues, 2009[32] Prospective cohort France Positive	N=275 Various	Weight loss PG-SGA[f] score NRI[g] score	BW[h] loss ≥10% significantly (P=0.02) correlated with LOS (P<0.05) BW loss ≥15% significantly correlated with LOS (P<0.001) PG-SGA score and NRI significantly correlated with LOS (P<0.001 and P=0.001, respectively)
Barlow and colleagues, 2011[33] Randomized, controlled trial United Kingdom Positive	N=121 Upper GI	NRI score Unintended weight loss	NS[i] differences in hospital readmissions between patients receiving early enteral nutrition vs nil by mouth within 6 wk of discharge or between 6 and 12 wk after discharge Patients receiving early enteral nutrition had shortened LOS (16 vs 19 d; P=0.023)
Bauer and colleagues, 2005[34] Noncontrolled trial Australia Neutral	N=7 Pancreatic or NSCLC[j]	PG-SGA score	Over 8 wk, change in nutritional status (PG-SGA score) was significantly associated with change in QoL (R=−0.835; P=0.020)
Braga and colleagues, 1998[35] Randomized, controlled trial Italy Positive	N=166 Gastric or pancreatic	Weight loss	Length of postoperative stay was significantly reduced in the enriched early enteral nutrition group compared with the parenteral nutrition group (13.7±4.8 d vs 17.5±6.1 d; P<0.05)

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Author(s), year Study design Location Quality rating	Population Cancer site	Nutritional status indicator(s)	Results
Capuano and colleagues, 2008[36] Noncontrolled trial Italy Neutral	N=40 Head and neck	Weight loss	84% of patients who were noncompliant with nutrition program were admitted to hospital due to severe malnutrition and dehydration Rate of hospital readmission was 53% in patients with BW loss >20% vs 13% in patients with BW loss <20% ($P<0.003$) Treatment interruptions were correlated with BW loss percentage (Spearman test: $r=0.484$; $P=0.003$) Infections were higher in patients with BW loss >20% vs <20% BW loss (47% vs 4%; $P=0.002$) Mortality was higher in patients with BW loss of >20% (35% vs 4%; $P=0.029$)
Carey and colleagues, 2011[37] Case series Australia Positive	N=30 Upper GI	SGA[k] score Low BMI[l] % Weight change Nutritional intake Suboptimal triceps skinfold, midarm muscle circumference, hand grip strength	Malnourished participants had poorer QoL and more symptoms SGA and the Gastrointestinal Symptom Rating Scale were significant in explaining 50.3% of variance in global QoL ($F=13.646$; $P<0.001$)
Correia and colleagues, 2007[38] DVR Portugal Positive	N=48 Gastric	PG-SGA score Suboptimal hand grip strength TNF-α[m] Loss of fat-free mass, subcutaneous fat Weight loss	TNF-α values showed an excellent discriminative power to identify malnourished patients with a sensitivity of 93% and a specificity of 94%; TNF-α was the more significantly associated to worse QoL in both functional and symptom scales and also to anorexia

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Author(s), year Study design Location Quality rating	Population Cancer site	Nutritional status indicator(s)	Results
Dewys and colleagues, 1980[39] Cross-sectional United States Neutral	N=3,047 Non-Hodgkin's lymphoma, breast, leukemia, sarcoma, colon, prostate, SCLC[n], NSCLC pancreatic, gastric	Weight loss	Survival was shorter in patients with BW loss compared with those without BW loss; statistically significant for 9 of 12 comparisons; when analyzed by weight loss categories, the greatest difference was between the no weight loss and the 0% to 5% weight loss categories for prostate and colorectal cancer; for each tumor extent category, survival was shorter in colon cancer patients with BW loss compared with those without
Eriksson and colleagues, 1998[40] Retrospective cohort Sweden Neutral	N=52 Acute leukemia	Suboptimal albumin Weight loss	BW change was statistically significant related to the number of days with fever ($R=-0.35$; $P=0.026$) Lowest recorded serum albumin value correlated negatively with the number of infections and number of days with fever ($R=-0.33$; $P=0.03$ and $R=-0.4$; $P=0.002$) 16% had unplanned breaks in RT, all of which experienced greater BW loss than those without treatment breaks (median change −3.1% vs −1.6%, respectively; $P<0.05$)
Fearon and colleagues, 2006[41] Prospective cohort Not specified Positive	N=175 Pancreatic	Cachexia Suboptimal hand grip strength Loss of LBM[o] Weight loss	QoL function variables ($P<0.001$), health status ($P<0.001$), Karnofsky performance scale ($P<0.001$), grip strength ($P<0.001$), and LBM ($P=0.003$) were significantly lower in patients meeting the cachexia profile definition (ie, BW loss, food intake, and inflammatory status) LBM (hazard ratio 1.028; $P=0.018$) and the 3-factor cachexia profile itself (hazard ratio 2.959; $P<0.001$) were prognostic
Gioulbasanis and colleagues, 2011[42] DVR Greece Positive	N=115 Metastatic lung	Mini Nutrition Assessment score	Mini Nutrition Assessment score was significantly associated with overall survival ($P=0.004$)

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Author(s), year Study design Location Quality rating	Population Cancer site	Nutritional status indicator(s)	Results
Gupta and colleagues, 2010[43] Retrospective cohort United States Positive	N=98 Ovarian	SGA score	At baseline, median survival for SGA-A (n=46) was 20.3 mo whereas SGA-B/C (n=52) was 9.8 mo (P=0.03); at 3 mo, median survival for SGA-A (n=63) was 19.9 mo, whereas SGA-B/C (N=35) was 3.7 mo (P<0.001) Patients with improved nutritional status at 3 mo had a significantly better survival than those with deteriorated nutritional status independent of age, stage at diagnosis, prior treatment history, and tumor response, as determined by cancer antigen 125
Hammerlid and colleagues, 1998[44] Descriptive Sweden Neutral	N=58 Head and neck	Weight loss Weight Index Low BMI Suboptimal albumin	When comparing malnourished with normal nutritional status, malnourished patients scored worse for 12 of the 16 functions/symptoms (NS) Patients with >5% BW loss vs those with no BW loss scored worse for 11 of 16 QoL functions, with significant differences between groups for "swallowing difficulties" and "problems swallowing food" (both P values <0.01) Survivors scored better than deceased patients for all 16 QoL functions/symptoms. Significant difference between the 2 groups for appetite loss (P<0.01), fewer problems swallowing food (P<0.01)
Hill and colleagues, 2011[45] Prospective cohort Australia Positive	N=73 GI cancers	PG-SGA score Weight loss	Toxicity severity was higher in those who experienced unplanned hospital admission compared with those without admission (42.1% vs 9.3%, respectively; P<0.001). Severity of RT toxicity was strongly correlated with PG-SGA score (R=0.839; P<0.001) 16% of patients had unplanned breaks in RT and experienced greater weight loss than those without treatment breaks (median change −3.1% vs −1.6%, respectively; P<0.05) Patients not completing prescribed CT had a significantly greater change in PG-SGA score throughout RT, than those who did complete CT (median increase 17 vs 3; P<0.05)

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Author(s), year Study design Location Quality rating	Population Cancer site	Nutritional status indicator(s)	Results
Horsley and colleagues, 2005[46] Case Australia Positive	N=66 Patients requiring peripheral blood stem cell transplantation	PG-SGA score Suboptimal albumin	Significant correlation between PG-SGA score and LOS posttransplant (R=0.308; P=0.013) and serum albumin (R= −0.338, P=0.006) Well-nourished 16.9±6.3 days vs malnourished 23.9±9.9 d; P<0.002; −7.0±2.1 d difference between groups in posttransplant LOS
Hyltander and colleagues, 2005[47] Randomized, controlled trial Sweden Positive	N=126 Upper GI	Preoperative weight loss >10% Suboptimal triceps skinfold, arm muscle circumference Loss of LBM	LOS among groups was NS The Psychological General Well-Being Index improved more in the patients receiving parenteral nutrition, than enteral and oral nutrition postoperatively. The difference in the Psychological General Well-Being Index total emerged from 6 mo onward after the operation (P<0.05) The Psychological General Well-Being Index dimensions of anxiety and positive well-being followed the same pattern with less anxiety and more positive well-being in the parenteral nutrition group (P<0.05) Emotional functioning according to EORTC QLQ-C30[p112] had improved significantly more in the parenteral nutrition group compared with the oral group after 6 mo (P<0.01), but after 12 mo difference was NS Survival did not differ significantly between patients receiving parenteral, enteral, or oral nutrition
Ionescu and colleagues, 2009[48] Randomized, controlled trial Romania Positive	N=96 Colorectal	Prolonged time to mobilization postsurgically and delayed feeding	Fast Track protocol vs Conventional (fluid and solid food intake): High-dependency unit stay 0.92±1.11 d (fast track) vs 1.77±1.46 d (conventional); LOS 6.43±3.41 d (fast track) vs 9.16±2.67 d (Conventional); (P=0.001 for both)
Isenring and colleagues, 2003[25] DVR Australia Positive	N=60 Head and neck, abdominal, rectal	PG-SGA score	Significant correlation between PG-SGA score and global QoL at baseline (R=−0.66; P<0.001) and after 4 wk of RT (R=−0.61; P<0.001)

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Author(s), year Study design Location Quality rating	Population Cancer site	Nutritional status indicator(s)	Results
Iversen and colleagues, 2010[49] Prospective cohort Norway Neutral	N=15 Multiple myeloma	Suboptimal albumin, handgrip strength, triceps skinfold Low BMI Weight loss	All 4 symptoms scores (nausea or vomiting, appetite loss, fatigue, and pain) for global health-related QoL rose significantly during therapy, indicating worsening of symptoms before returning to pretherapy levels at the end of the observation
Kathiresan and colleagues, 2011[50] Retrospective cohort US Neutral	N=300 Gynecologic	Suboptimal albumin Low BMI/underweight	Malnutrition reflected by low albumin levels is associated with significantly higher post readmission (3.9 vs 3.5 g/dL; $P=0.01$), more reoperations (3.8 vs 3.4 g/dL; $P=0.03$), more intensive care unit admissions (3.9 vs 3.0 g/dL; $P<0.001$) Underweight significantly correlated with more hospital readmissions (0.8 vs 17.4%; $P=0.001$)
Laky and colleagues, 2010[51] Prospective cohort Australia Positive	N=157 Gynecologic	PG-SGA score	Malnutrition and low QoL predicted prolonged LOS; Stage III or IV ovarian cancer associated with prolonged LOS
Martin and Lagergren, 2009[52] Prospective cohort Sweden Neutral	N=176 Esophageal, gastric	Weight loss Obesity	Preoperative BW loss was more pronounced in those who died between 6 mo and 3 y. However, postoperative BW loss was similar in the 2 groups.

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Author(s), year Study design Location Quality rating	Population Cancer site	Nutritional status indicator(s)	Results
Martin and colleagues, 2010[53] Prospective cohort Canada Neutral	N=1,164 Advanced cancer	PG-SGA score	Shortened survival was associated with increasing BW loss and BW gain compared with stable weight; survival was shorter for all BMI <30.0 Shortened survival was associated with the 3 low food-intake categories ("little solid food," "only liquids/nutritional supplements," "very little of anything") that were subsequently categorized as "abnormal food intake" Median survival fitness for patients with "normal intake" (5.0 mo; 95% CI 3.7-6.2 mo), "normal food at reduced amounts" (3.4 mo; 95% CI 3.0-3.8 mo) and "abnormal intake" (2.1 mo; 95% CI 1.7-2.4 mo) were different (P=0.001) Nutrition impact symptoms associated with shorter survival were no appetitive, feel full quickly, altered taste, dry mouth, and dysphagia Patient-reported PG-SGA performance status scores of 0-2 had longer median survival (4.3 mo [95% CI 3.8-4.8 mo]) than patients with PS 3 (2.5 mo [95% CI 2.2-2.8 mo]) or patients with PS 4 (1.3 mo [95% CI, 0.05-2.0 mo]; P<0.001)
Nourissat and colleagues, 2008[54] Cross-sectional France Positive	N=907 Various; at different management stages	NRI score PG-SGA score Weight loss	Patients who lost <10% BW since the start of their illness had a significantly higher QoL score compared with those who had lost >10% BW (62.8 vs 48.8; P<0.001) A significant difference in the QoL score was observed between patients who had and those who had not modified their diet at the time of the study (65.3 vs 52.5; P<0.001) A significant association was observed between the performance status score and percent BW loss (P<0.001)

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Author(s), year Study design Location Quality rating	Population Cancer site	Nutritional status indicator(s)	Results
Odelli and colleagues, 2005[55] Retrospective Australia Neutral	N=48 Esophageal	Nutrition assessment Weight loss	Control group patients were more likely to have an unplanned hospital admission during therapy than nutrition pathway patients ($P<0.04$) Total days of unplanned hospital admissions were less in nutrition pathway (3.2±5.4) than in the control group (13.5±14.1) ($P<0.002$) Nutrition pathway patients were more likely to receive nutrition intervention than control patients ($P<0.05$); nutrition pathway patients lost less BW over the treatment period ($P<0.03$); significant difference was observed between groups in the number who completed the prescribed course of RT: 92% (nutrition pathway) vs 50% (control) ($P<0.003$); Nutrition pathway group received a greater percentage of the desired RT dose ($P<0.004$). NS difference between groups in dose of CT received, although there was a trend toward fewer dose reductions in the nutrition pathway group ($P<0.33$)
Ollenschlager and colleagues, 1992[56] Randomized, controlled trial Germany Neutral	N=29 Acute leukemia	Weight loss	Fatigue/malaise correlated exclusively with BW loss, whereas nutrient intake correlated closely with tumor therapy side effects
Persson and colleagues, 1999[29] DVR Sweden Neutral	N=87 Various	PG-SGA score	Significant difference in survival between SGA-A and SGA B+C for total group, ($P<0.001$), GI tumors ($P<0.01$), and metastatic GI cancer ($P<0.05$)
Phippen and colleagues, 2011[57] DVR United States Neutral	N=58 Gynecologic	PG-SGA score	PG-SGA score of 7.5 predicted febrile neutropenia during CT

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Author(s), year Study design Location Quality rating	Population Cancer site	Nutritional status indicator(s)	Results
Piquet and colleagues, 2002[58] Retrospective cohort Switzerland Neutral	N=90 Oropharyngeal	Weight loss	Significant differences were seen between patients receiving nutrition intervention and those who did not for BW loss (3.5%±0.7% intervention vs 6.1±0.7 control; $P<0.01$) and hospital admissions for dehydration (0% intervention group [n=0] vs 18% control group [n=8]; $P<0.01$). Overall hospital admissions were 9 intervention vs 14 control (NS)
Prado and colleagues, 2007[59] Prospective cohort Canada Neutral	N=95 Colon	Loss of LBM	Mean 5-fluorouracil/kg LBM values of the population varied with regard to presence or absence of toxicity ($P=0.036$); a cutpoint of 20 mg 5-fluorouracil/kg LBM was identified as a threshold and predictor for developing toxicity ($P=0.005$) (odds ratio 16.5; $P=0.013$)
Prado and colleagues, 2008[60] Prospective cohort Canada Positive	N=250 Lung, colorectal, or other GI sites	Sarcopenia Obesity	Sarcopenic obesity was shown to be a significant independent predictor of survival ($P<0.0001$) Survival was ~10 mo shorter for patients with sarcopenic obesity
Prado and colleagues, 2009[61] Prospective cohort Canada Neutral	N=55 Breast	Sarcopenia	Prevalence of dose-limiting toxicity was 50% in patients with sarcopenia (7 of 14) and 19.5% in the nonsacropenia (8 of 41; $P=0.039$; hazard ratio for toxicity 4.1); toxicity prevalence (sarcopenia vs nonsarcopenia): stomatitis 36% vs 4.9% ($P=0.008$); diarrhea 29% vs 2.4% ($P=0.01$) The administered dose of capecitabine was highly variable (range=67.4-137 mg/kg LBM); thus, patients with sarcopenia received a raised amount of capecitabine dose per unit of LBM
Prado and colleagues, 2011[62] Prospective cohort Canada Neutral	N=132 Breast	Loss of LBM	Mean LBM was lower for patients presenting with treatment toxicity compared with those where toxicity was absent (41.6 kg vs 56.2 kg; $P=0.002$)

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Author(s), year Study design Location Quality rating	Population Cancer site	Nutritional status indicator(s)	Results
Pressoir and colleagues, 2010[63] Prospective cohort France Positive	N=1,545 Various (breast, head and neck, and other)	Weight loss Low BMI	Moderate or severe malnutrition was associated with prolonged LOS (median LOS 19.3+19.4 d); malnourished vs 13.3±19.4 d well-nourished ($P<0.0001$) Mortality (18.4%) was significantly higher in malnourished patients than in the other group (26.7 vs 11.8%; $P<0.0001$; odds ratio 2.7 [1.9-3.9], especially in severe malnutrition (37.1%; odds ratio 4.4 [2.8-6.9]) compared with mild symptoms (20.2%; odds ratio 1.9 [1.2-2.9]) Multivariate analysis showed that only severe malnutrition was independently associated with mortality
Ravasco and colleagues, 2003[64] Before-after study Portugal Positive	N=125 Head and neck, GI (high risk) Prostate, breast, lung, brain, gallbladder, uterus (low risk)	PG-SGA score	QOL was always better in low-risk patients than in high-risk patients ($P<0.01$); patients with gastric and head and neck cancers reported the lowest QoL; for high-riskpatients, QoL improved throughout the study, and improvement was statistically correlated with a rise in nutritional intake ($P<0.001$); QoL remained stable throughout the study in low-risk patients Individualized nutrition counseling is able to overcome predicted nutrition deterioration associated with RT, but only high risk patients appear to benefit
Ravasco and colleagues, 2005[65] Randomized, controlled trial Portugal Positive	N=75 Head and neck	PG-SGA score	After RT, QOL scores improved proportionally with improved nutritional status and intake in dietary counseling and nutrition supplement groups ($P<0.05$) and ↓ in the ad libitum group ($P<0.05$); at 3 mo, diet counseling group maintained or improved overall QOL, whereas patients in the other 2 groups maintained or worsened 90% of patients experienced RT-induced toxicities; trend for reduced toxicity in the dietary counseling group ($P<0.07$); at 3 mo, grade 1 and 2 symptoms of anorexia, nausea and vomiting, xerostomia, and dysgeusia were improved in 90% of these patients, 67% of nutrition supplements patients, and 51% of ad libitum patients ($P<0.001$)

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Author(s), year Study design Location Quality rating	Population Cancer site	Nutritional status indicator(s)	Results
Ravasco and colleagues, 2005[66] Randomized, controlled trial Portugal Positive	N=111 Colorectal	PG-SGA score	Following RT completion, QoL function scores improved proportionally to adequate food intake or nutritional status ($P<0.05$) in those receiving dietary counseling; in patients receiving protein supplements, only half of the function scores improved ($P=0.04$). In the ad libitum group all scores worsened ($P<0.05$) Patients receiving dietary counseling had significantly fewer toxicity symptoms than the other groups. After RT and at 3 mo, rates of anorexia, nausea, vomiting, and diarrhea were highest in the ad libitum group ($P<0.05$) Overall greater toxicity symptoms were correlated with poorer nutritional status (ie, PG-SGA score) ($r\geq-0.63$; $P\leq0.002$)
Robinson and colleagues, 2008[67] Randomized, controlled trial United States Neutral	N=86 Pancreatic	FACIT-F and FAACT[q111] score Weight loss Loss of LBM	Patients reported impaired health across all Short Form-36 general health survey measures at baseline with the lowest mean scores being in physical role (32.1), physical component summary (36.2), physical functioning (37.9), and pain (37.8) Patients who lost ≤5% BW within 30 d of treatment had a median overall survival of 7.3 mo (95% CI 6.3-9.1); Patients who lost >5% BW survived for a median of 6.5 mo (95% CI 4.6-10.7; log rank $P=0.44$) Median overall survival was 9.1 mo (95% CI 7.2-11.4) for patients having low fatigue (indicated by higher scores >30, n=48 and 5.2 mo, 95% CI 4.0-7.2) for those with high fatigue (indicated by score ≤30; n=32; log rank $P=0.002$)

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Author(s), year Study design Location Quality rating	Population Cancer site	Nutritional status indicator(s)	Results
Ross and colleagues, 2004[68] Cohort United Kingdom Positive	N=780 Lung	Weight loss	Fewer patients with BW loss (n=315; 67%) completed 3 cycles of CT than those without BW loss (n=210; 81%; $P<0.001$); treatment was delayed significantly more frequently in patients with BW loss associated with NSCLC than those without (9.0% vs 4.0%; $P=0.04$) Overall survival was significantly shorter for patients with BW loss compared with those without BW loss with SCLC (6 vs 11 mo; $P=0.0003$), NSCLC (6 vs 9 mo; $P<0.0001$), and mesothelioma (5 vs 12 mo; $P=0.025$) Weight stabilization for patients with NSCLC resulted in significant improvement in both progression free (rise from 5-7 mo; $P=0.01$) and overall survival (rise from 7-9 mo; $P=0.006$)
Shahmoradi and colleagues, 2009[69] Cross-sectional Malaysia Neutral	N=61 receiving home hospice care Advanced cancer	PG-SGA score	PG-SGA scores significantly correlated with total QoL scores and the 3 subscale scores Patients with a higher PG-SGA score or poorer nutritional status exhibited a lower QoL
Sorensen and colleagues, 2008[70] Prospective cohort Various countries in Eastern and Western Europe, and the Middle East Positive	N=5,051 Various	NRS-2002 score	Death was more frequent in nutritionally at-risk patients than not at risk ($P<0.001$) LOS $\geq$28 d was related to age $\geq$70 y ($P<0.0001$), cancer ($P<0.0001$), and specialties other than intensive care unit ($P<0.0001$); longer LOS predictors: at-risk status, age $\geq$70 y, cancer, comorbidity, and complications; LOS was significantly related to the nutrition screening components when adjusted for confounding variables Six days (range=3-11 d) not-at-risk patients vs 9 d (range=5-16 d) at-risk patients, $P<0.001$; LOS <28 d not-at-risk patients: 7.3±0.1 d vs 9.7±0.2 d at-risk patients; $P<0.01$ LOS >28 d marginally associated with nutritional status; $P=0.053$

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Author(s), year Study design Location Quality rating	Population Cancer site	Nutritional status indicator(s)	Results
Tan and colleagues, 2009[71] Prospective cohort Canada Positive	N=111 Ampullary carcinoma, cholangio-carcinoma, neuroendocrine tumors	Sarcopenia Overweight/obesity	Median survival for patients who were both overweight/obese and sarcopenic was 55 days (interquartile range=43-207 d) compared with 148 d (interquartile range=80-369 d) for those without overweight/obese sarcopenia (log-rank test P=0.003) On multivariate analysis, age ≥59 y (hazard ratio 1.71, 95% CI 1.10-2.66; P=0.018) and overweight/obese sarcopenia (hazard ratio 2.07, 95% CI 1.23-3.50; P=0.006) retained independent prognostic value
Yoon and colleagues, 2011[72] Retrospective cohort United States Positive	N=778 Esophageal	Obesity	For disease-specific survival the BMI and smoking interaction term was significant (P=0.023), indicating that the prognostic impact of excess BMI differed significantly on the basis of smoking status. Among never smokers (n=236), univariate analysis revealed that obese patients had significantly shorter disease-specific survival compared with normal-weight patients (hazard ratio 1, 0.62, 95% CI 1.03-2.53; P=0.034). In multivariable analysis among never smokers, obesity was significantly associated with adverse disease-specific survival (hazard ratio 2.11, 95% CI 1.31-3.43; P=0.002), DFS (hazard ratio 2.03, 95% CI 1.30- .18; P=0.002), and OS (hazard ratio 1.97, 95% CI 1.24 to 3.14; P=0.004) compared with normal weight, after adjusting for tumor stage, grade, age, presurgical BW loss, and sex

[a]GI=gastrointestinal.
[b]DVR=diagnostics, validity, or reliability study.
[c]MST=Malnutrition Screening Tool.
[d]MUST=Malnutrition Universal Screening Tool.
[e]NRS-2002=nutritional risk screening 2002.
[f]PG-SGA=patient generated subjective global assessment.
[g]NRI=nutrition risk index; not validated in oncology populations.
[h]BW=body weight.
[i]NS=not significant.
[j]NSCLC=non–small cell lung cancer.
[k]SGA=Subjective Global Assessment.
[l]BMI=body mass index.
[m]TNF-α=tumor necrosis factor α.
[n]SCLC=small cell lung cancer.
[o]LBM=lean body mass.
[p]EORTC QLQ-C30=European Organization for Research and Treatment of Cancer quality of life questionnaire.
[q]FACIT-F and FAACT=Fatigue and Nutritional Health Assessment and Functional Assessment of Anorexia/Cachexia Therapy.

Table 3. Summary of studies included in the Academy of Nutrition and Dietetics Evidence Analysis Library systematic review of the effectiveness of medical nutrition therapy (MNT) in adult oncology patients undergoing chemotherapy and radiation treatment

Author(s), year Study design Location Quality rating	Population Cancer site	MNT Intervention by an RDN/FNP[a] Duration	Results
Block and colleagues, 2009[73] Case series United States Neutral	N=90 Final N=78 Metastatic breast cancer; attending community-based facility (Block Center for Integrative Cancer Treatment) undergoing CT[b]	**MNT:** Personalized nutrition and supplement regimen developed by oncologist; RDN provided patient education and hands-on training[c] **Duration:** Followed for survival for 5 y	Median survival time from metastasis: 38 mo (range=7-137 mo; 95% CI 27-48). 3-y survival: 52%; 5-y survival: 27% Disease-free interval <18 mo, age group (<40, 41-50, >50 y) and estrogen receptor status (positive or negative) significantly predicted survival Disease-free interval of >18 mo had a significantly longer survival than those with a shorter disease-free interval (P=0.007).
Danielson and Fairchild, 2011[74] Noncontrolled trial Canada Neutral	N=40 Final N=29 Nonhematologic cancers with brain metastases; planned for RT[d]	**MNT:** A multidisciplinary palliative RT clinic (radiation oncologist, radiation therapist, registered nurse, nurse practitioner, pharmacist, social worker, occupational therapist, and FNP) to optimize symptom control and QoL[ce] **Control:** None **Duration:** 4 wk	A total of 11 clinic patients (33%) were assessed by FNP for symptoms of weight loss, ↓ appetite, and 1 for ↑ appetite and weight gain Patient satisfaction: 86% reported very satisfied with the clinic experience; 97% would recommend the clinic although these data are not specific to patients being seen by an FNP. Too few patients were able to complete the QoL questionnaires at 4 wk for meaningful statistics.
Dawson and colleagues, 2001[75] Non-RCT[f] Scotland Neutral	N=71 (MNT: n=45 consecutive patients; Control: n=26) Final N=NR[g] Squamous cell carcinoma of the oral cavity; undergoing RT 4 wk following surgery	**MNT:** Continuous dietary supervision per FNP postsurgery through post-RT as frequently as required, usually once a week[c] **Control:** Historical controls received dietary supervision once every 2 wk, with a 4-wk gap postsurgery through pre-RT **Duration:** 1 y	Weight loss postsurgically: MNT group 2.42% vs control 3.67% (P<0.05) Weight loss post-RT: MNT group 4.83% vs 6.56% control (P<0.05) Weight loss after surgery and RT: MNT group 6.6% vs 9.83% control (P<0.05). Weight loss at 1 y: MNT group 5.9% vs 7.82% control (NS)
Dintinjana and colleagues, 2008[76] Non-RCT Croatia Neutral	N=388 (MNT: n=215; Control: n=173) Final N=388 CRC[h]; undergoing CT[i]	**MNT:** Counseling by FNP + enteral or parenteral nutrition or MGA,[j] as appropriate **Control:** No nutrition support; monitored retrospectively **Duration:** 12 visits according to CT schedule; time course unclear	Number of patients with BMI[i] <20: 15.3% ↓ MNT group vs 12.1% ↑ control (P value=NR) 65% of MNT group ↑ BW[k]; greatest BW gain in patients on MGA. MNT group had ↑ appetite scores, especially in those receiving MGA 39% of control group had BW ↓ ≥2 kg/mo during treatment KPS[l] scores: NS[m] change for either group

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Table 3. Summary of studies included in the Academy of Nutrition and Dietetics Evidence Analysis Library systematic review of the effectiveness of medical nutrition therapy (MNT) in adult oncology patients undergoing chemotherapy and radiation treatment (*continued*)

Author(s), year Study design Location Quality rating	Population Cancer site	MNT Intervention by an RDN/FNP[a] Duration	Results
Glare and colleagues, 2011[77] Prospective cohort Australia Neutral	N=54 Final N=25 Lung, CRC, and upper GI[n] cancers with anorexia cachexia syndrome; 81% undergoing CT, RT, or combined therapy	**MNT:** Individualized treatment program, including symptom management, supplements if needed, and strength training, if needed Patients were evaluated by the physician, FNP, and physical therapist **Duration:** 8 wk	Those who stayed in the program for 2 mo lost less weight (10% vs 11.8%), were better nourished (Subjective Global Assessment category A 30% vs 0%), were fitter (median 6-min walk test in meters, 464 vs 390), and were less likely to have ↑ C-reactive protein value (≤10, 29% vs 9%) Of the 35 participants attending the baseline physical therapy assessment, >90% reported that the cancer nutrition rehabilitation program was important to them
Glimelius and colleagues, 1992[78] Case-control Sweden Neutral	N=58 (n=58 MNT; n=22 QoL-C; n=81 Survival nutrition control) Final N=36 MNT; 22 QoL-C; 81 Survival nutrition control SCLC; undergoing CT with curative intent	**MNT:** Individualized nutrition counseling, MFS[o] and symptom management **Control 1:** Survival nutrition control historical controls **Control 2:** QoL-C preproject control group **Duration:** 8 treatment courses	NS differences in survival, responses to treatment, duration of responses to treatment, number of treatment days in hospital, number of days before patients reached course 8 or 16, septic episodes, or erythrocyte transfusions BW during treatment ($P<0.01$), BMI during treatment ($P<0.05$), and proportion of patients who experienced a ↓ in BW >10% ($P<0.05$) was significantly better in the MNT group Proportion of patients with a marked ↓ in serum albumin was higher in the Survival nutrition control group ($P<0.01$) PRO[p] intake improved during the study period to an average 50-80 g/d/patient; no patient reached desired PRO level Global scores for the CIPS[q] were improved for the MNT group ($P<0.001$) vs QoL-C group Significant differences for the study group were seen in physical ($P<0.001$), psychosocial ($P<0.001$), CIPS groups subsets MNT group: Strong trend toward ↓ CT-related adverse effects ($P=0.07$)

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Table 3. Summary of studies included in the Academy of Nutrition and Dietetics Evidence Analysis Library systematic review of the effectiveness of medical nutrition therapy (MNT) in adult oncology patients undergoing chemotherapy and radiation treatment (*continued*)

Author(s), year Study design Location Quality rating	Population Cancer site	MNT Intervention by an RDN/FNP[a] Duration	Results
Goncalves Dias and colleagues, 2005[79] Non-RCT Brazil Neutral	N=64 (n=32 oral group; n=16 Nasoenteral feeding; n=16 MFS) Final N=NR H&N[r] cancer; undergoing RT following surgery	3 MNT groups **MNT Oral:** Oral diet of appropriate consistency with 5-6 small meals/d **MNT NEF:** Home nasoenteral feeding **MNT MFS:** Oral diet+MFS 3/d All groups counseled by specialized nutritionist to maintain 40 kcal/kg intake during treatment period **Duration:** 70 d	Caloric ingestion ↑ pre- to postintervention in all 3 groups ($P<0.001$). Greatest ↑ occurred in MNT nasoenteral feeding group ($P<0.05$) PRO ingestion ↑ in all 3 groups ($P<0.001$) NS pre- and postintervention differences in anthropometric values between the 3 groups NS differences in lab values between the 3 groups, with the exception of total lymphocytes, which ↓ significantly for all groups following RT
Isenring and colleagues, 2003[80] RCT Australia Positive	N=36 (MNT: n=15; Control: n=21) Final N=32 H&N cancer; undergoing RT	**MNT:** Regular intensive nutrition counseling by FNP according to the ADA[s] MNT protocol for radiation oncology **Control:** General nutrition advice from the nursing staff, a nutrition booklet, and MFS samples **Duration:** 3 mo	Control lost significantly more BW than MNT group (4.3 kg vs 1.1 kg, 6.1% vs 1.1%, respectively; $P<0.019$). Control lost significantly more fat-free mass than MNT group (2.2 kg vs 0.3 kg; $P<0.029$). Control lost more fat mass than MNT group (NS)
Isenring and colleagues, 2004[81] RCT Australia Positive	N=60 (MNT: n=29; Control: n=31) Final N=NR GI or H&N cancers; undergoing RT	**MNT:** Regular, intensive counseling by an FNP **Control:** UC[t] educated by nurses; received nutrition booklet and MFS samples **Duration:** 12 wk	MNT group differed from control in BW change (MNT −0.4 kg, UC −4.7 kg; $P<0.001$), deterioration in nutritional status per PG-SGA[u] score ($P<0.02$); deterioration in and recovery of global QoL score ($P<0.009$); physical function over time ($P<0.012$); number who remained weight stable during treatment (MNT 24% vs UC 11%; $P<0.016$). MNT group maintained fat-free mass; control group lost fat-free mass (+0.4 kg vs −1.4 kg; $P<0.195$)
Isenring and colleagues, 2004[82] RCT Australia Neutral	N=60 (MNT: n=29; UC: n=31) Final N=53 GI or H&N cancers; undergoing RT	**MNT:** Early, intensive nutrition support by the same FNP + high energy/PRO MFS if required **UC:** Education from nurses, a nutrition booklet, and MFS samples **Duration:** 12 wk	MNT group scored staff interpersonal skills higher than the UC group ($P<0.001$), perceived health benefits ($P<0.008$), staff presentation skills ($P<0.044$), and overall patient satisfaction ($P<0.002$) Overall patient satisfaction measures remained significant regardless of age or level of family support

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Table 3. Summary of studies included in the Academy of Nutrition and Dietetics Evidence Analysis Library systematic review of the effectiveness of medical nutrition therapy (MNT) in adult oncology patients undergoing chemotherapy and radiation treatment (*continued*)

Author(s), year Study design Location Quality rating	Population Cancer site	MNT Intervention by an RDN/FNP[a] Duration	Results
Isenring and colleagues, 2007[83] RCT Australia Positive	N=60 (MNT: n=29; UC: n=31) Final N=54 GI or H&N cancers; undergoing RT	**MNT:** Regular intensive nutrition counseling by FNP using the ADA MNT radiation oncology protocol **Control:** General nutrition talk by nurses, nutrition and cancer booklet, high energy/PRO MFS sample. **Duration:** 12 wk	MNT group had a smaller ↓ and faster recovery in global QoL (P=0.009) and physical function (P=0.012) over time than the control group. MNT group lost less BW over the treatment period (P<0.03) MNT group ↑ mean energy and PRO intake/day vs control group (P=0.029 and P<0.0001, respectively) Similarly mean energy and PRO intake/kg BW/d was ↑ for the MNT group (P=0.022 and P=0.001, respectively) MNT group had a trend toward a ↑ in fiber intake (P=0.083) MNT group had ↑ PG-SGA scores 8 wks (P=0.02) and trended higher at 12 wks (P=0.065)
Odelli and colleagues, 2005[55] Retrospective cohort Australia Neutral	N=48 (MNT: n=24; Control: n=24) Final N=48 Esophageal cancer; undergoing CT/RT	**MNT:** Nutrition pathway at initiation of treatment by FNPs and weekly **Control:** Nutrition treatment in a reactive manner by FNP, only when problems occurred **Duration:** NR	Number of patients assessed at severe risk who received enteral nutrition was greater in the MNT group (P<0.003), than control NS difference between the 2 groups in dose of CT received, although there was a trend toward fewer dose reductions in the MNT group (P<0.33) MNT group completed the prescribed number of RT treatments (92% MNT vs 50% control group; P<0.003) and received a greater % of desired RT dose (P<0.004) MNT group had fewer unplanned hospital admissions (P<0.04) and shorter unplanned hospital length of stay (3.2±5.4 vs 13.5±14.1 days) (P<0.002) during therapy than the control group Hospitalization for nutrition support was less in MNT than control (1 vs 6; NS)

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Table 3. Summary of studies included in the Academy of Nutrition and Dietetics Evidence Analysis Library systematic review of the effectiveness of medical nutrition therapy (MNT) in adult oncology patients undergoing chemotherapy and radiation treatment (*continued*)

Author(s), year Study design Location Quality rating	Population Cancer site	MNT Intervention by an RDN/FNP[a] Duration	Results
Ollenschlager and colleagues, 1992[56] RCT Germany Neutral	N=32 (MNT: n=16; Control: n=16) Final N=29 Acute lymphocytic leukemia; acute nonlymphocytic leukemia; undergoing poly-CT: LAM-6; ULM; TAD	**MNT:** Intensive oral nutrition + FNP intervention **Control:** Diet ad libitum **Duration:** Mean 25.5 wk	Days with temperature >38.5°C, remission rate, or in-study mortality between groups (NS) No difference between groups in BW during the induction period, and nutritional status of all patients was highly impaired Both groups experienced a BW ↓of 8% of pretreatment weight up to third to seventh study week; one-third lost >10% After the period of weight loss, the MNT group demonstrated benefits MNT group receiving LAM 6 treatment showed more weeks with BW gain to the end of the induction phase than the control (BW ↑ during 33.8% vs 13.2% of the induction phase) Dietetic intervention resulted in many more weeks of stable BW in the study groups (48.7% vs 18.3% of the induction phase for the TAD groups; 53.1% vs 31.5% of the induction phase for the ULM groups; $P<0.05$) At the end of the induction period, 5 out of 16 MNT group and 11 out of 16 control group weighed <95% prestudy weight (*P* value NR). During the consolidation period, MNT group ↓ weight for 22.8% of treatment weeks; control groups ↓ weight during 49% of treatment weeks A significant correlation was found between nutritional intake and tumor-therapy side effects (eg, anorexia and fatigue) (*P* values <0.01)

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Table 3. Summary of studies included in the Academy of Nutrition and Dietetics Evidence Analysis Library systematic review of the effectiveness of medical nutrition therapy (MNT) in adult oncology patients undergoing chemotherapy and radiation treatment (*continued*)

Author(s), year Study design Location Quality rating	Population Cancer site	MNT Intervention by an RDN/FNP[a] Duration	Results
Ovesen and colleagues, 1993[84] RCT Denmark Positive	N=137 (MNT: n=57; Control: n=48) Final N=105 Breast, ovary, or lung (small cell) tumors; undergoing CT with curative intent	**MNT:** Individual counseling by FNP as desired + MFS to achieve nutrition goals **Control:** Nutrition education at physician's discretion **Duration:** 5 mo	No difference in BW between groups (NS) although energy and PRO intake was ↑ for MNT group ($P<0.5$) Both groups ↑ QoL ($P<0.05$) but no difference between groups (NS) No differences in tumor response or overall survival rate
Pituskin and colleagues, 2010[85] Prospective cohort study Canada Neutral	N=82 Final N=23 Prostate, breast, non−small cell lung cancer malignancies with painful bone metastases; attending Rapid Access Palliative RT program	**MNT:** Recommendations were made by multidisciplinary team including pharmacy, occupational therapist, FNP, and social worker for relief of symptoms*[c] **Duration:** 4 wk	FNP recommendations (n=24) included weight loss or gain counseling (n=21); nutritional education (n=21); tips on symptom management (n=16); and information related to physical problems limiting intake (n=9). ESAS[v] for all available patients at 4 wk showed improvements in pain relief ($P=0.001$), tiredness ($P=0.001$), depression ($P=0.013$), anxiety ($P=0.000$), drowsiness ($P=0.022$), and overall well-being ($P=0.035$) No difference from baseline in shortness of breath, ($P=0.383$), nausea ($P=0.196$), or appetite ($P=0.062$)
Ravasco and colleagues, 2003[64] Before−after study Portugal Positive	N=125 Final N=125 H&N, gastric, esophageal, CRC, prostate, breast, lung, brain, gallbladder, uterus; undergoing RT	**MNT:** Assessment, dietary intake, dietary counseling by FNP **HR:** High-risk group: H&N, gastric, esophageal, CRC **LR:** Low-risk group: prostate, breast, lung, brain, gallbladder, uterus **Duration:** End of RT, not otherwise specified	High-risk group ↑ in severity of symptoms during therapy ($P<0.0001$) High-risk group ↑ energy intake during therapy ($P<0.03$) High-risk group ↑ PRO intake during therapy, although remained substandard throughout the study High-risk group attributed ↑ in energy and PRO intake to individualized nutrition counseling provided by FNP QoL ↑ for high-risk group; improvement was correlated with ↑ nutritional intake ($P<0.001$) Baseline energy and PRO intake were ↑ in the low-risk group ($P<0.002$ and $P<0.003$, respectively) Baseline nutritional status was associated with nutritional intake ($P<0.007$; Kruskal-Wallis analysis adjusted by tumor staging)

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Table 3. Summary of studies included in the Academy of Nutrition and Dietetics Evidence Analysis Library systematic review of the effectiveness of medical nutrition therapy (MNT) in adult oncology patients undergoing chemotherapy and radiation treatment (*continued*)

Author(s), year Study design Location Quality rating	Population Cancer site	MNT Intervention by an RDN/FNP[a] Duration	Results
Ravasco and colleagues, 2005[65] RCT Portugal Positive	N=75 (MNT: n=25; MFS: n=25; Control: n=25) Final N=NR H&N cancer; undergoing RT	**MNT:** Counseling by FNP+regular diet **MFS:** Usual diet+MFS **Control:** Usual diet **Duration:** 3 mo	MNT and MFS: At end of RT energy ↑ ($P<0.002$ and $P<0.05$, respectively) with MNT intake greater than MFS group ($P=0.005$). PRO intake ↑ (26 g/d, $P<0.006$; and 35 g/d, $P<0.001$, respectively) and ↓ in control group (15 g/d, $P<0.01$) At 3-mo follow-up, MNT maintained energy intake; MFS and control groups returned to or below baseline levels ($P=0.005$); PRO intake patterns were similar Most patients experienced RT-induced toxicities (90%); however, there was a trend for ↓ toxicity in MNT group ($P<0.07$) At 3 mo, nausea, vomiting, xerostomia, and dysgeusia were improved in 90% of MNT group, 67% of MFS, and 51% of control group ($P<0.001$), despite controlling for adequate and appropriate medications to improve symptoms After RT, MNT group showed ↑ QoL function scores ($P<0.003$), which were proportional with improved nutrition status and intake. MFS group showed similar results ($P<0.009$) although proportional only to PRO intake. At 3 mo, MNT group showed ↑ global QoL. QoL 6 of 6 function scales, 3 of 3 symptom scales, with mixed results for single item symptoms. At 3 mo, MFS group showed ↑ global QoL, 6 of 6 function scales but ↓ symptom scales and single item symptoms. At end of RT and 3 mo, the control group showed ↓ global QoL scores in all areas.

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Table 3. Summary of studies included in the Academy of Nutrition and Dietetics Evidence Analysis Library systematic review of the effectiveness of medical nutrition therapy (MNT) in adult oncology patients undergoing chemotherapy and radiation treatment (*continued*)

Author(s), year Study design Location Quality rating	Population Cancer site	MNT Intervention by an RDN/FNP[a] Duration	Results
Ravasco and colleagues, 2005[66] RCT Portugal Positive	N=111 (MNT: n=37; MFS: n=37; Control: n=37) Final N=111 CRC; undergoing RT	**MNT:** Counseling by FNP+regular diet **MFS:** MFS+regular diet **Control:** Ad libitum intake **Duration:** 3 mo	MNT and MFS: At end of RT, energy ↑ ($P<0.002$ and $P<0.04$, respectively); MNT intake greater than MFS group ($P=0.001$). Control group ↓ energy intake ($P<0.01$). At 3-mo follow-up, MNT maintained energy intake; MFS and control groups ↓ energy intake ($P=0.05$). PRO intake ↑ in MNT and MFS groups (27 g/d, $P<0.007$ and 30 g/d, $P<0.001$, respectively). Control group ↓ PRO intake by 10 g/d ($P<0.01$). At 3-mo follow-up MNT maintained PRO intake; MFS and control groups ↓ PRO intake ($P=0.06$). MNT group had less nutrition-related decline based on PG-SGA and BMI at the end of RT and at 3 mo ($P<0.001$) vs MFS or control MNT group experienced ↓ anorexia, nausea, vomiting, diarrhea ($P<0.001$, $P<0.0001$, and $P<0.0001$, respectively) vs MFS or control MNT group significantly ↑ global QoL, 6 of 6 function scales, 3 of 3 symptom scales, and improved or maintained single item symptoms at RT completion, which remained at 3 mo (specific *P* values for each item not shown for brevity)

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Table 3. Summary of studies included in the Academy of Nutrition and Dietetics Evidence Analysis Library systematic review of the effectiveness of medical nutrition therapy (MNT) in adult oncology patients undergoing chemotherapy and radiation treatment (*continued*)

Author(s), year Study design Location Quality rating	Population Cancer site	MNT Intervention by an RDN/FNP[a] Duration	Results
van den Berg and colleagues, 2010[86] Non-RCT The Netherlands Neutral	N=38 (MNT: n=20; Control: n=18) Final N=38 H&N cancer; undergoing RT	**MNT:** Individualized, intensive counseling by FNPs **Control:** FNP visits before initiation of RT; nurse for nutrition care during RT **Duration:** 20 wk	2 wk following treatment: Both groups had 3% unintended weight loss (*P* value NR) and 0 out of 20 in MNT group vs 5 out of 18 in the control were malnourished (defined as unintended weight loss ≥5%) (*P*=0.02) 8 wk following treatment: MNT group had a 1% BW ↑ vs 1.5% BW ↓ for control (*P*=0.03). Total BW loss was 2% for MNT and 4.5% for control; 3 out of 18 controls remained malnourished NS difference in BMI between groups at any time during the study

[a]RDN=registered dietitian nutritionist; FNP=food and nutrition practitioner.
[b]CT=chemotherapy.
[c]Multimodal therapy.
[d]RT=radiation therapy.
[e]QoL=quality of life.
[f]RCT=randomized controlled trial.
[g]NR=not reported.
[h]CRC=colorectal cancer.
[i]MGA=megestrol acetate.
[j]BMI=body mass index.
[k]BW=body weight.
[l]KPS=Karnofsky performance status.
[m]NS=not significant.
[n]GI=gastrointestinal.
[o]MFS=medical food supplement.
[p]PRO=protein.
[q]CIPS=Cancer Inventory of Problem Situations.
[r]H&N=head and neck.
[s]ADA=American Dietetic Association, former name of the Academy of Nutrition and Dietetics.
[t]UC=usual care.
[u]PG-SGA=patient-generated subjective global assessment.
[v]ESAS=Edmonton Symptom Assessment System.

Table 4. Summary of studies included in the Academy of Nutrition and Dietetics Evidence Analysis Library systematic review of effectiveness of medical food supplements and dietary supplements containing fish oil (ie, eicosapentaenoic acid) on body weight and lean body mass in adult oncology patients

Author(s), Year Study design Location Quality rating	Population Cancer site	Intervention Dosage Duration	Actual consumption per day: MFS[a] 8 oz (240 mL) Containers[b] or DS[c] (capsules/liquid) EPA[d] intake	Results
Dietary supplements containing fish oil				
Bonatto and colleagues, 2012[87] RCT[e] Single center, outpatient Brazil Positive	N=38 (EPA-DS: n=19; SOC[f]: n=19) Final N: NR[g] GI[h] (n=28) and other cancers (n=10) undergoing 5 fluorourocil/leucovorin CT[i] 3 times a week	**Experimental:** EPA-DS **Control:** SOC, no DS **Dosage:** 0.3 g EPA/d **Duration:** 8 wk	**DS:** NR **EPA:** NR; 0.3 g EPA planned	**Weight:** EPA-DS group ↑ (mean±SEM[j]) 1.7±0.9 kg; control group ↓ 2.5±0.8 kg; difference between groups ($P<0.002$)
Burns and colleagues, 2004[88] Before-after study Single center, outpatient United States Positive	N=43 Various cancers, including leukemia	**Experimental:** EPA-DS (ethyl ester form of EPA) **Dosage:** First 13 patients consumed 0.3 g/kg/d n-3 from fish oil twice a day for a minimum of 2 mo Dose ↓ for remainder of study to 0.15 g/kg/d (11 capsules for 70-kg patient=4.7 g EPA) due to patients unwilling or unable to take requested dose **Duration:** 1.5 mo	**DS:** NR; 0.3 g/kg planned; dose ↓ to 0.15 g/kg due to side effects. Authors stated "patients typically received assigned dose" **EPA:** Median 4.7 g[k] for a 70-kg patient (range=2.6-5.97 g[k]) **Actual duration:** Median 1.2 mo (range=0.5-3.1 mo)	**Weight:** Weight stabilization was associated with taking EPA-DS; many patients who did not respond were unable to tolerate capsules Median BW[l] ↓ of 0.8 kg or 1.2% (*P* value or range NR); n=12 ↑ BW; n=22 lost BW. (Includes patients who took only a few capsules and patients who had a truncated treatment course) Predicted BW loss would be at least a further 4.6%

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Table 4. Summary of studies included in the Academy of Nutrition and Dietetics Evidence Analysis Library systematic review of effectiveness of medical food supplements and dietary supplements containing fish oil (ie, eicosapentaenoic acid) on body weight and lean body mass in adult oncology patients (*continued*)

Author(s), Year Study design Location Quality rating	Population Cancer site	Intervention Dosage Duration	Actual consumption per day: MFS[a] 8 oz (240 mL) Containers[b] or DS[c] (capsules/ liquid) EPA[d] intake	Results
Fearon and colleagues, 2006[89] RCT Multicenter, outpatient United Kingdom Positive	N=518 Final N=270 Lung; Upper/lower GI; Unclassified GI not undergoing surgery, CT or RT[m] in the past 4 wk	**2 Experimental groups:** EPA-DS2: (2 g EPA diester/d) EPA-DS4: (4 g EPA diester/d) **Control:** Medium-chain triglyceride oil blended with unspecified diester oil capsules Compliance=80% of planned dose **Duration:** 8 wk	**DS: (% of patients taking prescribed capsules)** EPA-DS2: 68% took >80% of prescribed capsules EPA-DS4: 75% Control: 72% **EPA:** EPA-DS2: NR EPA-DS4: NR Control: 0 g	**Weight:** At baseline, mean BW ↓ was 18% Relative to placebo, at 8 wk mean BW↑ was 1.2 kg for the EPA-DS2 group (95% CI 0 to 2.3 kg) and 0.3 kg for the EPA-DS4 group (−0.9 to 1.5 kg); trend in favor of EPA-DS (P=0.066) **LBM**[n]: The EPA-DS2 group ↑ 0.9 kg LBM vs placebo (95% CI −0.3 to 2.0; NS[o]), whereas the EPA-DS4 group ↓ an average of 0.1 kg LBM (1.3-1.1 kg; NS)
Finocchiaro and colleagues, 2012[90] RCT Multicenter, outpatient Italy Positive	N=33 (EPA-DS: n=19; olive oil: n=14) Final N: 27 (EPA-DS: n=13; olive oil: n=14) Lung cancer, undergoing cisplatin/ gemcitabine CT	**Experimental:** EPA-DS (510 mg EPA/capsule) **Control:** 850 mg olive oil capsules **Dosage:** 4 capsules/d (2.04 g EPA) **Duration:** 66 d	**DS:** EPA-DS: NR; authors stated compliance "was good" Control: NR **EPA:** EPA-DS: NR; Based on dosing, estimate 2.04 g[k] EPA planned Control: 0 g	**Weight:** EPA-DS group experienced mean ↑ in BW of 3.4 kg (P<0.05); Control group (NS)
Gogos and colleagues, 1995[91] Non-RCT Single center, Outpatient Greece Negative	N=64 GI, lung, and breast cancer 4 mo from any treatment Final N: (EPA-DS: n=30 sugar tablet: n=30); both groups: well-nourished (n=15); malnourished (n=15); healthy control (n=15)	**Experimental:** EPA-DS (170 mg EPA/capsule) **Control:** Sugar tablets **Dosage:** 18 capsules/d (3.06 g EPA) **Duration:** 40 d	**DS:** NR **EPA:** EPA-DS: NR; Based on dosing, estimate 3.06 g[k] EPA planned Control: 0 g	**Weight:** Weight remained stable

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Table 4. Summary of studies included in the Academy of Nutrition and Dietetics Evidence Analysis Library systematic review of effectiveness of medical food supplements and dietary supplements containing fish oil (ie, eicosapentaenoic acid) on body weight and lean body mass in adult oncology patients (*continued*)

Author(s), Year Study design Location Quality rating	Population Cancer site	Intervention Dosage Duration	Actual consumption per day: MFS[a] 8 oz (240 mL) Containers[b] or DS[c] (capsules/liquid) EPA[d] intake	Results
Murphy and colleagues, 2011[92] Non-RCT Single center, outpatient Canada Positive	N=41 (EPA-DS: n=17; SOC: n=24; Reference group: n=104) Final N: 40 (EPA-DS: n=16; SOC: n=24) NSCLC[p] undergoing first-line platinum-based doublet CT	**Experimental:** EPA-DS (2.2 g EPA/d either as 1-g fish oil capsules or 7.5 mL liquid/d) **Control:** SOC, no DS **Reference group**: Data were aggregated and used as a point of comparison for expected body composition changes during CT; not included in statistical analysis **Dosage:** 4 capsules or 7.5 mL liquid/d (2.2 g EPA) **Duration:** ≥60 d or 2 cycles of CT	**DS:** NR; Patients consuming <80% of planned dose of 2.2 g EPA/d were withdrawn, lowest dose=3.2 capsules or 6 g liquid fish oil **EPA:** Lowest dose 1.76 g[k] (range=1.76-2.2 g[k])	**Weight:** EPA-DS group ↑ 0.5±1.0 kg (mean ±SEM) BW, whereas control group ↓2.3±0.9 kg ($P<0.05$) BW 69% of patients in EPA-DS group vs 29% in control group ↑ or maintained BW (*P* value NR) **LBM:** EPA-DS group maintained muscle mass throughout 10 weeks of CT, despite a mean BW ↓ of 6.3% over 6 mo before study entry Positive linear relation noted between changes in plasma EPA concentration and rate of muscle change from baseline to end of study ($R^2=0.55$; $P=0.01$). Loss of skeletal mass was evident in the control group; some lost up to 5.2 kg muscle from baseline to end of treatment 69% of patients in EPA-DS group vs 29% in control group maintained or ↑ muscle (*P* value NR) Note: % of patients gaining weight and muscle is similar to the BW gain, suggesting that BW gain is muscle Loss of skeletal muscle occurred concurrently with ↑ in muscle fat mass content in the control group

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Table 4. Summary of studies included in the Academy of Nutrition and Dietetics Evidence Analysis Library systematic review of effectiveness of medical food supplements and dietary supplements containing fish oil (ie, eicosapentaenoic acid) on body weight and lean body mass in adult oncology patients (*continued*)

Author(s), Year Study design Location Quality rating	Population Cancer site	Intervention Dosage Duration	Actual consumption per day: MFS[a] 8 oz (240 mL) Containers[b] or DS[c] (capsules/ liquid) EPA[d] intake	Results
Persson and colleagues, 2005[93] Randomized, nonplacebo controlled trial (pilot study) Single center, outpatient Sweden Positive	N=24 (EPA-DS: n=13 Melatonin: n=11) Final N: 16 (EPA-DS: n=10; Melatonin: n=6) Weight-losing advanced GI cancer; CT was allowed	**2 Experimental groups:** First intervention period: EPS-DS (30 mL fish oil providing 4.9 g EPA) Melatonin: 18 mg/d melatonin **Dose:** 4.9 g EPA/d **Duration:** 4 wk Second intervention period: All patients took combined interventions above **Duration**: 4 wk	**DS:** 62% compliance for both intervention periods (compliance represents number of patients, not amount of fish oil) **EPA:** NR	**Weight:** In the first intervention period, BW loss was attenuated, with both groups experiencing median weight ↓ (0.6 kg in EPA-DS group and 1.8 kg in melatonin group; both NS) In the second intervention period, a small median BW ↑ was observed in both groups (0.2 kg in EPA-DS group and 0.8 kg in melatonin group; both NS)
Pratt and colleagues, 2002[94] RCT Single center, outpatient Canada Negative	N=29 (EPA-DS: n=13; Olive oil: n=10; Healthy: n=6, details NR) Final N: 19 (EPA-DS: n=9; olive oil: n=10) Various advanced cancers; high-dose CT	**Experimental:** EPA-DS (180 mg EPA/1g capsule) **Control:** 1 g olive oil in capsules **Duration:** 14 d **Dosage:** 18 capsules/d (3.24 g EPA)	**DS:** (mean±SEM) 12±1.0 capsules **Control:** 10±1.0 **EPA:** EPA-DS: 2.16±0.18 g[k] Control: 0 g	**Weight:** After 2 wk EPA-DS, the change in BW was directly related to the ↑ in plasma phospholipid EPA levels (r=0.86; P=0.006)
Silva and colleagues, 2012[95] RCT Single center, outpatient Brazil Positive	N=23 (EPA-DS: n=11; SOC: n=12) Final N: 18 (EPA-DS: n=10; SOC: n=8) Colorectal cancer eligible for CT	**Experimental:** EPA-DS (150 mg EPA + DHA[q]/ capsule); EPA NR separately **Control:** SOC, no DS **Dosage:** 4 capsules/d; 2 g fish oil (600 mg EPA + DHA) **Duration:** 9 wk	**DS:** 4 capsules **EPA:** NR 600 mg EPA+DHA (definition of non-compliance <80% of EPA-DS)	**Weight:** Before study entry, all patients were weight-losing Median BW ↑ of 0.5 kg (range=−0.6 to 1.0 kg) over 9 wk in EPA-DS group; control group had a median BW ↓ of 1.6 kg [range=−2.4 to 0.3 kg (P=0.01)]

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Table 4. Summary of studies included in the Academy of Nutrition and Dietetics Evidence Analysis Library systematic review of effectiveness of medical food supplements and dietary supplements containing fish oil (ie, eicosapentaenoic acid) on body weight and lean body mass in adult oncology patients (*continued*)

Author(s), Year Study design Location Quality rating	Population Cancer site	Intervention Dosage Duration	Actual consumption per day: MFS[a] 8 oz (240 mL) Containers[b] or DS[c] (capsules/liquid) EPA[d] intake	Results
Taylor and colleagues, 2010[96] Time study Single center, outpatient Germany Neutral	N=31 Solid tumors Kidney, ureter prostate (n=3); Breast, ovary, cervix (n=8); GI (n=12); Other (N=8) Final N=17	**Experimental:** EPA-DS (0.278 g EPA/capsule) **Dosage:** 3 (500 mg) soft gel capsules/d: 1.5 g fish oil (0.834 g EPA[k]) **Duration:** 6 wk	**DS:** 94% (±2%) of capsules were consumed **EPA:** Range ~0.77-0.80 g[k]	**Weight:** 10 of 17 gained BW during EPA-DS intervention [median BW ↑ of 0.6% ($P<0.37$; NS)] Plasma phospholipid EPA correlated positively with BW change ($r=0.64$; $P=0.006$) **LBM:** LBM[b] remained stable during EPA-DS intervention (mean±SD[r] 39±8.6 kg baseline vs 39.6±7.7 kg Week 6)
Wigmore and colleagues, 2000[97] Time study Single center, outpatient Scotland Neutral	N=26 (Stage II: N=5; Stage III: N=8; Stage IV: N=13) Final N=14 Unresectable pancreatic cancer 4 weeks from any treatment	**Experimental:** EPA-DS (500 mg EPA/capsule; 95% pure EPA) - Week 1: 1 g/d - Week 2: 2 g/d - Week 3: 4 g/d - Week 4-12: 6 g/d **Dosage:** Escalating dose to 12 capsules/d **Duration:** 12 wk	**DS:** NR **EPA:** 6 g	**Weight:** EPA-DS group experienced a significant ↓ in the rate of BW loss, resulting in BW stabilization from baseline to 3 mo. At 1 mo intervals expressed as median and IQR[s]: Baseline= −2.0 kg (1.4-2.8 kg) vs 1 mo=0.5 kg (1.5-2.0 kg) 2 mo=0.2 kg (1.4-0.9 kg) 3 mo=0.3 kg (0.2-0.8 kg); $P<0.005$ for all **LBM:** Change in LBM[b] in EPA-DS group surviving at 4, 8, and 12 wk (NS)

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Table 4. Summary of studies included in the Academy of Nutrition and Dietetics Evidence Analysis Library systematic review of effectiveness of medical food supplements and dietary supplements containing fish oil (ie, eicosapentaenoic acid) on body weight and lean body mass in adult oncology patients (*continued*)

Author(s), Year Study design Location Quality rating	Population Cancer site	Intervention Dosage Duration	Actual consumption per day: MFS[a] 8 oz (240 mL) Containers[b] or DS[c] (capsules/liquid) EPA[d] intake	Results
Wigmore and colleagues, 1996[98] Time study Single center, outpatient Scotland Negative	N=18 (Stage II: n=2; Stage III: n=7; Stage IV: n=9) Final N: NR Unresectable pancreatic cancer 4 wk from any treatment	**Experimental:** EPA-DS (180 mg EPA/capsule as 1-g fish oil capsule) - Week 1: 2 g/d - Week 2: 4 g/d - Week 3: 6 g/d - Week 4: 8 g/d - Week 5: 10 g/d - Week 6: 12 g/d - Week 7: 14 g/d - Weeks 8-12: 16 g/d **Dosage:** 12 capsules/d (2 g EPA) **Duration:** 12 w k	**DS:** Median tolerated dose=12 capsules **EPA:** 2.16 g[k]	**Weight:** Prior to study entry, median BW ↓ was 2.9 kg/mo. EPA-DS group demonstrated BW stabilization with ↑ of 0.3 kg/mo (IQR=0-0.5; $P<0.002$) **LBM:** EPA-DS group demonstrated LBM[b] stabilization or anthropometric body composition measures (*P* value=NS)
MFS containing fish oil				
Barber and colleagues, 2000[99] Non-RCT Single center, outpatient; Scotland Neutral	N=22 (EPA-MFS: n=16; Controls: n=6) Final N=NR Pancreatic cancer 4 wk from any treatment vs healthy, weight stable	**Experimental:** EPA-MFS (Per Container: 1.1 g EPA, 305 kcal, 16.1 g pro[t]) **Dosage:** 2 containers/d **Duration:** 3 wk	**MFS:** Median 1.9 (range=1.25 -2.0g) containers **EPA:** Median 2.07 g[k] (range=1.36-2.18 g[k])	**Weight:** Median BW ↑ of 1.0 kg (−0.25 to 1.75; $P<0.05$) **LBM:** Median LBM ↑ of 0.75 kg (0.1-1.6; $P<0.05$), whereas fat mass remained unchanged (NS)
Barber and colleagues, 1999[100] Time study Single center, outpatient Scotland Neutral	N=20 At 3 wk n=18 Final N=13 Pancreatic cancer 4 wk from any treatment	**Experimental:** EPA-MFS (per container: 1.1 g EPA, 305 kcal, 16.1 g pro) **Dosage:** 2 containers/d **Duration:** 7 wk	**MFS:** Median 1.9 (range=1.2-2.0g) containers **EPA:** Median 2.07 g[k] (range=1.36-2.18 g[k])	**Weight:** Before study entry, median BW↓ of 2.9 kg/mo (−4.4 to 2.2). At 3 wk, median ↑of 1.0 kg BW (−0.1 to 2.0; $P=0.024$). At 7 wk median BW ↑of 2.0 kg BW (−0.4 to 4.6; $P=0.033$) **LBM:** At 3 wk, median LBM ↑ of 1.0 kg (0.6-1.4; $P=0.0064$). At 7 wk, median LBM ↑ of 1.9 kg (1.0- 3.0; $P=0.0047$)

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Table 4. Summary of studies included in the Academy of Nutrition and Dietetics Evidence Analysis Library systematic review of effectiveness of medical food supplements and dietary supplements containing fish oil (ie, eicosapentaenoic acid) on body weight and lean body mass in adult oncology patients (*continued*)

Author(s), Year Study design Location Quality rating	Population Cancer site	Intervention Dosage Duration	Actual consumption per day: MFS[a] 8 oz (240 mL) Containers[b] or DS[c] (capsules/liquid) EPA[d] intake	Results
Bauer and colleagues, 2005[101] RCT Outpatient 12 international sites Positive	N=200 Final N=110 Weight-losing, unresectable pancreatic cancer 4 wk from any treatment divided into Compliant (C: n=87) or Noncompliant (NC: n=98) Compliant=average consumption over 4 wk of >1.5 containers MFS/d	**Experimental:** EPA-MFS (per container: 1.1 g EPA, 305 kcal, 16.1 g pro) **Control:** Identical MFS without EPA (N=105) **Dosage:** 2 containers/d **Duration:** 8 wk	**MFS:** **4 wk** C=51.4% reached recommended dose of ≥1.5 containers NC=48.6% did not reach recommended dose **8 wk** C=53.6% reached recommended dose of ≥1.5 containers NC=46.4% did not reach recommended dose **EPA:** NR	**Weight:** Significant BW ↑ of 1.7 kg (SEM±0.4; $P<0.001$) in C group **LBM:** Change in LBM (NS)
de Luis and colleagues, 2008[102] RCT Single center, outpatient Spain Positive	N=65 (EPA-MFS high n-3:n-6: n=31; EPA-MFS low n-3:n-6: n=34) Final N=65 Postsurgical oral and laryngeal cancer	**2 Experimental groups:** EPA-MFS/H (per container: 1.01 g EPA, 295 kcal, 16 g pro [high n-3:n-6]) EPA-MFS/L (per container: 0.92g EPA, 310 kcal, 18 g pro) (low n-3:n-6) **Dosage:** 2 containers/d **Duration:** 12 wk	**MFS:** (mean±SD) 1.6±0.62 containers in both groups **EPA:** (mean±SD) EPA-MFS/H: 1.61±0.63[k] EPA-MFS/L: 1.47±0.57g[k]	**Weight:** BW maintained; between or within-group change (NS) **LBM:** LBM maintained; between or within-group change in LBM, triceps skinfold, or arm circumference (NS)

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Table 4. Summary of studies included in the Academy of Nutrition and Dietetics Evidence Analysis Library systematic review of effectiveness of medical food supplements and dietary supplements containing fish oil (ie, eicosapentaenoic acid) on body weight and lean body mass in adult oncology patients (*continued*)

Author(s), Year Study design Location Quality rating	Population Cancer site	Intervention Dosage Duration	Actual consumption per day: MFS[a] 8 oz (240 mL) Containers[b] or DS[c] (capsules/liquid) EPA[d] intake	Results
de Luis and colleagues, 2005[103] RCT Single site Spain Positive	N=73 (EPA-MFS: n=38; ARG-MFS: n=35) Final N=NR Postsurgical oral and laryngeal cancer	**Experimental:** EPA-MFS (per container: 1.01 g EPA, 295 kcal, 16 g pro [high n-3:n-6]) **Control:** ARG-MFS (per container: 7.4 g free arginine, 0.598 g EPA, 303 kcal, 16.7 g pro) **Dosage:** 2 containers/d **Duration:** 12 wk	**MFS:** (mean±SD) 1.5±0.52 containers in both groups **EPA:** EPA-MFS: 1.52±0.52 g[k] Control: 0.89±0.31 g[k]	**Weight:** EPA-MFS group showed significant improvement from baseline in BW, fat mass, and triceps skinfold ($P<0.05$); control group (NS)
Fearon and colleagues, 2003[104] RCT Outpatient 12 international sites Positive	N=200 (EPA-MFS: n=95; MFS: n=105) Final N: 110 (EPA-MFS: n=50; MFS: n=60) Pancreatic cancer (no cancer treatment in 4 wk; no plans to undergo treatment) Trend toward more Stage IV patients in the EPA-MFS group over control (52% vs 41%)	**Experimental:** EPA-MFS (per container: 1.1 g EPA, 310 kcal, 16 g pro) **Control:** Identical MFS without EPA **Dosage:** 2 containers/d **Duration:** 12 wk	**MFS:** Mean 1.4 (SD NR) containers for both groups **EPA:** EPA-MFS: 1.54 g[k] Control: 0 g	**Weight:** At 8 wk, both MFS were equally effective in halting BW loss (EPA-MFS group: −0.25kg/ mo; control group: −0.37 kg/mo; $P=0.74$). **LBM:** In the EPA-MFS group, significant associations were found between plasma EPA and LBM ↑ ($P=0.043$) and between 8-wk plasma EPA and ↑ in BW ($P<0.001$) and LBM ($P=0.001$). No such associations were seen in the control group
Guarcello and colleagues, 2007[105] RCT Single site, outpatient Italy Neutral	N=46 (EPA-MFS: n=26; MFS: n=20) Lung cancer eligible for CT (SCLC[u] [n=5]; NSCLC [n=41]) Final N: 25 (EPA-MFS: n=14; MFS: n=11)	**Experimental**: EPA-MFS (per container: 1.1 g EPA, 310 kcal, 16 g pro) **Control:** MFS without EPA (per container: 275 kcal, 15 g pro) **Dosage:** 2 containers/d **Duration:** 60 d	**MFS:** Median 2 (range=1.5-2) containers for both groups **EPA:** EPA-MFS: Median 2.2[k] (range=1.65-2.2g)[k] Control: 0 g	**Weight:** EPA-MFS group showed significant ↑in BW (57.7 kg at T0 vs 58.6 kg at Day 30 and Day 60; $P<0.05$) whereas MFS group showed no ↑ in BW (59.1 kg at T0 vs 57.0 at Day 30 and 59.1 at Day 60; NS)

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Table 4. Summary of studies included in the Academy of Nutrition and Dietetics Evidence Analysis Library systematic review of effectiveness of medical food supplements and dietary supplements containing fish oil (ie, eicosapentaenoic acid) on body weight and lean body mass in adult oncology patients (*continued*)

Author(s), Year Study design Location Quality rating	Population Cancer site	Intervention Dosage Duration	Actual consumption per day: MFS[a] 8 oz (240 mL) Containers[b] or DS[c] (capsules/liquid) EPA[d] intake	Results
Jatoi and colleagues, 2004[106] RCT Multicenter outpatient United States, Canada Positive	N=421 (EPA-MFS/placebo MA[v]: n=141; EPA-MFS/MA: n=140; MFS/MA: n=140) Final N: NR Variety of cancers (lung, GI, and others excluding hormone-sensitive tumors) vs MA alone	**3 Experimental groups:** EPA-MFS/placebo MA: EPA-MFS (per container: 1.1 g EPA, 300 kcal, 16 g pro) + placebo liquid suspension appearing identical to MA EPA-MFS/MA: EPA-MFS (per container: 1.1 g EPA, 300 kcal, 16 g pro) with MA liquid suspension 600 mg/d MFS/MA: 300 kcal, 16 g pro MFS without EPA + MA liquid suspension 600 mg/d **Duration:** ≥3 months	**MFS:** NR **EPA:** NR	**Weight:** 18% of patients in MFS/MA group showed a 10% ↑ in BW (P=0.01), whereas in the EPA-MFS group, 22% of patients showed a 1%-4% ↑ in BW and 9% of patients showed a 5%-9% ↑ in BW; no difference among the 3 treatment arms (P=0.24 and P=0.69, respectively) The area under the curve for absolute weight ↑ at 1 mo was also similar among the treatment arms
Read and colleagues, 2007[107] Prospective cohort Single center, outpatient Australia Positive	N=23 At 3 wk N=20 Final N=15 Advanced colorectal cancer treated with FOLFIRI[w]; life-threatening toxicities	**Experimental:** EPA-MFS (per container: 1.1 g EPA, 310 kcal, 16 g pro) **Control:** MFS without EPA (per container: 275 kcal, 15 g pro) **Dosage:** 2 containers/d **Duration:** 9 wk	**MFS:** Mean 1.7 (SD NR) containers in both groups **EPA:** EPA-MFS: 1.9 g[k] Control: 0 g	**Weight:** Mean BW ↑ of 2.5 kg from baseline to end of Week 3 (P=0.03) in EPA-MFS group; BW remained stable during 3 cycles CT or 9 wk **LBM:** EPA-MFS group maintained LBM; change from baseline (NS)

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Table 4. Summary of studies included in the Academy of Nutrition and Dietetics Evidence Analysis Library systematic review of effectiveness of medical food supplements and dietary supplements containing fish oil (ie, eicosapentaenoic acid) on body weight and lean body mass in adult oncology patients (*continued*)

Author(s), Year Study design Location Quality rating	Population Cancer site	Intervention Dosage Duration	Actual consumption per day: MFS[a] 8 oz (240 mL) Containers[b] or DS[c] (capsules/liquid) EPA[d] intake	Results
Ryan and colleagues, 2009[108] RCT Outpatient followed by inpatient surgery, single center Ireland Neutral	N=53 (EPA-MFS: n=28; MFS: n=25) Final N=53 Resectable esophageal cancer Patients unable to go on to surgery were discontinued	**Experimental:** EPA-MFS (per container: 1.1 g EPA, 310 kcal, 16 g pro) orally for 5 d before surgery and identical enteral nutrition via jejunostomy postoperatively **Control:** MFS without EPA (1.5 kcal/mL, 0.054 g pro/mL) **Dosage:** 2 containers/d + standard enteral nutrition **Duration:** 21 d	**MFS:** Mean 2.0 (SD=NR) containers in both groups **EPA:** EPA-MFS: 2.2 g[k] Control: 0 g	**Weight:** 8% of EPA-MFS group vs 39% of control group lost ≥5% of BW during study period (P=0.03) **LBM:** EPA-MFS group demonstrated NS differences from baseline to 21 d postoperatively for any body composition measurement EPA-MFS group maintained fat-free mass (55 kg preoperatively vs 55.3 kg postoperatively; P=0.9), whereas there was a significant ↓ of fat-free mass in the control group (−1.9 kg±3.7 kg; P<0.03; 95% CI 0.17-3.6)
van der Meij and colleagues, 2010[109] RCT Single center The Netherlands Positive	N=40 (EPA-MFS: n=20; MFS: n=205) Final N: 33 (EPA-MFS: n=14; MFS: n=19) Stage III NSCLC undergoing concurrent chemoradiation treatment	**Experimental**: EPA-MFS (per container: 1.1 g EPA, 300 kcal, 16 g pro) **Control:** Isocaloric MFS without EPA and DHA **Dosage:** 2 containers/d **Duration:** 5 wk	**MFS:** (mean±SD) EPA-MFS: 1.1±1.0 containers Control: 1.0±0.9 containers **EPA:** EPA-MFS: 1.2±1.1 g[k] Control: 0 g	**Weight:**, The EPA-MFS group had better BW maintenance than control group; difference of 1.1 kg (P=0.07) at 1 wk, 1.3 kg (P=0.02) at 2 wk, and 1.7 kg (P=0.04) at 4 wk Mean BW ↑was 0.71 kg (n=27; P=0.245) from baseline to admission; ↑ 0.66 kg (n=30; P=0.519) from baseline to discharge **LBM:** Over time, fat-free mass ↓ in both groups, but less in EPA-MFS group vs control. Between-group differences after Weeks 3 and 5 (1.5 kg; P=0.05 and 1.9 kg; P=0.02, respectively)

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Author(s), Year Study design Location Quality rating	Population Cancer site	Intervention Dosage Duration	Actual consumption per day: MFS[a] 8 oz (240 mL) Containers[b] or DS[c] (capsules/liquid) EPA[d] intake	Results
Weed and colleagues, 2011[110] Prospective cohort Outpatient followed by inpatient surgery, single center United States Neutral	N=38 Final N=31 Head and neck (stage II, III, IV) scheduled for surgical resection with curative intent	**Experimental:** EPA-MFS (per container: 1.1 g EPA, 300 kcal, 16 g pro) starting 2 wk before surgery until discharge N=24 of 31 consumed the EPA-MFS before admission **Dosage:** 2 containers/d **Duration:** Trial entry to hospitalization: (mean ±SEM) 23±2.5 d **Hospitalization:** Median 10 d (range=4-19 d)	**MFS:** Mean 1.8 containers presurgery; 1.5 containers postsurgical hospitalization **EPA:** Mean 1.98 g[k] presurgery; 1.65 g[k] postsurgical hospitalization	**Weight:** 70% of 27 patients ↑ or maintained BW (0.71 kg; P=0.245) for the 2 wk before surgery; 57% of 30 ↑ or maintained BW (0.66 kg; P=0.519) from baseline to discharge (~11 d postsurgery). **LBM:** Significant ↑ in LBM (3.20 kg or ±7%) from baseline to discharge (N=23; P<0.001); significant ↓ in fat mass (3.19 kg; P<0.001)

[a]MFS=medical food supplement.
[b]As measured by multiple-frequency bioelectric impedance analysis.
[c]DS=dietary supplement.
[d]EPA=eicosapentaenoic acid.
[e]RCT=randomized controlled trial.
[f]SOC=standard of care.
[g]NR=not reported.
[h]GI=gastrointestinal.
[i]CT=chemotherapy.
[j]SEM=standard error of the mean.
[k]Calculated.
[l]BW=body weight.
[m]RT=radiation therapy.
[n]LBM=lean body mass.
[o]NS=not significant.
[p]NSCLC=non–small cell lung cancer.
[q]DHA=docosahexaenoic acid.
[r]SD=standard deviation.
[s]IQR=interquartile range.
[t]Pro=protein.
[u]SCLC=small cell lung cancer.
[v]MA=megestrol acetate.
[w]FOLFIRI=irinotecan with fluorouracil and folinic acid.

Obesity and tumor growth: inflammation, immunity, and the role of a ketogenic diet

Christopher Wright[a] *and Nicole L. Simone*[b]

Purpose of review
This article reviews the impact the obese state has on malignancy through inflammation and immune dysregulation using recent excerpts from the medical literature.

Recent findings
The obese state creates a proinflammatory endocrinologic milieu altering cellular signaling between adipocytes, immunologic cells, and epithelial cells, leading to the over-activation of adipose tissue macrophages and the upregulation of compounds associated with carcinogenesis. Obesity correlates with a deficiency in numerous immunologic cells, including dendritic cells, natural killer cells, and T cells. In part, this can be attributed to a recent finding of leptin receptor expression on these immune cells and the upregulation of leptin signaling in the obese state. A number of clinical trials have demonstrated the feasibility of a high-fat, low-carbohydrate diet as an adjuvant treatment for cancer, and current trials are investigating the impact of this intervention on disease outcomes. In preclinical trials, a ketogenic diet has been shown to impede tumor growth in a variety of cancers through anti-angiogenic, anti-inflammatory, and proapoptotic mechanisms.

Summary
Obesity is becoming more prevalent and its link to cancer is clearly established providing a rationale for the implementation of dietary interventions as an adjuvant therapeutic strategy for malignancy.

Keywords
immune dysregulation, inflammation, ketogenic diet, obesity

INTRODUCTION

Cancer cells modify their metabolism in an effort to achieve unregulated cellular proliferation and to build new biomass, but this transformation renders tumor cells vulnerable because of their reliance on a constant supply of nutrients and energy. Although cancer's dependence on an anaerobic energy source was first observed more than 90 years ago by the 1931 Nobel laureate Otto Warburg [1], the field of cancer metabolism has become a topic of renewed interest in the 21st century. A reason for this renewed interest is the ever-increasing obesity pandemic here in America. The Centers for Disease Control and Prevention currently estimates that over 69% of the American adult population is overweight or obese (BMI $\geq$ 25) [2]. Epidemiologic studies have demonstrated a link between obesity and metabolic syndromes to elevated cancer incidence and worse disease outcomes, leading to the estimation that 14 and 20% of all cancer deaths, respectively, are a result of being overweight and obese [3]. Extensive population-based studies, including a recent UK population-based prospective cohort of 5.2 million patients with 166 966 incident cancers, have demonstrated convincingly reliable increased cancer incidences per 5 kg/m^2 increases in BMI [4▪].

We maintain the position, along with the American Society of Clinical Oncology, that obesity-related cancers will be amongst the most urgent issues that the oncologic field faces over the next decade [5▪]. Despite this significant impact, the molecular underpinnings of this relationship remain poorly understood. Here, we attempt to

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KEY POINTS

- Obesity-related cancers will be amongst the most urgent issues that the oncologic field faces over the next decade.
- Obesity creates a state characterized by chronic systemic inflammation and immune dysregulation.
- Leptin is a key adipokine upregulated in the obese state and is intricately involved in the cellular signaling between adipocytes and immunologic cells.
- Dietary intervention has been demonstrated as a feasible adjuvant therapy for a variety of cancers and its impacts on disease progression are a current topic of study.
- Ketogenic diets impede tumor growth through anti-angiogenic, anti-inflammatory, and proapoptotic mechanisms.

explore the impact obesity has on tumorigenesis via chronic systemic inflammation and immune dysregulation, recently completed and ongoing clinical trials investigating this relationship, and the role of the ketogenic diet as an alternative therapeutic option (Table 1).

OBESITY AND TUMORIGENESIS: INFLAMMATION

The proliferation inducing proinflammatory environment of the obese state alters cellular signaling between adipocytes, immune cells, and epithelial cells through various hormones and cytokines. Visceral adipocytes secrete a variety of endocrinologically active molecules, including the adipokines leptin and adiponectin, the cytokines tumor necrosis factor (TNF)α, and interleukin-6 (IL-6), augmenting insulin resistance and stimulating immunologic cells [6▪].

The resulting endocrine milieu leads to an over-activation of monocytes and macrophages contributing to the localized and systemic inflammatory state that has been implicated in cancer [7]. Subsequently, adipose tissue macrophages produce additional inflammatory mediators, including TNFα, IL-6, and IL-1b, in addition to the chemoattractant monocyte chemoattractant protein 1 (MCP-1) and macrophage migration inhibitory factor (MIF) [8▪]. The IL-6 family of cytokines is highly upregulated in many cancers and is considered to be one of the most important cytokines involved in tumorigenesis and metastasis [9]. Proangiogenic factor MCP-1 has been demonstrated to be upregulated in preclinical mouse models consuming a high-fat diet leading to a higher tumor-associated microvascular density [10]. Additionally, paracrine interactions between adipocytes and macrophages also play a pivotal role in carcinogenesis, as prostaglandin E2 (PGE2) produced under the influence of elevated cyclooxygenase-2 (COX-2) activity stimulates growth of estrogen receptor-positive breast epithelial cells. The list of adipokines that potentially contribute to malignancy continues to grow as other less well studied peptides that signal the functional status of adipose tissue, including chemerin, lipocalin 2, and fibroblast growth factor 21, are linked to inflammation, angiogenesis, and other tumorigenic pathways [11▪].

Table 1. Hazard ratios for cancer incidence per 5 kg/m^2 increase in BMI

Cancer type (incident cases)	HR	CI (99%)	*P*-value
Uterine (2758)	1.62	1.56–1.69	<0.0001
Gallbladder (303)	1.31	1.12–1.52	<0.0001
Renal (1906)	1.25	1.17–1.33	<0.0001
Liver (1859)	1.19	1.12–1.27	<0.0001
Colon (13 465)	1.10	1.07–1.13	<0.0001
Cervical (1389)	1.10	1.03–1.17	0.00035
Ovarian (3684)	1.09	1.04–1.14	<0.0001
Thyroid (941)	1.09	1.00–1.19	0.088
Leukemia (5833)	1.09	1.05–1.13	<0.0001
Pancreas (3851)	1.05	1.00–1.10	0.012
Breast-postmenopausal (28 409)	1.05	1.03–1.07	<0.0001
Rectum (6123)	1.04	1.00–1.08	0.017
Primary brain and CNS (2974)	1.04	0.99–1.06	0.053
Esophageal (5213)	1.03	0.99–1.08	0.056
Stomach (3337)	1.03	0.98–1.09	0.16

CNS, central nervous system; HR, hazard ratio [4▪].

OBESITY AND TUMORIGENESIS: IMMUNE DYSREGULATION

Tumor microenvironment has been established as an important determinant of progression and outcome of cancer. Utilizing histopathologic assessment of the tumor inflammatory cell infiltrate as a prognostic aid has shown superior predictability compared with traditional histopathological staging methods, highlighting the importance of immunosurveillance in tumorigenesis [12▪]. Murine models consuming a high-fat diet display a characteristic immunologic makeup consisting of a reduction in dendritic cells, natural killer (NK) cells, cytotoxic CD8+ T cells, and gamma delta (γδ) T cells, leading to an impairment of immune responses to infection and immunization [13]. γδ T cells represent a subset of T cells that possess a distinct T cell receptor on

their surface. They play a key role in tumor immunosurveillance by producing interferon gamma (IFN-γ) and lysing abnormal cells. γδ T cell quantities in peripheral blood are inversely correlated with BMI, with obese donors having four-times fewer circulating γδ T cells than nonobese donors [14▪]. The remaining γδ T cells demonstrate a phenotype resembling aged populations with a blunted IFN-γ response and reduced IL-2 receptor alpha chain expression, suggesting a lack of T cell growth factor signaling.

The aforementioned proinflammatory state may act as a potential driver for immune dysregulation, as elevated amounts of circulatory cytokine can profoundly alter adaptive immunity, although through unclear mechanisms. Increases in leptin signaling via visceral adipocytes may be a substantial contributory factor to immune dysregulation as various immune cells express the leptin-receptor [15]. An activator of both adaptive and innate immunity, leptin promotes inflammatory cytokine production in macrophages and signals granulocyte chemotaxis [16▪▪]. In the presence of elevated levels of leptin, T cells are inhibited from differentiating into regulatory T (Treg) cells, whereas NK cells exhibit reduced cytotoxicity. Additionally, leptin receptor signaling is required for CD4+ T cell differentiation into T helper-17 (Th17) cells [17]. A high number of Th17 cells found in tumor-associated fluids have been significantly correlated with improved overall survival (OS) [18]. NK cells exhibit decreased cytotoxicity in the obese state, and this correlated with prolonged increased leptin signaling [19]. A comparison of NK cells from adult males before and after weight loss indicated that following weight loss NK cells produced more IFN-γ, a critical effector cytokine [20▪]. Further substantiating adipokine signaling involvement in carcinogenesis, patients with premalignant oral lesions were shown to have increased systemic levels of leptin [21].

OBESITY AND TUMORIGENESIS: CLINICAL TRIALS

Observational evidence has established a correlation between obesity and cancer risk. Overweight and obese patients with cancer develop a more aggressive disease with worse outcomes than do patients of normal weight [6▪], yet dietary interventions are not a routine part of therapy. The American Society of Clinical Oncology recently called for the designing and implementation of studies incorporating weight management and physical activity programs into standard oncology practice [22]. Diet and exercise as a therapeutic intervention are feasible, as demonstrated by the Lifestyle Intervention Study in Adjuvant Treatment of Early Breast Cancer (LISA), in which the lifestyle intervention arm had a significantly greater mean weight loss at 6 and 24 months as compared with a control [23]. A subsequent study demonstrated that both in-person and telephone counseling were effective weight loss strategies, with a mean weight loss of 6.4 and 5.4%, respectively, versus the standard of care [24]. Our group is currently recruiting early stage breast cancer patients undergoing breast-conserving therapy for enrollment in the CaReFOR trial combining surgery, radiation therapy, and a 25% reduction in caloric intake as a multifaceted treatment modality [25]. Additional randomized clinical trials are currently exploring whether dietary and exercise interventions can improve cancer survival. The Colon Health and Life-Long Exercise (CHALLENGE) trial is determining how participation in a physical activity program alters disease-free survival in survivors of high-risk stage II or stage III colon cancer [26]. The Lifestyle Intervention for Ovarian Cancer Enhanced Survival (LIvES) study is a randomized phase III trial evaluating the impact of dietary intervention and physical activity on progression-free survival in patients with previously treated stage II, III, or IV ovarian, fallopian tube, or primary peritoneal cancer [27]. A number of groups are exploring the impact of specific dietary alterations on disease progression. The Men's Eating and Living (MEAL) study is a randomized, phase III clinical trial assessing the impact of increased vegetable intake on clinical progression in men with clinically localized prostate cancer on active surveillance [28]. Additionally, the influences of a ketogenic diet on cancer outcomes is currently being studied in pancreatic, lung, breast, head and neck, and primary brain cancers [29–34].

KETOGENIC DIET/CALORIC RESTRICTION

Cancer cells have been shown to have an altered metabolism when compared with normal nonmalignant cells by preferentially metabolizing glucose by glycolysis. Although this 'Warburg effect' was first discovered more than 90 years ago, further exploration has revealed that increased glucose metabolism promotes several hallmarks of cancer, such as excessive proliferation, anti-apoptotic signaling, cell cycle progression, and angiogenesis [35]. A more recent study has shown that a modest reduction (41% from 55% of total calories) in dietary carbohydrates has beneficial effects on body composition, insulin sensitivity, and TNFα expression [36▪]. Given these findings, it is logical to consider diets for patients that restrict carbohydrate consumption in the setting of cancer.

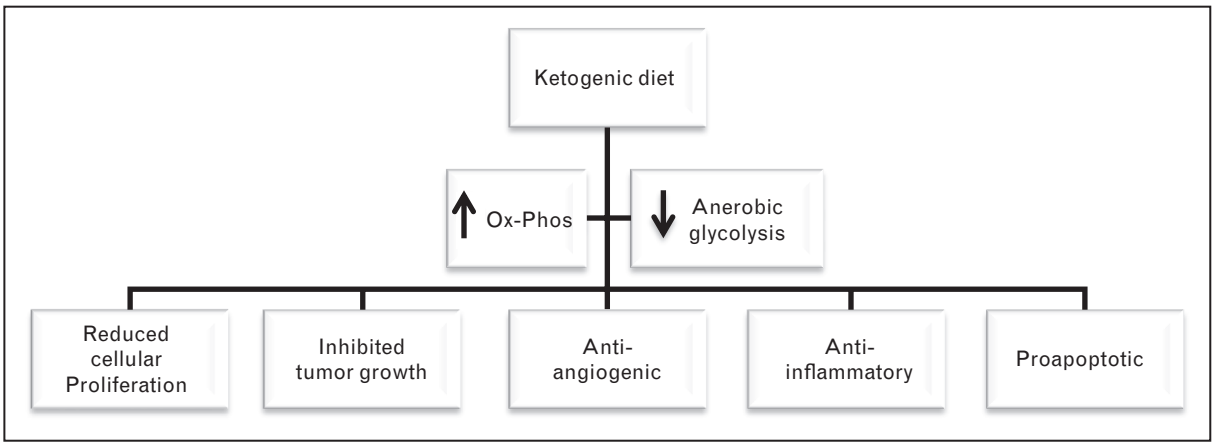

FIGURE 1. Rationale for ketogenic diets as adjuvant cancer therapy. Ketogenic diets consist of high-fat, modest protein, and low-carbohydrate components, classically in a 4 : 1 ratio (fat : carbohydrates + protein). Ketogenic diets in the setting of tumor burden are associated with a reduction in cellular proliferation, impeded tumor growth, a reduction in both inflammation and neovascularization, and an increase in programmed cellular death via apoptosis.

Mimicking the metabolic state of starvation, the high in fat and low in carbohydrate ketogenic diet forces cells to utilize fatty acids as their primary source of energy. Furthermore, tumors are typically deficient in mitochondrial enzymes required to metabolize fatty acids and ketone bodies as a primary energy source. As cancer cells notoriously favor anaerobic glycolysis and down-regulate oxidative phosphorylation enzymes, there is increasing data implicating the ketogenic diet as an effective adjuvant cancer therapy [37]. Preclinical studies have demonstrated that the ketogenic diet-induced metabolic changes improve survival in animal models of malignant gliomas and can potentiate the antitumor effect of chemotherapies and radiation treatment [38▪]. Both a ketogenic diet and calorie restriction significantly reduced tumor growth and prolonged survival in a neuroblastoma murine model [39]. Neuroblastoma growth reduction correlated with decreased blood glucose concentrations and was characterized by a significant decrease in the cellular proliferation marker Ki-67. These findings have been recapitulated in several additional central nervous system (CNS) cancers, including glioblastoma multiforme (GBM) [40], and have been attributed to the ketogenic diet's targeting of the metabolic malady of aerobic fermentation common to all tumor cells. Therapies that increase inflammation and energy metabolites in the GBM microenvironment have been shown to enhance tumor progression, whereas the calorie-restricted ketogenic diet is an anti-angiogenic, anti-inflammatory, and proapoptotic metabolic therapy that also reduces fermentable fuels in the tumor microenvironment [41▪▪] (Fig. 1).

The ketogenic diet alters the transcription of a number of proteins involved in angiogenesis, epithelial invasion, and vascular permeability, including a reduction in expression of hypoxia marker carbonic anhydrase 9, hypoxia inducible factor 1-alpha, and decreased activation of nuclear factor kappa B [42]. Additionally, tumors from animals maintained on a ketogenic diet had reduced tumor microvasculature and decreased expression of vascular endothelial growth factor receptor 2, matrix metalloproteinase-2, and vimentin. Additionally, the application of an unrestricted ketogenic diet delayed tumor growth in non-CNS disease, including cancers of the breast and colon [43,44]. The glucose ketone index, a ratio describing the data published on blood glucose and ketone levels in humans and mice as a single value, demonstrated a clear relationship with the therapeutic efficacy of ketogenic diets for cancers [45▪]. These findings provide a rationale for the utilization of a ketogenic diet as an adjuvant therapeutic strategy to impede tumor growth.

CONCLUSION

Obesity is becoming more prevalent and its link to cancer clearly established rendering obesity-related cancers amongst the most urgent of issues that the oncologic field will face over the next decade. Given that the obese state alters the tumor microenvironment through inflammatory and immunologic mechanisms, targeting the underlying metabolic dysfunction is a logical therapeutic strategy. The feasibility and efficacy of ketogenic diets as an intervention to malignancy have been validated by clinical trials and preclinical studies, respectively.

Current and future clinical trials are needed to explore the safety and the impact of dietary interventions as an adjuvant therapy to conventional radiation and chemotherapies and to further elucidate the mechanisms by which diets may enhance cancer cell therapeutic responses.

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Conflicts of interest

There are no conflicts of interest.

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Papers of particular interest, published within the annual period of review, have been highlighted as:

- ■ of special interest
- ■■ of outstanding interest

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This study pioneered a unique tool, the Glucose Ketone Index Calculator (GKIC) that tracks the ratio of blood glucose to ketones as a single value. A clear relationship between GKIC and the therapeutic efficacy of ketogenic diets was shown in humans and mice with brain tumors, suggesting this simple tool may be utilized to monitor the efficacy of metabolic therapy in preclinical animal models and in clinical trials.

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CANCER (MF LEITZMANN, SECTION EDITOR)

The Impact of Diet on Breast Cancer Outcomes

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Abstract

Purpose of Review Breast cancer is the most common cancer in women, yet conclusive evidence of the effects of dietary modification in breast cancer survivors is lacking. Here, we summarize the literature and highlight important data regarding the association between dietary interventions and breast cancer outcomes.

Recent Findings Long-term follow-up and secondary analysis of the Women's Health Initiative study demonstrated a significant improvement in overall survival for women who were randomized to the low-fat diet pattern compared with those in the usual-diet group. Dietary quality as measured by Healthy Eating Index score was also associated with both a decrease in cancer-specific mortality and overall mortality.

Summary Despite current evidence on the role of diet and nutrition in breast cancer outcomes, conclusive data to translate current findings to clinical practice is lacking and requires multidisciplinary prospective research to advance the field.

Keywords Breast cancer · Breast cancer survivors · Diet intervention · Diet quality · Low-fat diet · High-quality diet · Mortality

Introduction

Breast cancer (BC) is the most common cancer among women worldwide and is a major global health priority. In the USA, an estimated 268,670 new cases (women and men) of BC were diagnosed in 2018 [1]. As of January 1, 2016, more than 3.5 million women with a history of BC were alive in the USA and this number is expected to rise to approximately 4.5 million in 2026, accounting for approximately 45% of all women cancer survivors [2]. There is a significant interest in lifestyle modification, including dietary interventions, weight loss, and physical activity in improving BC outcomes. There is ample data supporting the benefits of physical activity in mitigating and recovering from treatment-related side effects and consistent observational data in its ability to improve cancer outcomes [3]. However, the role of dietary modification in BC outcomes is less clear and requires further study.

Studies of dietary modification in BC have shown conflicting results, with some but not all showing an association between dietary modification and improved prognosis. The prospective, randomized controlled trial is the gold standard study design to provide direct evidence whether an intervention, such as dietary modification, can affect oncologic outcomes. However, this is methodologically difficult for several reasons. First, adopting and adhering to a lifestyle change such as dietary modification is challenging, as is monitoring adherence in the context of a clinical trial. Second, early prognostic biomarkers which might be used as short-term endpoints are lacking, making larger most costly studies with longer follow-up and traditional outcomes such as disease-free survival necessary. Finally, it is difficult to isolate the effect of diet from factors such as weight loss and physical activity, which often accompany dietary modification.

In this review, we will summarize data from observational studies and prospective randomized controlled trials that provide direct and indirect evidence of dietary modification in BC outcomes. We will review the current dietary guidelines for cancer survivors. We will also discuss the ongoing clinical trials and translational research in the field, and highlight potential areas for future research.

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Diet and Breast Cancer Incidence

Descendants of those who migrate from countries with a low incidence of BC to countries with a high incidence will have a higher rate of BC [4,5] emphasizing the impact of environmental factors on BC development. There are significant geographical differences in dietary patterns and components which suggest that diet may be a factor in BC risk and has led to the investigation of dietary interventions and BC incidence and outcomes. The evidence for diet and BC risk is complicated, but it has provided some rationales and foundation to understand and study dietary modification after a diagnosis of BC.

One difference between Western and Eastern diets is the high fat component in the Western diet. Several mechanisms of how a high-fat diet may affect BC incidence have been proposed [6]. High fat intake leads to the accumulation of adipose tissue, where the conversion of androstenedione to estrone occurs, increasing the concentrations of estrogens which subsequently activate BC growth. Polyunsaturated fatty acid (PUFA) is metabolized to arachidonic acid, which activates P450 aromatase, further promoting the conversion of androstenedione to estrone [7]. Furthermore, PUFA can reduce the binding of estrogens to serum binding proteins, increasing the proportion of circulating free estrogens that are biologically more potent [6].

Additionally, more than a decade ago, researchers noted the similarity between the risk factors for cancers with higher incidence in Western countries and risk factors for insulin resistance. This has led to the insulin-cancer hypothesis [8], which has been supported by both animal and human data [9]. Chronic hyperinsulinemia downregulates insulin-like growth factor binding proteins (IGFBP), leading to an increase of free IGF-1. Free IGF-1 is the bioavailable form and may promote mutagenic change in tissues including breast tissue [9]. Insulin can also activate intracellular signaling pathways implicated in BC, including the PI3K/mTOR pathways, promoting cancer cell growth [10].

One meta-regression analysis, including a total of 21 eligible studies, 3609 cases and 7137 controls, demonstrated that high concentrations of IGF-1 and its binding protein, IGFBP-3, is associated with increased risk of BC in premenopausal women [11]. In an analysis of 21,103 women in the Women's Health Initiative (WHI) with 14.7 years of follow-up and 1185 BC cases, [12] fasting baseline insulin level was positively associated with BC risk (multivariable-adjusted HR for highest vs. lowest quartile 1.41, 95% CI 1.16–1.72, $p < 0.0003$) [12]. This study provided support for an association between hyperinsulinemia and BC risk.

In animal models, high-fat diets were shown to increase the occurrence of mammary tumors in rodents [13]. However, individual human case-control and cohort studies have shown mixed results. A meta-analysis of 45 published studies (including 31 case-control and 14 cohort studies with a total of 25,015 cases of BC and over 580,000 control subjects) examined the role of dietary fat in relation to BC risk and found that higher fat intake is associated with an increased risk of BC [14]. Subjects consuming the highest vs. lowest levels of total fat had a 13% increase in BC risk (RR 1.13, 95% CI 1.03–1.25) [15]. This increase in BC risk was most pronounced for saturated fat (RR 1.19, 95% CI 1.06–1.35) [15]. The WHI Dietary Modification (WHI-DM) trial is a randomized controlled study evaluating a low-fat diet intervention for prevention of breast and colorectal cancer. It demonstrated a numerical but non-significant reduction in BC incidence after a mean of 8.3 years intervention (during intervention HR 0.92, 95% CI 0.84–1.01; post-intervention HR 0.97, 95% CI 0.89–1.05) [16].

Epidemiological studies have linked the Mediterranean diet to a reduced risk of BC. Adherence to the Mediterranean diet can be measured by a Mediterranean Diet Score (MDS), ranging from 0 (lowest adherence) to 9 (highest adherence). As demonstrated in a case-control study performed in Italy and Switzerland, the odds ratio for BC was 0.82 (95% CI, 0.71–0.95) in women with a MDS of 6–9 compared with those with a MDS of 0–3 [17]. In the PREDIMED (Prevención con Dieta Mediterránea) study conducted in Spain, participants were randomized to a Mediterranean diet supplemented with extra-virgin olive oil, a Mediterranean diet supplemented with mixed nuts, or a control diet (advised to reduce dietary fat). Breast cancer incidence was lower in the two Mediterranean diet groups combined than in the control group (HR, 0.43, 95% CI, 0.21–0.88) [18], suggesting a beneficial effect of a Mediterranean diet in the primary prevention of BC.

Our knowledge on diet and BC risk has provided the rationale and foundation to investigate and apply dietary modification in women with a diagnosis of BC. The data has suggested potential mechanisms by which diet may impact BC survival, including various signaling pathways essential in the pathogenesis of primary BC which may be important in the development of BC recurrence or a second primary cancer.

Associations Between Dietary Patterns and Quality and Breast Cancer Outcomes

Dietary Pattern

The two largest prospective randomized studies in the USA to investigate the association between dietary pattern and BC outcomes are the WHEL (Women's Healthy Eating and Living) and WINS (Women's Intervention Nutrition Study) studies (Table 1).

The WINS trial enrolled 2437 postmenopausal women between 48 and 79 years of age who were treated for early stage BC. They were randomized into a dietary intervention

Table 1 Observational studies and clinical trials assessing the association between diet and mortality and/or survival

Study Author Year	Patients	Follow-up (years)	Study design	Breast cancer specific mortality	All-cause mortality	Non-breast cancer mortality	Survival	Comments
Nurses' Health Study (NHS) Kroenke 2005	2619	9	Observational, Western diet vs. Prudent diet[a]	Prudent pattern $p = 0.57$ Western pattern $p = 0.87$	Prudent pattern $p = 0.25$ Western pattern $p = 0.13$	Prudent pattern $p = 0.03$ Western pattern $p = 0.03$	N/A	A higher intake of the prudent diet pattern and a lower intake of the Western pattern diet were associated with decreased mortality from non-breast cancer causes.
Life After Cancer Epidemiology (LACE) Study Kwan 2009	1901	5.93	Observational, Western diet vs. Prudent diet	Prudent pattern $p = 0.57$ Western pattern $p = 0.60$	Prudent pattern $p = 0.02$ Western pattern $p = 0.05$	Prudent pattern $p = 0.003$ Western pattern $p = 0.02$	N/A	A higher intake of the prudent diet pattern and a lower intake of the Western pattern diet were associated with decreased overall mortality and mortality from non-breast cancer causes.
Health, Eating, Activity, and Lifestyle (HEAL) Study George 2011	670	6	Observational, High-quality vs. poor-quality diet	HR 0.12 (95% CI 0.02–0.99)	HR 0.40 (95% CI 0.17–0.94)	N/A	N/A	A high-quality diet assessed by HEI score was associated with decreased risk of overall mortality and breast cancer–specific mortality when compared with a poor-quality diet.
The Third National Health and Nutrition Examination Survey (NHANES III) Deshmukh 2018	1191	17.2	Observational, High-quality vs. poor-quality diet	HR 0.35 (95%CI 0.19–0.63)	HR 0.59 (95%CI 0.45–0.77)	N/A	N/A	A high-quality diet assessed by HEI score was associated with decreased risk of overall mortality and breast cancer-specific mortality when compared with a poor-quality diet.
Women's Healthy Eating and Living (WHEL) Study Pierce 2007	3088	7.3	Prospective, randomized controlled trial, plant-based diet vs. usual diet	N/A	10.1% vs. 10.3% (intervention group vs. control group)	N/A	Disease-free survival: 16.7% vs. 16.9% with events, HR 0.96 (95% CI 0.80–1.14)	High vegetable/fruit and low-fat diet intervention did not lead to significant improvement in disease-free survival or overall mortality.
Women's Intervention Nutrition Study (WINS) Chlebowski 2006	2437	5	Prospective, randomized controlled trial, plant-based diet vs. usual diet	N/A	N/A	N/A	Relapse-free survival: 9.8% vs. 12.4% with events, HR 0.78 (95% CI 0.60–0.98, p = 0.03)	Low-fat diet intervention was associated with improved breast cancer relapse-free survival during the 5-year intervention period.
Women's Intervention Nutrition Study (WINS) Chlebowski 2008	2437	8.1	Prospective, Randomized controlled trial, Low-fat diet vs. usual diet	N/A	All: 9.1% vs. 11.1% (RR 0.83, $p = 0.146$); ER-/PR-subgroup: 7.5% vs. 18.1% (RR 0.41, $p = 0.003$)	N/A	N/A	Low-fat diet intervention was not shown to significantly decrease mortality in all participants during the 8-year follow-up, but may benefit women with ER-/PR- disease.
Cancer Prevention Study-II (CPS-II) McCullough	4452	9.9	Observational, diet score based on ACS recommendations	Post-diagnosis diet score of 6–9 compared with a score of 0–2: RR	N/A	Post-diagnosis diet score, per 2-point increase in score:	N/A	There was no association found between post-diagnosis diet score based on ACS recommendations and breast cancer–specific

Table 1 (continued)

Study Author Year	Patients	Follow-up (years)	Study design	Breast cancer specific mortality	All-cause mortality	Non-breast cancer mortality	Survival	Comments
2016				1.44 (95% CI 0.90–2.30)		RR 0.88 (95%CI 0.79 to 0.99)		mortality, but there was an inverse relationship with mortality from other causes.
Women's Health Initiative (WHI) Study Chlebowski 2017, 2018	48,835	16.1	Prospective, Randomized controlled trial, dietary intervention initiated before breast cancer diagnosis	HR 0.91 (95% CI 0.72–1.15)	HR 0.82 (95%CI 0.70–0.96)	N/A	10-year overall survival: 82% vs. 78%, HR 0.78 (95% CI 0.65–0.94)	Dietary intervention was associated with few deaths after breast cancer diagnosis and improved overall survival, probably mediated by non-breast cancer causes.

[a] Prudent dietary pattern was characterized by a diet high in fruits, vegetables, whole grains, legumes, poultry, and fish, and the Western pattern was characterized by high intake of refined grains, processed and red meats, desserts, high-fat dairy products, and French fries

(targeting fat intake reduction) or a usual-diet control group. Women in the intervention group were given a fat gram goal by centrally trained, registered dieticians and received eight biweekly individual counseling sessions and subsequent contacts every 3 months, while the control group received general dietary guidelines and dietician contacts every 3 months. After a median follow-up of 60 months, fat intake was significantly reduced in the intervention group (29.2–20.3% of calories, $p < 0.0001$), but not in the control group [19•]. Importantly, participants in the intervention group achieved a 2.7 kg lower mean body weight than the control group ($p = 0.005$) [19•]. Relapse-free survival, the primary endpoint, was significantly lower in the intervention group compared with the control group (9.8% vs. 12.4% with events, HR 0.78, 95% CI 0.60–0.98, $p = 0.03$) [19•]. Exploratory subgroup analysis by receptor status revealed a particularly favorable impact in women with hormone receptor-negative BC (HR 0.44, 95% CI 0.25–0.77), but not in women with hormone receptor-positive cancer. Although there were fewer deaths observed in the intervention group, the difference between the two groups was not statistically significant (9.1% vs. 11.1% cumulative mortality, RR 0.83, $p = 0.146$) [20].

The WHEL study used a slightly more comprehensive approach by targeting a diet high in vegetables, fruits, and fiber in addition to being low in fat. Three thousand eighty-eight women (2448 were postmenopausal at participation) were randomly assigned to the intervention or comparison group. Participants in the intervention group received telephone counseling from trained counselors, 12 cooking classes in the first year, and monthly newsletters throughout the study. Women in the comparison group were provided general guidelines on dietary intake, 4 optional cooking class in the first year (average attendance: 1 out of 4), and 24 newsletters during the first 4 years. After a mean of 7.3-year follow-up, there was no significant difference in invasive BC events (intervention vs. control 16.7% vs. 16.9%, HR 0.96, 95% CI 0.80–1.14, $p = 0.63$) or all-cause mortality (intervention vs. control 10.1% vs. 10.3%, HR 0.91, 95% CI 0.72–1.15, $p = 0.43$) [21•]. It is noteworthy, however, that women in the intervention arm of this study did not lose weight compared with the control arm, which is in contrast to the WINS study. A subsequent analysis focusing on women without baseline hot flashes (more likely to have higher circulating estradiol concentrations) was conducted and demonstrated a decrease in BC recurrence, predominantly distant recurrence (intervention vs. control 83.9% vs. 76.4% BC free, $p = 0.002$), regardless of hormone receptor status ($p = 0.63$) [22].

Although the conflicting results of these studies have made it difficult to translate dietary modification into practice, there are some fundamental differences between these two studies which may in part explain the contradictory results. [23] The WINS focused on a low-fat diet whereas the WHEL study adopted a plant-based diet including high vegetable, fruits,

and fiber in addition to the low-fat component. Decrease in dietary fat intake was less pronounced in the WHEL study than in WINS. There were also differences in study populations. WINS included only postmenopausal women 48–79 years old, while the WHEL study included both pre- and postmenopausal women 18–70 years old. WINS participants had more favorable prognosis compared with those in WHEL with more than 50% of patients having stage I disease while only one-third of patients in the WHEL had stage I disease. Furthermore, the WINS enrolled all participants within 1 year of diagnosis and the WHEL study enrolled patients up to 4 years post diagnosis. Finally, women in WINS study intervention arm lost weight (2.7 kg between-group difference at year 5), while there was no between-group difference in the WHEL study, suggesting weight loss may be necessary to improve prognosis.

Importantly, both studies relied on self-reported dietary assessment. The survey completion rate in the WINS declined with time (~ 70% at year 3 and ~ 40% at year 5), while the completion rate in the WHEL study only experienced a small decline, about 85% at year 6. The authors of both studies reported a completers-only analysis in terms of dietary data, BC free survival, and mortality. This assumed that diets of the non-responders were similar to the diets of the responders, which may lead to selection bias; however, in WINS, there was an improvement in relapse-free survival in this analysis (9.8% vs. 12.4% with events, HR 0.78, 95% CI 0.60–0.98, $p = 0.03$) [19•].

Most recently, a secondary post-hoc analysis of WHI-DM was published, which focused on overall survival and mortality in postmenopausal women who developed BC during the low-fat dietary intervention. After an average of 11.5 years' follow-up, overall survival was improved in women in the intervention group than in the usual-diet comparison group (10-year survival of 82% and 78%, respectively; HR 0.78, 95%CI 0.65–0.94; $p = 0.01$) [24••] (Table 1). Although there were fewer deaths from BC, other cancers, cardiovascular disease, and other causes in the dietary intervention group than the control group, only deaths from cardiovascular disease were statistically different between the two groups (HR 0.62, 95% CI 0.39–0.99). This study highlighted that a low-fat diet may reduce cardiovascular deaths in women diagnosed with BC, which is a major source of mortality for this population [25, 26].

Additional dietary patterns have been investigated. For example, the prudent diet (as characterized by a diet high in fruits, vegetables, whole grains, legumes, poultry, and fish) and the Western pattern (as characterized by high intake of refined grains, processed and red meats, desserts, high-fat dairy products, and French fries) were analyzed in the NHS (Nurses' Health Study) and LACE (Life After Cancer Epidemiology) trials [27, 28] (Table 1). The NHS demonstrated an inverse relation between the prudent diet and non-BC mortality (relative risks of non-BC death were 0.85 [95% CI, 0.53 to 1.35], 0.74 [95% CI, 0.45 to 1.21], 0.70 [95% CI, 0.42 to 1.17], and 0.54 [95% CI, 0.31 to 0.95]; $p = 0.03$, from lowest to highest quintile of intake of prudent diet). Likewise, LACE confirmed an inverse association between the prudent diet and non-BC mortality. In addition, adhering to a prudent dietary pattern was shown to correlate with all-cause mortality (p trend 0.02; HR for highest quartile 0.57; 95% CI, 0.36 to 0.90).

Diet Quality and Inflammation

Recent studies and guidelines have emphasized the quality of "total diet," most often assessed by the Healthy Eating Index (HEI). The HEI score is calculated based on a variety of food components according to recommendations of the Dietary Guidelines for Americas, with higher HEI scores correlating with better-quality diet. The Health, Eating, Activity, and Lifestyle (HEAL) Study demonstrated that women who consumed better-quality diets (as defined by the highest-quartile HEI-2005 score) had a 60% reduction in all-cause mortality compared with those who consumed mixed-quality diet (middle quartiles HEI-2005 score) or poor-quality diet (lowest quartile HEI-2005 score) (HR 0.40, 95% CI 0.17–0.94), and an 88% reduction in BC related mortality (HR 0.12, 95% CI 0.02–0.99) [29]. The NHANES (National Health and Nutrition Examination Survey) III study showed similar results with a 41% reduction in overall mortality (HR 0.59, 95% CI 0.45–0.77) and a 65% reduction in all cancer-related mortality (HR 0.35, 95% CI 0.19–0.63) in women with high-quality diets (highest-quartile HEI score) [30••] (Table 1). Another diet score was developed based on the ACS (American Cancer Society) recommendations, which was used in the Cancer Prevention Study-II (CPS-II) Nutrition Cohort [31]. The score was derived from summing three key food-based recommendations (fruits/vegetables, whole grains, and limited consumption of red and processed meats). The score ranges from 0 to 9, with a score of 9 reflecting the optimal adherence to the ACS guidelines. There was no association between post-diagnosis diet score with either BC-specific mortality (scores 6–9 vs. 0–2 RR 1.44, 95% CI 0.90–2.30) or cardiovascular disease mortality (scores 6–9 vs. 0–2 RR 0.81, 95% CI 0.47–1.39), but a higher score was associated with a borderline lower risk of other causes of death (scores 6–9 vs. 0–2 RR 0.78, 95% CI 0.56–1.07; continuous diet score RR 0.88, 95% CI 0.79–0.99, p trend = 0.03) [31] (Table 1). These studies demonstrate the need for further research on the "total diet" approach.

Interestingly, dietary quality is inversely associated with inflammatory potential of diet and high-quality diets have lower dietary inflammatory index (DII) scores [32]. The association between baseline dietary inflammatory potential and BC mortality was evaluated in the WHI. A total of

122,788 postmenopausal women with a mean of 16-year follow-up completed a baseline food frequency questionnaire, among which, 7495 developed BC and 667 died of BC[33]. DII was calculated based on a comprehensive literature review on the association between dietary factors and six inflammatory markers (IL-1β, IL-4, IL-6, IL-10, TNFα, and C-reactive protein [CRP]). Higher DII scores signify a more inflammatory diet and lower scores indicate a less inflammatory diet. Although there was no significant association between baseline DII scores with BC incidence, a higher risk of death from BC was noted in those with the highest vs. lowest DII scores (HR 1.33 95% CI 1.01–1.76 $p = 0.03$) [33]. A second analysis of the WHI evaluated the association between post-cancer diagnosis DII and mortality. A total of 2150 postmenopausal women were included in the study. Energy-adjusted DII (E-DII) scores were inversely associated with cardiovascular mortality (HR Q1VSQ4 0.44; 95% CI 0.24–0.82; p trend 0.005), but no association was found between E-CII scores and BC-specific mortality (HR Q1VSQ4 0.96; 95% CI 0.62–1.49; p trend 0.96) or all-cause mortality (HR Q1VSQ4 0.82; 95% CI 0.63–1.05; p trend 0.17) [34].

Post-diagnosis CRP, one of the six inflammation markers used in DII, was examined separately in an analysis of the WHEL study [35]. CRP was measured by high-sensitivity electrochemiluminescence assay and had a positive association with death due to any cause, death due to BC, and additional BC events. CRP, an acute phase protein in response to inflammation, thus may have potential as a prognostic biomarker for BC survival, modifiable by improving diet quality and adopting other healthy lifestyle modifications.

Prolonged Nightly Fasting

Another novel dietary intervention approach is to prolong the nightly fasting interval. Although patients enrolled in the WHEL study were randomized, the two cohorts were combined for the analysis of the effects of prolonged nightly fasting. A total of 2413 women reported a mean fasting duration of 12.5 h per night. Nightly fasting interval less than 13 h per night was associated with a 36% increase in BC recurrence compared with those reported nightly fasting interval more than 13 h. During a mean of 11.4 years of follow-up, there was no significant difference in BC mortality or all-cause mortality based on fasting interval [36].

Dietary Modification and Weight Loss

Two-thirds of BC survivors are overweight or obese at the time of diagnosis [37]. There is increasing evidence indicating that being overweight/obese increases the risk of BC recurrence and decreases survival [38–42]. Although it is currently unknown whether intentional weight loss will improve prognosis, there are some data supporting this hypothesis and a clinical trial to answer this question is ongoing [43]. Frequently, dietary intervention will result in weight loss, making it difficult to separate the effects of dietary change from those from weight loss. In the WHI-DM trial, body weight was 2.2 kg lower in the dietary group compared with the usual-diet control group ($p < 0.001$) [24••]. Similarly, participants in the WINS lost an average of 2.7 kg more weight in the intervention vs. control group [19•]. The LEAN (Lifestyle, Exercise and Nutrition) study randomized BC survivors to either a usual care or lifestyle intervention (by either in-person or telephone-based counseling on healthy diet, physical activity, targeting weight loss) [44]. The intervention led to a successful decrease in total fat/saturated fat intake and an increase in fiber and fruit intake. Participants in the intervention group who lost ≥ 5% body weight demonstrated a significant improvement in their HEI score compared with those who did not lose ≥ 5% body weight [44]. This, like the conflicting results of the WINS and WHEL studies, raises the question of whether the impact of dietary modification may be partially or entirely mediated by weight loss and whether outcomes can be affected by dietary modification without significant weight loss.

Ultimately, there is no conclusive evidence to support any one specific dietary pattern in BC survivors. The WINS study demonstrated an improved relapse-free survival in women on low-fat diet intervention, but this has not been confirmed by other large studies. Novel dietary interventions further evaluating fasting intervals, dietary inflammation, and overall quality are needed.

Clinical Trials and Future Research

Further investigation of the effects of dietary patterns and components on BC prognosis is needed to refine dietary recommendations for cancer survivors. There are dozens of active trials (clinicaltrials.gov) on dietary intervention either alone or combined with other behavioral therapies. These studies evaluate different dietary patterns (such as Mediterranean diet), compositions (such as low-carbohydrate diet), energy restrictions (such as low-calorie diet), supplements (such as fish oil), and include biomarker identification and analysis. Among these, the SUCCESS C trial uses a telephone-based lifestyle intervention to have patients adopt a hypocaloric diet with less fat, more whole grain products, and fruit and vegetables with a goal to lose weight, as well as a gradual increase in physical activity. Interim analysis reported at the 2018 San Antonio Breast Cancer Symposium demonstrated promising results for those who lost weight. At the end of the 2-year follow-up period, patients in the intervention group lost an average of 1.0 kg and those in the control

arm gained an average of 0.95 kg. Interestingly, those who completed the lifestyle intervention program had improved disease-free survival compared with those who completed a general program in the control arm (events 5.1% vs. 8.8% , HR 0.51, 95% CI 0.33–0.78, $p = 0.002$) [45].

One challenge we face is the lack of biomarkers to evaluate patients' adherence to dietary interventions and to predict long-term outcomes. Nutritional biomarkers may provide complementary information beyond self-reported food intake data. In the field of metabolic syndrome, dietary biomarkers (DB) including pentadecanoic acid /α-linolenic acid (markers for total fatty acid), EPA/DHA (markers for fatty fish), β-carotene, and alkylresorcinol (markers for vegetables and whole grains) were combined to create a DB score to assess dietary compliance [46]. This same study found that median DB score was 57% higher in the healthy Nordic diet group than the control diet group, suggesting this and/or other dietary biomarkers may be a useful tool to assess compliance in diet intervention studies.

Of equal importance is the need for prognostic biomarkers. Prognostic biomarkers would allow for shorter and more diverse studies (thus allowing for evaluation of a larger number of dietary interventions) to be conducted with biomarker endpoints. Such studies could then inform the design of larger clinical trials with traditional BC outcomes such as risk of recurrence and death. Post-diagnosis high-sensitivity CRP, as mentioned above, may serve as one such potential biomarker, but this requires validation. Obesity-related markers have also been investigated. In a nested case-control study using Cancer Prevention Study-II (CPS-II) Nutrition Cohort, low level of adiponectin and high levels of IGF-1, CRP, and C-peptide have been linked with postmenopausal BC risk, but only the association between C-peptide and BC risk was statistically significant (OR 1.63, 95% CI 0.08–2.45, p linear trend 0.001) [47]. In the HEAL study, fasting C-peptide in patients without type II diabetes was associated with increased all-cause mortality and BC-specific mortality. In patients with type II diabetes, the association between C-peptide levels and BC-specific mortality was stronger [48]. Another prospective cohort study including 512 women with early stage BC observed positive associations between fasting insulin levels and BC outcomes (distant recurrence HR for highest vs. lowest quartile 2.0, 95% CI, 1.2–3.3; all-cause mortality HR for highest vs. lowest quartile 3.1, 95% CI 1.7–5.7) [49]. Additional research is necessary to validate these inflammatory and obesity-related markers and to identify novel prognostic lifestyle linked biomarkers.

Current Guidelines

The American Institute for Cancer Research (AICR) publishes comprehensive guidelines for the prevention of cancer, yet guidelines for cancer survivors remain relatively vague. The AICR acknowledges the WINS and WHEL studies, but finds it difficult to translate these results into recommendations for BC survivors given the discrepancies in results and potential confounding factor of weight loss. With respect to micronutrient supplements, the AICR guidelines do not support their use as a means of improving outcome in cancer survivors, based on data from 39 randomized controlled trials [50].

The ACS also publishes guidelines on nutrition and physical activity in cancer survivors, with an emphasis on achieving and maintaining a healthy diet and weight [51]. The ACS guidelines stress the importance of eating a diet high in vegetables, fruits, and whole grains. Similar to the AICR guidelines, ACS states that dietary supplements are unlikely to improve prognosis or overall survival in cancer

Table 2 Resources for dietary intake and weight management in breast cancer survivors

Organization	Tool	Website
AICR	portion control	http://www.aicr.org/new-american-plate/reduce_diet_new_american_plate_portion.html
ACS	healthy recipes	https://www.cancer.org/healthy/eat-healthy-get-active/eat-healthy/find-healthy-recipes/main-dishes.html
USDA	food plate volumes	https://www.choosemyplate.gov/
CDC	BMI calculator	https://www.cdc.gov/healthyweight/assessing/bmi/adult_bmi/english_bmi_calculator/bmi_calculator.html
ASCO	toolkit on obesity and cancer	https://www.asco.org/practice-guidelines/cancer-care-initiatives/prevention-survivorship/obesity-cancer
LIVESTRONG	My Plate Calorie Tracker	https://www.livestrong.com/myplate/

AICR, American Institute for Cancer Research; *ACS*, American Cancer Society; *USDA*, United States Department of Agriculture; *CDC*, Centers for Disease Control and Prevention; *ASCO*, American Society of Clinical Oncology

survivors and importantly, some supplements may cause harm. With regard to diet composition, the ACS adopts both the Institute of Medicine and current Federal Guidelines, and the American Heart Association (AHA) guidelines that the spectrum of dietary composition for an adult cancer survivor should include fat 20 to 35% of energy (AHA 25–35%), carbohydrate 45 to 65% of energy (AHA 50–60%), and protein 10 to 35% of energy (at least 0.8 g/kg). Choices of food sources are also important. For example, healthy carbohydrate sources are foods like vegetables, fruits, whole grains, and legumes. A list of resources on nutrition and weight management is summarized in Table 2.

The National Comprehensive Cancer Network (NCCN) Survivorship guidelines (version 2.2018) address nutrition and weight management as well. The guidelines emphasize dietary patterns as well as eating habits including portion size, night grazing, snacking habits, and use of added fats and/or sugars to foods and beverages. Supplement use is again not recommended by NCCN, except in women with documented deficiencies, inadequate diet, or comorbid conditions, such as osteoporosis.

Conclusions

It is currently unknown whether weight loss is required to improve prognosis, as suggested by the conflicting results of WINS and WHEL, and the WHI-DM and LEAN studies, or whether specific diets alone may have an impact. Healthy diet and weight management should be encouraged for all BC survivors as it may decrease mortality from other causes, especially cardiovascular disease, a significant concern for BC survivors. In addition to dietary patterns and weight loss, dietary quality has been an emerging research topic. The HEAL and NHANES III studies substantiated the need for further research on "total diet" approach, perhaps a more feasible approach than specific diet patterns, in BC survivors. Identification of novel lifestyle linked prognostic biomarkers would allow us to design more versatile yet efficient studies to better define the role of dietary intervention in BC survivors and ultimately provide more personalized dietary recommendations for cancer survivors.

Compliance with Ethical Standards

Conflict of Interest Lai Xu declares that she has no conflict of interest.

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Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance
- •• Of major importance

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Review

HDL and LDL: Potential New Players in Breast Cancer Development

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Abstract: Breast cancer is the most prevalent cancer and primary cause of cancer-related mortality in women. The identification of risk factors can improve prevention of cancer, and obesity and hypercholesterolemia represent potentially modifiable breast cancer risk factors. In the present work, we review the progress to date in research on the potential role of the main cholesterol transporters, low-density and high-density lipoproteins (LDL and HDL), on breast cancer development. Although some studies have failed to find associations between lipoproteins and breast cancer, some large clinical studies have demonstrated a direct association between LDL cholesterol levels and breast cancer risk and an inverse association between HDL cholesterol and breast cancer risk. Research in breast cancer cells and experimental mouse models of breast cancer have demonstrated an important role for cholesterol and its transporters in breast cancer development. Instead of cholesterol, the cholesterol metabolite 27-hydroxycholesterol induces the proliferation of estrogen receptor-positive breast cancer cells and facilitates metastasis. Oxidative modification of the lipoproteins and HDL glycation activate different inflammation-related pathways, thereby enhancing cell proliferation and migration and inhibiting apoptosis. Cholesterol-lowering drugs and apolipoprotein A-I mimetics have emerged as potential therapeutic agents to prevent the deleterious effects of high cholesterol in breast cancer.

Keywords: Breast cancer; cholesterol; 27-hydroxycholesterol; HDL; LDL; cholesterol-lowering therapies

1. Introduction

Breast cancer is the third most common cancer overall, with an estimated incidence of 1.7 million cases in 2016 and a 29% increase in incident cases between 2006 and 2016. Moreover, breast cancer was the fifth leading cause of cancer deaths for both sexes in 2016 and the primary cause of death for women [1]. A substantial proportion of the worldwide burden of cancer could be prevented; however, improved primary prevention of cancer requires identification of risk markers [2]. Reproductive, hormonal factors, and unhealthy lifestyles that trigger obesity are considered significant risk factors for breast cancer [3]. Obesity represents a potentially modifiable risk factor

that could increase the risk of breast cancer in women [4,5]. The biological association between obesity and disease risk, at least in part, may be related to circulating lipid levels and tissue lipid metabolism [6].

Cancer cells show specific alterations in different aspects of lipid metabolism, which can affect the availability of structural lipids for the synthesis of membranes, contribution of lipids to energy homeostasis, and lipid signaling functions, including the activation of inflammation-related pathways. All these changes are related to important cellular processes, including cell growth, proliferation, differentiation, and motility [7]. The interplay among cholesterol, lipoproteins, proinflammatory signaling pathways, and tumor development has mainly been studied in breast cancer cells and experimental models in vivo. Furthermore, in humans, both benign and malignant proliferation of breast tissue were associated with changes in plasma lipid and lipoprotein levels [8], despite that epidemiological data on the association between lipoproteins and breast cancer showed inconclusive results [9–11]. This article reviews the progress to date in research on the role of cholesterol and its main lipoprotein transporters, the low-density and high-density lipoproteins (LDL and HDL), on breast cancer development, mainly focusing on recent findings in human trials and those obtained in experimental models of breast cancer. PubMed was searched comprehensively with combinations of the keyword Breast Cancer and the rest of keywords related with cholesterol and lipoproteins.

2. Association of Cholesterol in Breast Cancer Risk: Clinical and Epidemiological Studies

Study of the relationship between serum cholesterol levels and risk of cancer is of special interest and has sparked debate, especially with the expansion of lipid-modifying therapies and more aggressive cholesterol goals to reduce the risk of cardiovascular events [12]. However, different studies have produced divergent results. Indeed, one study found that total cholesterol was associated with the risk of breast cancer [13], but others failed in finding such an association [14–18], or they even found that total cholesterol was inversely associated with the risk of breast cancer [19].

Since cholesterol is mainly transported by LDL and HDL, several clinical trials have associated them with breast cancer. A clinical study in which the lipid profile was assessed in women with breast cancer showed that LDL cholesterol (LDL-C) levels at diagnosis was a prognostic factor of breast tumor progression. A systemic LDL-C level above 117 mg dL^{-1} was found to be a predictive factor of tumor stage, and it was positively associated with worse prognosis because of a higher histological grade, higher proliferative rate, and more advanced clinical stage [20] (Table 1). Moreover, patients with LDL-C above 144 mg dL^{-1} were also prone to have lymph node metastasis [20]. More importantly, a Mendelian randomization study found that genetically raised LDL-C was associated with a higher risk of breast cancer [11]. However, other meta-analyses and prospective studies found no association between LDL-C and breast cancer risk [9,10,16,21]; some trials even found that LDL-C or non-HDL were inversely associated with the risk of breast cancer [14,22] (Table 1).

Concerning HDL-C, discordant results were also found. One prospective study with a follow-up time of 11.5 years found an inverse association between HDL-C and breast cancer risk [19], and retrospectively collected clinical data showed that decreased HDL-C levels had a significant association with worse overall survival in breast cancer patients [23] (Table 1). In contrast, a Mendelian randomization study showed that raised HDL-C increased the risk of estrogen receptor (ER)-positive breast cancer [11] (Table 1). It should also be noted that other studies failed to find any association between HDL-C and breast cancer risk [10,21,24] or survival [24]. Moreover, controversy also exists when considering the menopausal status of patients (Table 1). Some studies have found that low HDL-C among premenopausal women increased breast cancer risk [9,25,26], while others found that low HDL-C was associated with an increased postmenopausal risk of breast cancer [16,27].

Table 1. Clinical and epidemiological studies linking low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) levels to breast cancer risk.

Reference	Year	Study design	Participants	Main findings
Nowak et al. [11]	2018	Mendelian randomization	>400,000	Raised LDL-C increased the risk of breast cancer (OR = 1.09 (1.02–1.18)) and ER-positive breast cancer (OR = 1.14 (1.05–1.24)). Raised HDL-C increased the risk of ER-positive breast cancer (OR = 1.13 (1.01–1.26)).
Ni et al. [16]	2015	Meta-analysis	1,189,635	Inverse association between HDL-C and breast cancer risk among postmenopausal women (RR = 0.45 (0.64–0.93)). No association in premenopausal women. No association between LDL-C and breast cancer risk.
Touvier et al. [9]	2015	Meta-analysis	1,489,484	Inverse association between HDL-C and breast cancer risk among premenopausal women (HR = 0.77 (0.31–0.67)). No association in postmenopausal women. No association between LDL-C and breast cancer risk.
Borgquist et al. [21]	2016	Prospective	5281	No evident associations between LDL-C or HDL-C and breast cancer incidence.
Chandler et al. [10]	2016	Prospective	15,602	No association between LDL-C or HDL-C and breast cancer risk.
His et al. [19]	2014	Prospective	7557	HDL-C was inversely associated with breast cancer risk (HR 1 mmol L^{-1} increment = 0.48 (0.28–0.83)).
Rodrigues dos Santos et al. [20]	2014	Prospective	244	Systemic levels of LDL-C correlated positively with tumor size (Spearman's r = 0.199, $p = 0.002$).
Kucharska-Newton et al. [25]	2008	Prospective	7575	Modest association of low HDL-C (<50 mg dL^{-1}) with breast cancer among premenopausal women (HR = 1.67 (1.06–2.63)). No association in postmenopausal women.
Furberg et al. [27]	2004	Prospective	30,546	The risk of postmenopausal breast cancer was reduced in women in the highest quartile of HDL-C (>1.64 mmol L^{-1}) compared with women in the lowest quartile (<1.20 mmol L^{-1}; RR = 0.73 (0.55–0.95)). No association was found in premenopausal women.
Li et al. [23]	2017	Retrospective	1044	Decreased HDL-C levels showed significant association with worse overall survival (HR = 0.528 (0.302–0.923)).
Li et al. [28]	2018	Case–control	Total: 3537 Cases: 1054 Controls: 2483	The levels of LDL-C and HDL-C were lower in breast cancer patients than controls ($p < 0.001$).
His et al. [24]	2017	Case–control	Total: 1626 Cases: 583 Controls: 1043	No association between LDL-C or HDL-C and breast cancer risk or survival.

Martin et al. [14]	2015	Case–control	Total: 837 Cases: 279 Controls: 558	HDL-C was positively associated (75th vs. 25th percentile: 23% higher, $p = 0.05$) and non-HDL-C was negatively associated (75th vs. 25th percentile: 19% lower, $p = 0.03$) with breast cancer risk.
Llanos et al. [22]	2012	Case–control	Total: 199 Cases: 97 Controls: 102	Increasing levels of LDL-C were inversely associated with breast cancer risk (OR = 0.41 (0.21–0.81)). Lower levels of HDL-C were associated with a significant increase in breast cancer risk (OR = 1.99 (1.06–3.74)).
Yadav et al. [29]	2012	Case–control	Total: 139 Cases: 69 Controls: 70	Postmenopausal breast cancer patients had higher LDL-C levels ($p < 0.001$) and lower HDL-C levels ($p = 0.025$) than controls. No significant changes in premenopausal women.
Kim et al. [26]	2009	Case–control	Total: 2070 Cases: 690 Controls: 1380	Protective effect of HDL-C on breast cancer was only observed among premenopausal women (OR = 0.49 (0.33–0.72) for HDL-C ≥ 60 vs. <50 mg dL^{-1} ($p < 0.01$)).
Owiredu et al. [30]	2009	Case–control	Total: 200 Cases: 100 Controls: 100	Increased LDL-C levels in postmenopausal breast cancer patients vs. controls ($p < 0.05$). No significant changes in premenopausal women. No changes in HDL-C levels between cases and controls.
Michalaki et al. [31]	2005	Case–control	Total: 100 Cases:56 Controls: 44	A decrease in HDL-cholesterol was observed in patients with breast cancer vs. controls ($p < 0.05$).

LDL-C = low-density lipoprotein cholesterol, HDL-C = high-density lipoprotein cholesterol, ER = estrogen receptor, OR = odds ratio, RR = risk ratio, and HR = hazard ratio. Between brackets, 95% confidence interval.

In summary, although some studies failed to find associations between lipoproteins and breast cancer, the results of some large clinical trials seem to point to a direct association between LDL-C and breast cancer risk as well as an inverse association between HDL-C and breast cancer risk. It is important to note that clinical or methodological differences in the design of the studies, including variation in geographic regions, menopausal status, number of cases, or follow-up length, could explain the discrepancies found in these studies (summarized in Table 1). For this reason, basic scientific research can contribute to determining potential underlying mechanisms that may explain these associations [12].

3. Hypercholesterolemia and Breast Cancer

Diet and obesity are important risk factors for breast cancer development [5,32]. High cholesterol intake was found to be positively associated with the risk of breast cancer, mainly among postmenopausal women [33,34]. To address interactions between body weight and dietary fat intake on subsequent mammary tumor development, a study was performed in which female murine mammary tumor virus (MMTV)-transforming growth factor α (TGFα) mice consumed a moderately high-fat diet [35]. The MMTV promoter specifically directs expression to the mammary epithelium [36], obtaining a model that recapitulates human breast cancer progression from early hyperplasia to malignant breast carcinoma [37]. These mice exhibited mammary tumor latency inversely related to their body fat, suggesting that body fat may be the mediating factor of the effect of a high-fat diet on mammary tumor development [35]. Moreover, the expression of a number of proteins associated with leptin and apoptosis signaling pathways were also affected by diet in the mammary tumors of these animals [38].

Some studies have specifically addressed the role of dietary cholesterol in the regulation of tumor progression in different experimental mouse models of breast cancer. Llaverias et al. studied the role of a high-fat/high-cholesterol (HFHC) diet administration in MMTV polyoma middle T (PyMT) oncogene transgenic mice and found that the HFHC diet accelerated and enhanced tumor progression in these mice [39]. Plasma cholesterol levels were reduced during tumor development but not prior to its initiation, providing new evidence for an increased utilization of cholesterol by tumors and for its role in tumor formation [39]. Another group administered an HFHC diet to female immunodeficient mice implanted orthotopically with MDA-MB-231 cells and found that diet induced angiogenesis and accelerated breast tumor growth in this model of breast cancer [40].

The role of dyslipidemia in breast cancer growth and metastasis was also explored in hypercholesterolemic apolipoprotein E knockout mice (apoE$^{-/-}$) fed an HFHC diet and injected with non-metastatic Met-1 and metastatic Mvt-1 mammary cancer cells derived from PyMT mice and c-Myc/vegf tumor explants, respectively [41]. The apoE glycoprotein is a structural component of all lipoprotein particles other than LDL, and it acts as a ligand of lipoprotein receptors and participates in the uptake of lipids into cells. The absence of apoE leads to the accumulation of cholesterol and triglycerides in plasma [42]. ApoE$^{-/-}$ mice exhibited increased tumor growth and displayed a greater number of spontaneous metastases to the lungs. The results in tumor growth were only observed when an HFHC diet was administered to the mice, not when they were fed a standard chow diet [41]. Therefore, although the uptake of cholesterol via apoE was blocked, other adipocyte apoE-independent receptors, such as LDL receptor (LDLR) [43], may be involved in the cholesterol uptake by cancer cells. Moreover, the phosphoinositide 3-kinase (PI3K)/Akt pathway, involved in proinflammatory and cell proliferation signals, was found to be one mediator of the tumor-promoting activity of hypercholesterolemia [41].

Whereas the selection of HFHC diets for these studies reflects current dietary trends, this approach has not allowed an evaluation of the specific effect of cholesterol on tumor biology [44]. To directly address this question, PyMT mice were administered a high-cholesterol diet from weaning and developed palpable tumors earlier than mice on a control chow diet were [45]. High-cholesterol diet administration to mice injected with different breast cancer cell lines (human breast cancer HTB20 and MDA-MB-231, and the mouse breast cancer cell line 4 T1) also promoted breast tumor growth. Tumors of animals in the high-cholesterol diet group showed a higher proliferative ratio than those from chow-fed mice, and lung metastasis was increased [46].

4. 27-Hydroxycholesterol and Breast Cancer

Estrogen receptor α-induced signal transduction controls the growth of most breast cancers [47]. 27-hydroxycholesterol (27-HC), one of the most prevalent oxysterols, was identified as an endogenous selective ER modulator (SERM) and liver X receptor (LXR) agonist [48]. This oxysterol is generated enzymatically from cholesterol by the P450 enzyme sterol 27-hydroxylase CYP27A1. CYP27A1 is abundant in the liver, but it is also expressed in the intestine, vasculature, brain, and macrophages. 27-HC is mainly transported in association with HDL and LDL, primarily in the esterified form [49]. Regarding its catabolism, 27-HC is hydroxylated by oxysterol 7α-hydroxylase CYP7B1, which is also abundant in the liver [50].

The first evidence for 27-HC's role in breast cancer began with studies that found that it stimulated the growth of ER-positive MCF-7 cells but not that of ER-negative MCF-10 cells. The effect of a concentration of 1–2 μM of 27-HC was similar to that of 1–2 nM of 17β-estradiol [51]. The proliferative role of 27-HC in vitro on MCF-7 cells was also confirmed by others, who also reported that 27-HC increased tumor growth in vivo in PyMT mice and in murine or human cancer cell xenografts [45,52]. 27-HC was also found to hasten metastasis to the lungs, an effect that implicated LXR activation [45]. 27-HC also hastened myeloid immune cell functions, as it was found that this oxysterol increased the number of polymorphonuclear neutrophils and γδ T cells as well as decreased cytotoxic CD8^{+} T cells within tumors and metastatic lesions [53].

In human breast cancer tissue, 27-HC concentration was found to increase because of decreased catabolism, since *CYP7B1* gene expression was downregulated, whereas *CYP27A1* remained

unchanged. Moreover, increased *CYP7B1* mRNA was correlated with better survival [52]. Consistently, Nelson et al. found increased CYP27A1 protein expression in higher grade tumors [45]. Nevertheless, the first prospective epidemiological study on prediagnosis of circulating 27-HC and breast cancer risk showed an inverse association between blood 27-HC and breast cancer risk among postmenopausal women. The authors hypothesized that 27-HC-associated inhibition of estradiol–ER binding outweighed 27-HC's agonistic effect in human breast cancer [54].

Unlike humans, mice do not normally become severely hypercholesterolemic when fed an HFHC diet [44]. To circumvent this limitation, breast cancer cells were implanted in mice in which the mouse *Apoe* gene was replaced with the human *APOE3* allele, which codes for the most frequent human isoform. The animals on an HFHC diet exhibited both increased cholesterol and 27-HC in plasma as well as promotion of larger tumors, effects that were partially reversed by treatment with the CYP27A1 inhibitor GW273297X [45].

Several studies investigated the potential mechanisms involved in 27-HC-induced breast cancer development. First, 27-HC inhibited p53 protein and activity in MCF-7 cells via ER. The oxysterol increased p53 regulator mouse double minute 2 (MDM2) levels and enhanced interaction between p53 and MDM2, suggesting that 27-HC proliferation depended on MDM2-mediated p53 degradation. Interestingly, estradiol, the main physiological endogenous ligand for ER, which had similar effects to 27-HC on cell proliferation, had no effect on p53 activity; this demonstrates that 27-HC may contribute to ER-positive breast cancer progression via different mechanisms compared with known estrogens [55]. Another study found that 27-HC increased Myc protein stability (a critical oncogene that can promote proliferation, migration, and invasion of cancer cells) by reducing its dephosphorylation and ubiquitination for proteasomal degradation [56]. Signal transducer and activator of transcription (STAT)-3 is an important transcription factor that can target c-Myc, vascular endothelial growth factor (VEGF), cyclin D1, matrix metalloproteinase (MMP) 2, and MMP9 to promote the development of cancer involving tumor proliferation, invasion, metastasis, and angiogenesis [57]. 27-hydroxycholesterol induced activation of STAT-3, which promoted the angiogenesis of breast cancer cells via proinflammatory-related reactive oxygen species (ROS)/STAT-3/VEGF signaling [58]. Moreover, it induced the epithelial–mesenchymal transition (EMT) [59], a mechanism that promotes migration and invasion, via STAT-3/MMP9 and STAT-3/EMT [60], in both ER-positive and ER-negative breast cancer cells. Furthermore, 27-HC causes greater macrophage infiltration and exacerbation of inflammation in the setting of hypercholesterolemia [61], thereby providing a link between inflammation and cancer development. Collectively, mechanisms involved in 27-HC-promoted progression of breast cancer are complex. Therefore, seeking effective measures to prevent 27-HC-caused pathogenicity is difficult, and further studies should be carried out with an emphasis on deeply investigating the potential mechanisms involved in 27-HC breast cancer promotion [58].

The discovery of 27-HC as an endogenous ER ligand that promotes ER-positive breast tumor growth could help explain why some breast cancer patients are resistant to aromatase inhibitors [62]. In this way, 27-HC may act as an alternate estrogenic ligand in a low-estrogen environment [63]. Assessments of 27-HC or their metabolic enzymes' abundance in tumors could aid in personalizing hormone-based therapy [64].

5. Low-Density Lipoprotein and Breast Cancer

Proliferating cancer cells have an increased cholesterol need. Increased LDLR expression was demonstrated in breast cancer tissue to increase the uptake of LDL-C from the bloodstream [65]. In vitro, LDLR gene and protein expression was found increased in ER-negative MDA-MB-231 cells in contrast to ER-positive MCF-7 cells [66,67]. Accordingly, LDL-C mainly promoted proliferation [68–70] and migration [46,71] in ER-negative cells, but this was not evident in ER-positive cell lines. This difference between the two cell types corresponded to a greater ability of ER-negative cells to take up, store, and utilize exogenous cholesterol because of the increased activity of acyl-CoA:cholesterol acyltransferase 1 (ACAT1) [68]. The Women's Intervention Nutrition Study (WINS) found that a low-fat diet mainly extended relapse-free survival in women with ER-negative breast cancer [72]. At least

in part, that ER-negative breast cancer cells differentially uptake and store cholesterol may explain the differential effect of a low-fat diet on human breast cancer recurrence [68]. Another study found that LDL-C also induced proliferation in ER-positive BT-474 breast cancer cells [46]. This discrepancy could be because BT-474 cells usually express the Her2 (ErbB2) receptor [73]; furthermore, high plasma LDL-C levels were found to be associated with Her2-positive breast cells [20]. It is noteworthy that the Her2-positive and triple-negative subtypes are the most aggressive breast cancers [74].

Beyond in vitro studies, tumors from breast cancer cells with high LDLR expression (murine MCNeuA (Her2-positive) and human MDA-MB-231 (triple-negative), respectively) have been incrementally grown in immunocompetent (LDLR$^{-/-}$ and apoE$^{-/-}$) and immunodeficient (Rag1$^{-/-}$/LDLR$^{-/-}$ and Rag1$^{-/-}$/apoE$^{-/-}$) mouse models of hyperlipidemia with increasing serum LDL concentrations. Importantly, silencing LDLR in the tumor cells reduced tumor growth [67].

Finally, in human samples, *LDLR* and *ACAT1* were also found to be increased in Her2-positive and triple-negative tumors compared with luminal A tumors. Her2-positive and triple-negative tumors were more cholesteryl ester-rich and had higher histological grades, Ki-67 expression, and tumor necrosis. Therefore, cholesteryl ester accumulation due to increased LDL-C internalization and esterification was associated with breast cancer proliferation [75]. In line with these findings, higher LDLR expression was found to be associated with a worse prognosis in patients who underwent systemic therapy [67]. Overall, elevated circulating LDL and breast cancer expression of LDLR have roles, at least in Her2-positive and triple-negative breast cancers, in disease progression and disease-free survival.

5.1. Oxidized Low-Density Lipoprotein and Breast Cancer

Lipid peroxidation is associated with carcinogenesis [76]. Lipid peroxidation metabolites cause structural alterations in DNA and decrease DNA repair capacity through their direct interaction with repair enzymes [77]. The oxidation of LDL affects both protein and lipid contents, resulting in the formation of peroxidation metabolites. Patients with breast cancer exhibited elevated serum levels of oxidized LDL (oxLDL) [78]. Moreover, serum oxLDL levels were associated with increased breast cancer risk [78]. Oxidized LDL was also reported to trigger pro-oncogenic signaling in MCF10A cells; concretely, cells treated with oxLDL showed a dose-dependent stimulation of proliferation mediated by stimulation of the microRNA miR-21, which, in turn, activated the related proinflammatory PI3K/Akt signaling pathways [79].

OxLDL lecithin-like receptor 1 (OLR1) is the main receptor for internalization of oxLDL. It is overexpressed in human breast cancer and positively correlates to tumor stage and grade [80]. A microarray analysis of hearts of *Olr1* KO mice compared with wild-type mice showed a reduction in the expression of nuclear factor κB (NF-κB) target genes involved in cellular transformation (regulation of apoptosis, proliferation, wound healing, defense response, immune response, and cell migration) as well as an inhibition of key enzymes involved in lipogenesis. The human breast cancer cell line HCC1143 showed increased *OLR1* expression compared with the normal mammary epithelial cell line MCF10A [81]. Forced overexpression of *OLR1* in both cell lines resulted in upregulation of NF-κB and its target pro-oncogenes involved in the inhibition of apoptosis (*BCL2*, *BCL2A1*, and *TNFAIP3*) and regulation of the cell cycle (*CCND2*) in HCC1143 cells. Moreover, upregulation of *OLR1* in breast cancer cell lines enhanced cell migration [81,82]. In line with these findings, *OLR1* depletion by siRNAs, or ORL1 inhibition by antibodies or a recombinant OLR1 protein, significantly suppressed the invasion and migration of breast cancer cells [81–83]. *TBC1D3* is a hominoid-specific oncogene that also regulates migration of human breast cancer cells. *TBC1D3* was found to stimulate the expression of *OLR1*, and this *TBC1D3*-induced *OLR1* expression was regulated by tumor necrosis factor α (TNFα)/NF-κB signaling [84]. Therefore, *OLR1* may function in special situations, such as obesity and chronic inflammation, to increase breast cancer susceptibility.

6. High-Density Lipoprotein and Breast Cancer

Controversy exists about the association between HDL-C levels and breast cancer risk, as detailed in Section 2. In the present section, experimental data evaluating the role of HDL in breast

cancer development are reviewed. In vitro analyses have shown that HDL stimulated proliferation in both ER-positive [69,85] and ER-negative breast cancer cell lines [69] in a dose-dependent manner, but ER-negative cells showed a higher response [69]. Human HDL3 also induced migration and activated Akt and extracellular signal-regulated kinases (ERK)1/2 signal transduction pathways in both MCF7 and MDA-MB-231 cells [86].

The scavenger receptor class B type I (SR-BI) acts as an HDL receptor and mediates its cholesterol uptake in breast cancer cells [87]. The receptor SR-BI is abundantly expressed in human breast cancer tissue compared with adjacent normal tissue [88]. Moreover, high SR-BI expression was found related to tumor aggressiveness and poor prognosis in breast cancer [75,89,90], whereas knockdown of SR-BI in vitro attenuated Akt activation and inhibited breast cancer cell proliferation and migration [86]. Moreover, HDL-induced proliferation was blocked in transfected MCF-7 cells with a mutant, nonfunctional SR-BI [88]. Beyond in vitro studies, mice injected with SR-BI-knockdown breast cancer cells showed a decreased tumor burden, accompanied with reduced Akt and ERK1/2 activation, reduced angiogenesis, and increased apoptosis [86]. Therefore, cholesteryl ester entry via HDL-SR-BI and Akt signaling seems to play a critical role in the regulation of cellular proliferation and migration and tumor growth. SR-BI was also found to increase concomitantly with an increased number and size of tumors in PyMT mice fed an HFHC diet compared with those fed a chow diet. However, cholesterol was not found accumulated in the mammary tumors, suggesting that even if tumor cholesterol uptake was increased, cholesterol was probably metabolized to sustain a high level of proliferation [39].

Serum HDL particles contain either a single copy or multiple copies of apolipoprotein A-I (apoA-I), the most abundant HDL apolipoprotein [91]. Apolipoprotein A-I plays a role in promoting cholesterol release from cells; possesses anti-inflammatory, antioxidant, and antiapoptotic properties; and influences innate immunity [92]. The levels of apoA-I have normally been found to be inversely associated with breast cancer risk [19,93,94], although one study found that apoA-I was positively associated with breast cancer [21]. Our group showed that human apoA-I-containing HDL could not hinder breast tumor development in PyMT mice. While overexpression of human apoA-I reduced the levels of oxLDL, 27-HC levels were increased, which could promote tumor growth [95]. Concerning apoA-II, the second major protein constituent of HDL [96], our research group showed that human apoA-II-containing HDL increased the breast tumor burden in PyMT mice (Figure 1A) (unpublished results). These results may be related with the apoA-II-mediated alteration in HDL remodeling, decreased capacity to protect against LDL oxidative modification and its proinflammatory actions, and postprandial hyperlipidemia (Figure 1B) [97,98].

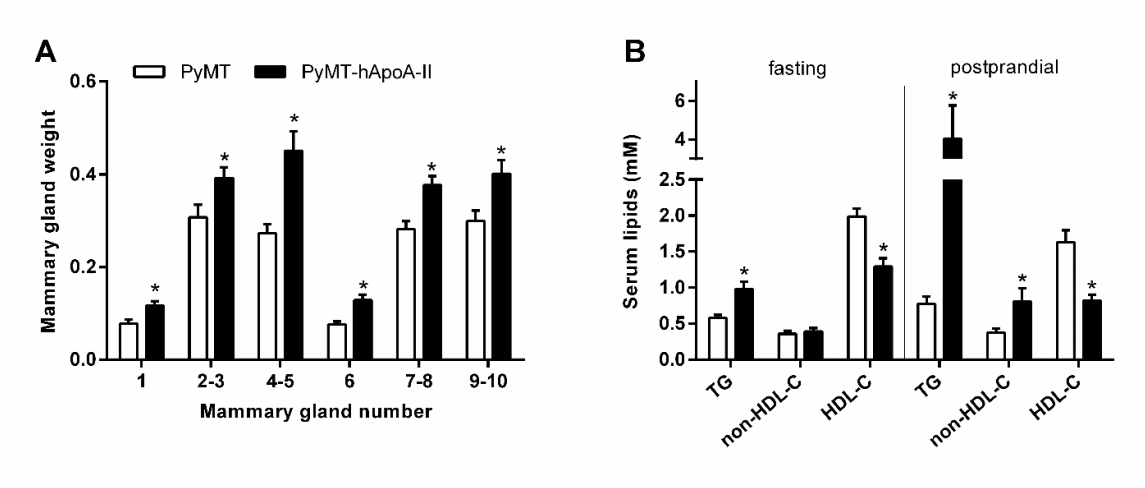

Figure 1. Effects of human apolipoprotein A-II (hApoA-II) overexpression on tumor development in polyoma middle T (PyMT) mice. PyMT mice were backcrossed with hApoA-II transgenic (TG) mice on a C57BL/6 background. The mice were maintained on a regular chow diet until 19 weeks of age,

when they were euthanized, and the mammary glands were excised and weighed. Serum lipids were determined after an overnight fasting period and 3 h after a 0.15 mL dose of olive oil by oral gavage. A) Mammary gland weight. B) Serum lipid levels in fasting and postprandial conditions (TG = triglycerides, and HDL-C = high-density lipoprotein cholesterol). Values shown represent the mean ± SEM. A *t*-test was performed to determine the statistical significance between groups. * $p < 0.05$ vs. PyMT mice.

6.1. Dysfunctional High-Density Lipoprotein and Breast Cancer

Under conditions of oxidative stress, HDL can be oxidatively modified, and these modifications may have an effect on HDL function. Hypochlorite-oxidized HDL was found to stimulate cell proliferation, migration, invasion, and adhesion in vitro, involving the protein kinase C (PKC) pathway, which regulates numerous cellular responses including cell proliferation and the inflammatory response. This modified HDL promoted breast cancer cell pulmonary and hepatic metastasis compared with normal HDL in vivo. Interestingly, in this study, normal HDL reduced the metastasis of MCF7 cells in the liver compared with control animals in which HDL was not injected [99].

In patients with type 2 diabetes mellitus (T2DM), HDL can be modified into dysfunctional glycated HDL and oxidized HDL [100]. Indeed, T2DM patients have a 20% increased risk of breast cancer compared with nondiabetic subjects [101]. In this context, diabetic HDL was found to have a stronger capability to promote cell proliferation, migration, and invasion of breast cancer cells through the Akt, ERK, and p38 mitogen-activated protein kinase (MAPK) pathways. These observations were also found in glycated and oxidized HDL produced in vitro, compared with normal HDL [102]. Pretreatment with diabetic, glycated, and oxidized HDL also promoted the metastasis capacity of breast cancer cells in vivo, and it increased their capacity of adhesion to human umbilical vein endothelial cells (HUVECs) and attachment to the extracellular matrix in vitro, compared with normal HDL. These effects mainly were due to elevated PCK activity, which, in turn, could stimulate secretion of integrins, which are important in promoting breast cancer metastasis [103]. Similarly, HDL isolated from patients with breast cancer complicated with T2DM promoted an increase in breast cancer cell adhesion to HUVECs and stimulated higher intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) expression on the cell surfaces of breast cancer cells and HUVECs, along with the activation of PKC, compared with HDL isolated from breast cancer patients. However, in breast cancer patients complicated with T2DM, a lower expression of ICAM-I and VCAM-I was found in their tumor tissue, which may contribute to the metastasis of tumor cells [104]. Collectively, associations between T2DM and breast cancer could be attributed, in part, to alterations in HDL structure and composition and their proinflammatory actions.

7. Effects of Cholesterol-Lowering Therapies on Breast Cancer

The studies reviewed indicate that cholesterol and its main metabolite, 27-HC, may increase breast cancer development and metastasis. To address this, cholesterol-lowering drugs have emerged as potential therapies to reverse the deleterious effects of impaired cholesterol metabolism in breast cancer.

7.1. Statins

Statins are inhibitors of the enzyme hydroxy-methyl-glutaryl-coenzyme A reductase (HMGCR), which catalyzes the conversion of HMG-CoA to mevalonate, the rate-limiting step of cholesterol synthesis [105]. In humans, the effect of statins in cancer prevention and treatment remains controversial (Table 2). The use of lipid-lowering drugs, and more concretely, statins, was found to be associated with a reduced risk of breast cancer in older women [106]. Specifically, the use of lipophilic statins but not hydrophilic statins were found to significantly reduce the risk of breast cancer in Thai women [107]. Conversely, other studies, including a large Mendelian randomization study, failed to find a protective effect of statins against breast cancer risk [108–113], or they even

found a positive association between long-term use of statins and increased risk of breast cancer [114]. In contrast, treatment with statins seems to have a more important effect in protecting against breast cancer recurrence and death [115–122]. Considering the type of statin, lipophilic statins were mainly found to be associated with a reduced risk of breast cancer recurrence or mortality [123–125], although hydrophilic statin use was also found to be associated with improved progression-free survival compared with no statin use in inflammatory breast cancer patients [126]. Taken together, HMGCR inhibitors do not seem to protect against breast cancer development, but statins, and more concretely, lipophilic statins, could be a good strategy for protecting against breast cancer recurrence and death.

Statins also exert antiproliferative and cytotoxic effects on breast cancer cells in vitro by increasing apoptosis, autophagy, and cell cycle arrest [127,128]. However, only lipophilic statins show anticancer activity [129], and the ER-negative phenotype seems to be more sensitive than those that overexpress ER [129,130]. ER-positive cell resistance to statin treatment is associated with high expression of cholesterol biosynthesis genes [131].

In vivo studies have also reported controversial results. Atorvastatin was able to reduce the level of circulating cholesterol, and it attenuated enhanced tumor growth and lung metastasis associated with an HFHC diet in a transgenic model in which the murine *Apoe* gene was replaced with the human *APOE3* allele and injected with ER-positive E0771 murine mammary cancer cells [45,53]. Moreover, simvastatin and fluvastatin treatments were found to inhibit tumor growth in mice inoculated with breast cancer cells [129,132], and fluvastatin was also found to reduce the metastatic burden in a murine breast cancer metastasis model [133]. The mechanisms of action of simvastatin included the inhibition of NF-κB transcription factor, which attenuated expression of antiapoptotic Bcl_{XL} and derepressed expression of the antiproliferative/proapoptotic tumor suppressor PTEN, which reduced the phosphorylation of Akt, resulting in decreased cancer cell proliferation and survival [132]. In contrast, statin treatment failed to reduce plasma cholesterol levels or tumor growth in mice injected with breast cancer cells on an HFHC diet [46] or other models of breast cancer in mice and rats [134]. An explanation for these negative results could be that mice are generally unresponsive to statins [135].

Finally, an interesting study investigated the biological effect of short-term lipophilic fluvastatin exposure on in situ and invasive breast cancer through paired tissue, blood, and imaging-based biomarkers in women with a diagnosis of ductal carcinoma in situ or stage 1 breast cancer. Fluvastatin exposure showed reduced tumor proliferation and increased apoptotic activity in high-grade breast cancer, concomitant with a reduction of cholesterol levels [136]. An upregulation of HMGCR was observed in breast cancer patients after two weeks of atorvastatin treatment, which was interpreted as activation of the negative feedback loop controlling cholesterol synthesis. Moreover, in tumors expressing HMGCR before treatment with atorvastatin, the proliferation marker Ki67 was found to be downregulated. In summary, these results suggested that HMGCR was targeted by statins in breast cancer cells in vivo, and that statins could have antiproliferative effects, mostly in HMGCR-positive breast cancers [137]. Importantly, atorvastatin was also found to decrease serum 27-HC and CYP27A1 expression in tumors of breast cancer patients [138].

Table 2. Clinical and epidemiological studies linking statin treatment to breast cancer risk.

Reference	Year	Study design	Participants	Main findings
Ference et al. [113]	2019	Mendelian randomization	654,783	Genetic inhibition of *HMGCR* did not affect breast cancer risk.
Islam et al. [109]	2017	Meta-analysis	121,399	There was no association between statin use and breast cancer risk.
Liu et al. [123]	2017	Meta-analysis	197,048	Significant protective effects of lipophilic statin use, but not hydrophilic statins, against cancer-specific mortality (HR = 0.57 (0.46–0.70)).
Mansourian et al. [116]	2016	Meta-analysis	124,669	Significant reduction in breast cancer recurrence (OR = 0.792 (0.735–0.853)) and death (OR = 0.849 (0.827–0.870)) among statin users.
Manthravadi et al. [124]	2016	Meta-analysis	75,684	Lipophilic statin use was associated with improved recurrence-free survival (HR = 0.72 (0.59–0.89)).
Wu et al. [119]	2015	Meta-analysis	144,830	There was a significantly negative association between prediagnosis statin use and breast cancer mortality (for overall survival: HR = 0.68 (0.54–0.84), and for disease-specific survival (HR = 0.72 (0.53–0.99)). There was also a significant inverse association between postdiagnosis statin use and breast cancer disease-specific survival (HR = 0.65 (0.43–0.98)).No significant association was detected between statin use and breast cancer risk.
Undela et al. [111]	2012	Meta-analysis	>2.4 million	Statin use and long-term statin use did not significantly affect breast cancer risk.
Bonovas et al. [108]	2005	Meta-analysis	327,238	Statin use did not significantly affect breast cancer risk.
Dale et al. [112]	2005	Meta-analysis	86,936	Statins did not reduce the incidence of breast cancer.
Borgquist et al. [115]	2017	Prospective	8010	Initiation of cholesterol-lowering medication in postmenopausal women with early stage, hormone receptor-positive invasive breast cancer during endocrine therapy was related to improved disease-free survival (HR = 0.79 (0.66–0.95)), breast cancer-free interval (HR = 0.76 (0.60–0.97)), and distant recurrence-free interval (HR = 0.74 (0.56–0.97)).
Murtola et al. [122]	2014	Prospective	31,236	Both postdiagnostic and prediagnostic statin uses were associatedwith a lowered risk of breast cancer death (HR = 0.46 (0.38–0.55) and HR = 0.54 (0.44–0.67), respectively).
Brewer et al. [126]	2013	Prospective	723	Hydrophilic statins were associated with significantly improved progression-free survival compared with no statin (HR = 0.49 (0.28–0.84)) in inflammatory breast cancer patients.

Ahern et al. [125]	2011	Prospective	18,769	Significant reduction in breast cancer recurrence among patients using simvastatin after 10 y of follow up (adjusted HR = 0.70 (0.57–0.86)).
Cauley et al. [106]	2003	Prospective	7528	Older women who used statins had a reduced risk of breast cancer (RR = 0.28 (0.09–0.86), adjusted for age and body weight) compared with nonusers.
Shaitelman et al. [120]	2017	Retrospective	869	Statin use was significantly associated with overall survival (HR = 0.10 (0.01–0.76)) in triple-negative breast cancer.
Smith et al. [121]	2017	Retrospective	6314	Prediagnostic statin use was associated with breast cancer-specific mortality (HR = 0.81 (0.68–0.96)). This reduction was greatest in statin users with ER-positive tumors (HR = 0.69 (0.55–0.85)).
Anothaisintawee et al. [107]	2016	Retrospective	15,718	Using lipophilic statins, but not hydrophilic statins, could significantly reduce the risk of breast cancer (risk difference = –0.0034 (–0.006,–0.001) lipophilic statin users vs. nonusers).
Mc Menamin et al. [139]	2016	Retrospective	15,140	There was no evidence of an association between statin use and breast cancer-specific death.
Sakellaki et al. [117]	2016	Retrospective	610	Statins may be linked to a favorable outcome in early breast cancer patients, especially in younger age groups (HR = 0.58 (0.36–0.94)).
Chae et al. [118]	2011	Retrospective	703	Significant reduction in breast cancer recurrence among patients who used statins (HR = 0.43 (0.26–0.70)). No association was found regarding overall survival.
Schairer et al. [110]	2018	Case–control	Total: 228,973 Cases: 30,004 Controls: 198,969	Statin use did not significantly affect breast cancer risk.
McDougall et al. [114]	2013	Case–control	Total: 2886 Cases: 916 IDC + 1068 ILC Controls: 902	Current users of statins for ≥10 y had increased risk of IDC (OR = 1.83 (1.14–2.93)) and ILC (OR = 1.97 (1.25–3.12)) compared with never users of statins.

OR = odds ratio, RR = risk ratio, HR = hazard ratio, y = years, IDC = invasive ductal carcinoma; and ILC = invasive lobular carcinoma. Between brackets, 95% confidence interval.

7.2. Ezetimibe

Ezetimibe is a drug that specifically targets intestinal Niemann-Pick C1-Like 1 (NPC1L1) and mediates the inhibition of intestinal sterol absorption [140]. Few studies have explored the effects of ezetimibe on breast cancer. However, considering that statins may have little effect on plasma cholesterol in mice [135], ezetimibe's action on breast cancer development is of interest. A study by Pelton et al. investigated the effects of ezetimibe administered in an HFHC diet on breast cancer development in an orthotopic breast tumor model, in which mice were implanted with MDA-MB-231 cells. Ezetimibe was able to reduce tumor volume, proliferation, and angiogenesis and increase

apoptosis compared with the HFHC-fed mice, achieving similar results to those in mice fed a low-fat/low cholesterol (LFLC) diet. These results were accompanied with a reduction in circulating cholesterol levels, but intratumoral cholesterol levels remained unchanged [40].

To our knowledge, the effects of ezetimibe treatment on breast cancer risk or mortality have not been studied. Only Kobberø Lauridsen et al. explored the effects of genetic variants of *NPC1L1* (–133A>G and V1296V T>C), mimicking treatment with ezetimibe, on breast cancer risk. These researchers found that *NPC1L1* variants were not associated with the risk of breast cancer [141].

7.3. Phytosterols

Plant sterols, or phytosterols, lower serum LDL-C levels by reducing intestinal cholesterol absorption [142]. Several in vivo studies have tested the efficacy of dietary phytosterol in breast cancer development. Female severe combined immunodeficiency (SCID) mice supplemented with 2% phytosterols and injected with MDA-MB-231 cells exhibited a reduction in serum cholesterol, accompanied with a reduction in tumor size and metastasis to lymph nodes and lungs [143]. In ovariectomized athymic mice injected with MCF-7 cells, supplementation with β-sitosterol, the most common phytosterol, was also able to reduce tumor size [144]. Furthermore, phytosterol supplementation could decrease both the development of mammary hyperplastic lesions and tumor burden in PyMT mice fed an HFHC diet. This protective effect was not observed in mice fed an LFLC diet. A potential mechanism of action of phytosterol was the prevention of lipoprotein oxidation [145].

Numerous experimental in vitro studies showed that phytosterols functioned as anticancer compounds acting on host systems to affect tumor surveillance or on tumors to affect tumor cell biology. Mechanisms affecting the tumors include slowing of cell cycle progression, induction of apoptosis, inhibition of tumor metastasis, altered signal transduction, and activation of angiogenesis. Host influences comprise enhancing immune recognition of cancer, influencing hormonal-dependent growth of endocrine tumors, and altering cholesterol metabolism (reviewed in [146,147]).

7.4. Other Therapies

Fibrates are agonists of the peroxisome proliferator-activated receptor α (PPARα), which stimulate the expression of genes involved in fatty acid and lipoprotein metabolism, resulting in a shift from hepatic fat synthesis to fat oxidation. Fibrates are used as therapeutic agents for treating dyslipidemia [148]. A meta-analysis of 17 long-term, randomized, placebo-controlled trials found that fibrates had a neutral effect on breast cancer and other cancer outcomes [149].

The levels of HDL-C and apoA-I are inversely related to cardiovascular risk [150]. The beneficial effects of HDL have largely been attributed to apoA-I, and researchers have sought apoA-I mimetic peptides as therapeutic agents based on physical–chemical and biological properties [151]. To our knowledge, a study from our group was the only one to analyze the effects of apoA-I mimetics on breast cancer. In that study, the apoA-I mimetic peptide D-4F was administered to PyMT female mice, and the treatment significantly increased tumor latency and inhibited the development of tumors. D-4F was unable to reduce the levels of 27-HC in the tumors, but it decreased the plasma levels of oxLDL and prevented the oxLDL-mediated proliferative response in MCF-7 cells, suggesting that D-4F inhibited breast cancer by protecting against LDL oxidative modifications [95].

8. Concluding Remarks

Results of some large clinical studies indicate a direct association for LDL-C and an inverse association for HDL-C and breast cancer risk; however, these findings have not been reproduced in all epidemiological studies and are still debated. Basic research studies have determined the important role of cholesterol, especially the 27-HC metabolite, and its transporters in breast cancer development. Both LDL and HDL, and their modified forms (oxLDL and oxidized and glycated HDL), may promote breast cancer via several mechanisms. Investigations in breast cancer cells and experimental models in vivo have demonstrated an interplay among modified lipoproteins,

proinflammatory signaling pathways, and breast cancer tumorigenic processes (summarized in Figure 2). Cholesterol can be esterified or metabolized to 27-HC, which has been hypothesized to be responsible for stimulating the proliferation of ER-positive breast cancer cells rather than cholesterol (Figure 2). Oxidized LDL as well as oxidized and glycated HDL induce different OLR1 and SR-BI downstream inflammation-related pathways, thereby inhibiting apoptosis and enhancing cell proliferation and migration. Therefore, considering the important role of cholesterol in breast cancer development, cholesterol-lowering drugs and apoA-I mimetics, which possess antioxidant and anti-inflammatory properties, could emerge as potential therapies for preventing the deleterious effects of high cholesterol in breast cancer. Lipophilic statins seem a good strategy for protecting against breast cancer recurrence and death. However, more studies in humans are necessary to evaluate the role of other therapies, such as ezetimibe, phytosterols or fibrates, on breast cancer risk and prognosis.

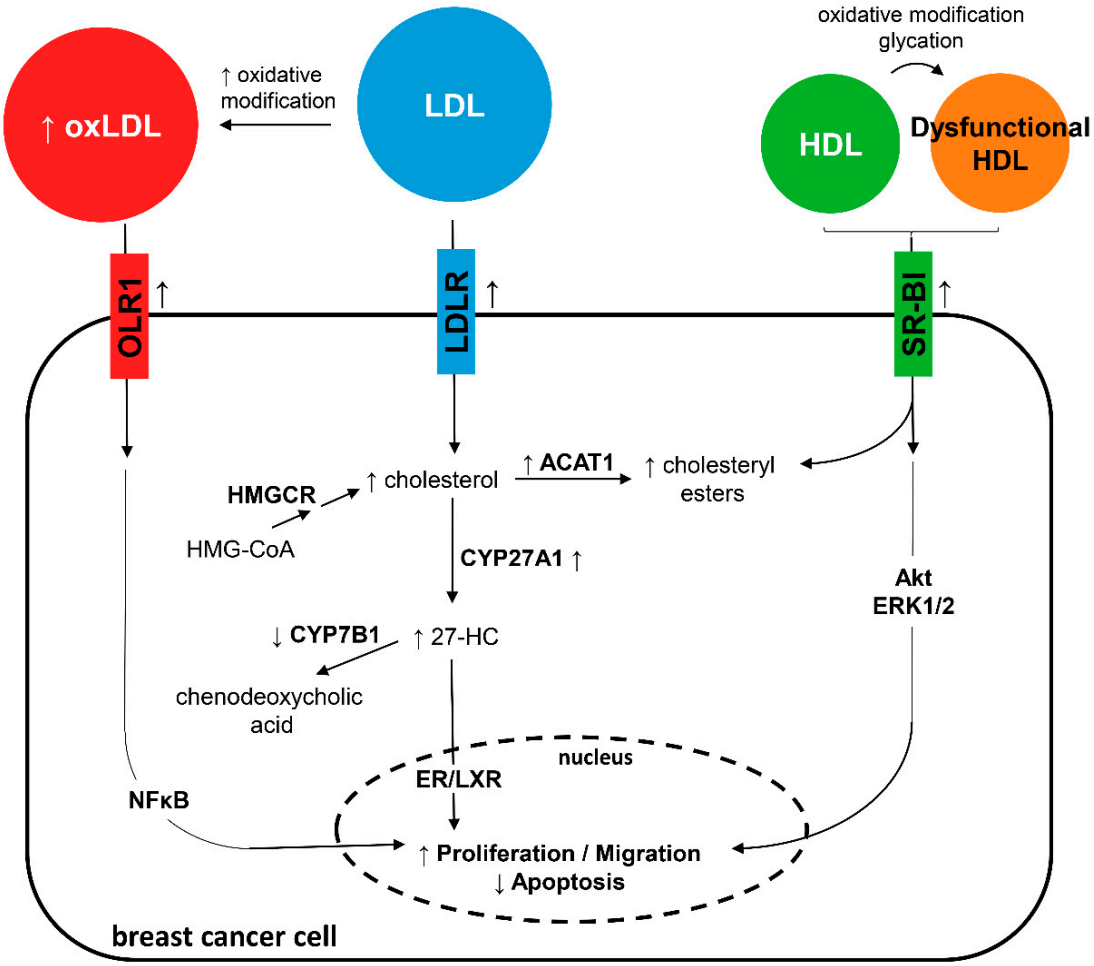

Figure 2. Mechanisms by which low-density lipoprotein (LDL), high-density lipoprotein (HDL), and their modified forms induce proliferation and migration and reduce apoptosis in breast cancer cells. OLR1 = OxLDL lecithin-like receptor 1, LDLR = LDL receptor, SR-BI = scavenger receptor class B type I, HMGCR = hydroxy-methyl-glutaryl-coenzyme A reductase, ACAT1 = acyl-CoA:cholesterol acyltransferase 1, 27-HC = 27-hydroxycholesterol, ERK1/2 = extracellular signal-regulated kinases ½, NFκB = nuclear factor κB, and ER/LXR = estrogen receptor/liver X receptor.

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COMPREHENSIVE REVIEW

Mediterranean Diet and cancer risk: an open issue

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ABSTRACT

The traditional Mediterranean Diet of the early 1960s meets the characteristics of an anticancer diet defined by the World Cancer Research Fund/American Institute for Cancer Research (WCRF/AIRC). A diet rich of whole grains, pulses, vegetables and fruits, limited in high-calorie foods (foods high in sugar or fat), red meat and foods high in salt, without sugary drinks and processed meat is recommended by the WCRF/AIRC experts to reduce the risk of cancer. The aim of this review was to examine whether Mediterranean Diet is protective or not against cancer risk. Three meta-analyses of cohort studies reported that a high adherence to the Mediterranean Diet significantly reduces the risk of cancer incidence and/or mortality. Nevertheless, the Mediterranean dietary pattern defined in the studies' part of the meta-analyses has qualitative and/or quantitative differences compared to the Mediterranean Diet of the early 1960s. Therefore, the protective role of the Mediterranean Diet against cancer has not definitely been established. In epidemiological studies, a universal definition of the Mediterranean Diet, possibly the traditional Mediterranean Diet of the early 1960s, could be useful to understand the role of this dietary pattern in cancer prevention.

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Introduction

In February 2015, the World Health Organization (WHO) affirmed that cancers figure among the leading causes of morbidity and mortality worldwide (https://who.int 2015). As previously pointed out, the increasing cancer burden needs efficient prevention strategies to curb the disease (https://med.unsw.edu.au 2014).

Only 5–10% of all cancer cases can be attributed to genetic defects, whereas the remaining 90–95% have their origins in the environment and lifestyle. In the US, diet contributes approximately 30–35% of all cancer-related deaths, while tobacco, infections, obesity and others factors contribute approximately 25–30%, 15–20%, 10–20% and 10–15%, respectively (Anand et al. 2008).

The most complete review of the evidence on diet and cancer is the 2007 WCRF/AIRC report (WCRF/AIRC 2007) that has been updated by the Continuous Update Project (www.wcrf.org). A set of 10 recommendations, both at personal and public health levels, was established by the experts of WCRF/AIRC in 2007 for cancer prevention. The recommendations regarding the diet emphasize the consumption of foods of plant origin such as non-starchy vegetables, fruits, unprocessed cereals and legumes in every meal; limit the intake of refined starchy vegetables, red meat, alcoholic drinks and energy dense foods; avoid processed meat and sugary drinks (WCRF/AIRC 2007).

The aim of this review was to examine whether Mediterranean Diet is protective or not against cancer risk.

Mediterranean Diet and cancer risk

The traditional Mediterranean Diet term was initially referred to dietary patterns of Mediterranean regions such as Crete, part of the rest of Greece and Southern Italy in the early 1960s (Fidanza 2001). It was characterized by a high consumption of plant foods (mostly whole grains products; vegetables; fruits including nuts; and legumes); a low to moderate amount of fish; a low intake of eggs, meat and full-fatty dairy. Refined carbohydrates were present in small quantities. Wine was drunk during the meals, in low or moderate amount. Olive oil was the only added fat in the diet (Fidanza et al. 2004, 2005). As a result, this dietary pattern was low in saturated fatty acids (SFAs) ($\leq$7–8% of energy) with total fat ranging from $<$25% to $>$35% of energy throughout the Mediterranean region (Willett et al. 1995). Habitual levels of physical activity were high

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(Willett et al. 1995). This dietary pattern has been associated with relatively low rates of coronary heart disease, cardiovascular disease and cancer (Keys et al. 1986; Menotti et al. 1999). It shares many characteristics with an anticancer diet that experts have defined (WCRF/AIRC 1997; Norat et al. 2015).

There is good epidemiological evidence that adherence to Mediterranean Diet has a protective effect against various cancers. Since the 2000s, several cohort studies have analyzed the relationship between adherence to the Mediterranean Diet and cancer risk and in the last three years, three meta-analyses were published. The first showed that a two-point increase in adherence to Mediterranean Diet was reported to determine a 4% reduction of neoplastic diseases in terms of incidence and/or mortality risk [summary relative risk (RR): 0.96; 95% CI: 0.95, 0.97] (Sofi et al. 2014). The second meta-analysis showed that the highest adherence to the Mediterranean Diet resulted in a significantly risk reduction for overall cancer mortality and/or incidence (summary RR: 0.90; 95% CI: 0.86, 0.95; $p < 0.0001$; $I^2 = 55\%$) (Schwingshackl & Hoffmann 2014a). The third meta-analysis reported that the highest adherence to the Mediterranean Diet was significantly associated with a lower risk of cancer mortality (summary RR: 0.87; CI: 0.81–0.93; $I^2 = 84\%$) (Schwingshackl & Hoffmann 2015).

Adherence to Mediterranean Diet

In the studies included in the three meta-analyses the adherence to the Mediterranean Diet was established by a priori indexes with one exception that used a factor analysis to derive a dietary pattern resembling the characteristics of the Mediterranean Diet (Menotti et al. 2012). The most used a priori indexes are those by Trichopoulou (t-MED) (Trichopoulou et al. 2003), the modified Mediterranean Diet index (m-MED) (Trichopoulou et al. 2005), the alternate Mediterranean Diet index (a-MED) (Fung et al. 2006) and the relative Mediterranean Diet index (r-Med) (Buckland et al. 2010). In the first three indexes, the medians of the intakes, which were gender specific, were used as cutoff points.

The t-MED has nine components. The subjects whose consumption of healthy components presumed to fit the Mediterranean Diet (cereals, legumes, vegetables and potatoes, fruit and nuts, fish) was below the median, were assigned a value of 0, while subjects whose consumption was at or above the median were assigned a value of 1. The score was inverted for components considered unhealthy and that did not fit the Mediterranean Diet (meat and meat products, dairy products). For alcohol intake a value of 1 was assigned to moderate consumption (from 10 to 50 g/day for men and from 5 to 25 g/day for women) and a value of 0 otherwise. For lipid intake a value of 1 was assigned to subjects with monounsaturated (MUFAs)/SFAs ratio at or above the median (Trichopoulou et al. 2003).

The m-MED was a modified t-Med in which the MUFAs + polyunsaturated (PUFAs)/SFA ratio was used as lipid intake (Trichopoulou et al. 2005).

The a-MED derived by t-MED excluded potatoes from vegetables, included whole grains products only, separated fruits from nuts, excluded dairy products, included red and processed meats only in the meat groups and considered 1 point for alcohol intake between 5 and 15 g/day. For lipid intake a value of 1 was assigned to subjects with MUFAs/SFAs ratio at or above the median (Fung et al. 2006).

The r-MED derived from the t-MED and consisted of nine components. Among these, six components were considered typical of Mediterranean Diet: fruit (including nuts and seeds but excluding fruit juices); vegetables (excluding potatoes); legumes; cereals (whole and refined grains); fresh fish and sea foods; and olive oil. Two components were considered not typical of the Mediterranean Diet: meat and meat products; and dairy products (including low-fat and high-fat milk, yogurt, cheese, cream desserts, and dairy and nondairy creams). Each component, except alcohol, was measured as grams per 1000 kcal/day and was divided into tertiles of dietary intakes. A value of 0, 1 and 2 to the first, second and third tertiles of intake, respectively, were assigned to typical components. Non-typical components were assigned with an inverted score. For alcohol, 2 points was assigned for an intake from 5 to 25 g per day for women and from 10 to 50 g/day for men. Intakes above or below these ranges were scored 0 points (Buckland et al. 2010).

The adapted relative Mediterranean Diet index (ar-MED) was derived from r-MED but excluded alcohol (Buckland et al. 2012).

The score by Bamia derived from m-MED but considered the origin of MUFAs. If they derived from meat the subjects were penalized by having 0 in the component of meat intake of the score (Bamia et al. 2013).

The score by Knoops was a t-MED modified because it did not consider alcohol (Knoops et al. 2004).

The Italian Mediterranean Index consisted of 11 components: six typical Mediterranean foods (pasta; typical Mediterranean vegetables; fruit; legumes; olive oil; and fish), four non-Mediterranean foods (soft drinks; butter; red meat; and potatoes) and alcohol.

Table 1. Studies that evaluated the adherence to Mediterranean Diet by a priori indexes in three meta-analyses.

Author, year	Country/cohort	Score	Score range	Number	Outcome
Benetou et al. 2008	Gr/EPIC	t-MED	0–9	25,623	Cancer incidence
Cuenca-García et al. 2014	US/ACLS	t-MED	0–9	12,449	Cancer mortality
Lagiou et al. 2006	Swe	t-MED	0–9	42,237	Cancer mortality
Martínez-González et al. 2012	Spa/SUN	t-MED	0–9	15,535	Cancer mortality
Couto et al. 2011	Eu/EPIC	m-MED	0–9	478,478	Cancer incidence
Couto et al. 2013	Swe/SWLHC	m-MED	0–9	42,258	Breast cancer risk
Bosire et al. 2013	US/NIH-AARP	a-MED	0–9	293,464	Prostate cancer risk
Fung et al. 2006	US/NHS	a-MED	0–9	71,058	Breast cancer risk
George et al. 2014	US/WHIOS	a-MED	0–9	63,805	Cancer mortality
Harmon et al 2015	US/Multiethnic Cohort	a-MED	0–9	215,782	Cancer mortality
Lopez-Garcia et al. 2014	US/HPFS-NHS	a-MED	0–9	17,415	Cancer mortality
Mitrou et al. 2007	US/NIH-AARP	a-MED	0–9	320,296	Cancer mortality
Reedy et al. 2014	US/NIH-AARP	a-MED	0–9	493,823	Cancer mortality
Buckland et al. 2010	Eu/EPIC	r-MED	0–18	485,044	Gastric adenocarcinoma risk
Buckland et al. 2011	Spa/EPIC	r-MED	0–18	40,622	Cancer mortality
Buckland et al. 2013	Eu/EPIC	ar-MED	0–16	335,062	Breast cancer risk
Bamia et al. 2013	Eu/EPIC	score by Bamia	0–9	397,641	Colorectal cancer risk
Knoops et al. 2004	Eu/HALE	score by Knoops	0–8	2339	Cancer mortality
Agnoli et al. 2013	I/EPIC	Italian Mediterranean Index	0–11	45,275	Colorectal cancer risk
Tognon et al. 2012	Swe/VIP	score by Tognon	0–8	77,151	Cancer mortality
Vormund et al. 2015	Sui/MONICA-NRP 1A	score by Vormund	0–9	17,861	Cancer mortality

EPIC: European Prospective Investigation into Cancer and nutrition; ACLS: Aerobics Center Longitudinal Study; SUN: Seguimiento Universidad de Navarra; SWLHC: Swedish Women's Lifestyle and Health Cohort; NIH-AARP: National Institutes of Health-AARP Diet and Health Study; NHS: Nurses' Health Study; WHIOS: Women's Health Initiative Observational Study; HPFS: Health Professionals Follow-up Study; HALE: Healthy Ageing: a Longitudinal study in Europe; VIP: Västerbotten Intervention Program; MONICA-NRP 1A: MONItoring of trends and determinants in CArdiovascular disease-National Research Program 1A; t-MED: Trichoupoulou Mediterranean Diet index; m-MED: modified Mediterranean Diet index; a-MED: alternate Mediterranean Diet index; r-MED: relative Mediterranean Diet index; ar-MED: adapted relative Mediterranean Diet index.

People whose consumption of typical Mediterranean foods were in the third tertile of distribution received 1 point whereas all others received 0 points. People whose consumption of non-Mediterranean foods was in the first tertile of the distribution, received 1 point and all others received 0 points. For alcohol, people whose consumption was up to 12 g per day received 1 point, whereas abstainers and people whose consumption was >12 g per day received 0 points. The theoretical score ranged from 0 to 11 (Agnoli et al. 2013).

The score by Tognon derived from m-MED considered vegetables and potatoes, fruit and juices, whole grain cereals only. One alternative index considered wine instead of alcohol (Tognon et al. 2012).

The score by Vormund consisted of nine components (salad, vegetables, fruits, dairy products, whole grains, meat, fish, MUFAs and wine). Whole grains, salad, vegetables, fruits, dairy products, white meat, fish and wine, were considered healthy and were assigned a value of 1 if they were preferred from the subject or 0 if they were not. Red and processed meat were considered unhealthy and received the value of 0 if they were preferred by the subject or 1 if they were not. MUFAs that included olive, groundnut or canola oil were given 0.5 score if they were preferred. The score was divided into three groups (Vormund et al. 2015).

The characteristics of the studies that evaluated the adherence to Mediterranean Diet by a priori indexes in three meta-analyses are shown in Table 1.

Preliminary conclusions

It is evident that the cutoff points established by the medians are different from a population to another so infinite types of Mediterranean Diets are possible. In all the studies the definition of Mediterranean Diet is etherogeneous as regards of the components of the diet and the medians of intake of food consumption. But we know the features of the Mediterranean Diet of the early 1960s and to follow a Mediterranean Diet it is not the same as eating some foods of this dietary pattern (Verberne et al. 2010). Besides, nowadays, we know that some anticancer qualities of olive oil and of red wine are linked to the polyphenolic compounds and that whole cereals and not refined cereals have anticancer properties. Only a few studies consider olive oil instead of MUFAs (Buckland et al. 2010, 2011, 2013; Agnoli et al. 2013), wine instead of alcohol (Tognon et al. 2012; Vormund et al. 2015), whole grains instead of cereals (Fung et al. 2006; Mitrou et al. 2007; Tognon et al. 2012; George et al. 2014; Lopez-Garcia et al. 2014; Reedy et al. 2014; Harmon et al. 2015; Vormund et al. 2015). Virgin olive oil, red wine and whole grains were typical of the Mediterranean Diet of the early 1960s.

Another important issue is that the medians used as cutoff points do not necessarily capture the level of consumption of a food that can be healthy or unhealthy.

Selected components of the Mediterranean Diet and cancer risk

Mediterranean foods

Whole cereals grains

Whole grains are cereals that contain the entire grain kernel (bran, germ and endosperm) in contrast with refined grains that contain the endosperm only. The benefit of whole cereal grains for cancer prevention is due to dietary fiber and bioactive compounds of germ and bran that are removed during the refining process (Fardet 2010).

In an Italian series of case-control studies, the high intake of whole grain foods consistently reduced the risk of neoplasms at several sites. The odds ratios (OR) for the highest category of intake of whole grain foods were 0.20–0.30 for upper digestive and respiratory tract neoplasms, 0.50 for stomach, colon and gallbladder, 0.70 for rectum, 0.60 for liver, 0.90 for breast, 0.60 for ovary, and 0.40 for bladder and kidney. The tests for trends in risks were significant for all these neoplasms (Chatenoud et al. 1998).

A systematic review of 40 case-control studies of 20 cancers and colon polyps suggested that eating whole grains $\geq$4 times/week reduced the chance of cancer by about 40% compared with never eating (summary OR 0.59; 95% CI: 0.51, 0.67; p for trend $<$0.0001) (Jacobs et al. 1998).

Colorectal cancer is the third most common type of cancer, after lung and breast cancer (Ferlay et al. 2015). A recent meta-analysis of six cohorts studies showed that an increment of three servings (90 g)/day of whole grains reduced the risk of colorectal cancer by 17% (summary RR: 0.83; 95% CI: 0.78, 0.89; $I^2 = 18\%$; $p_{\text{heterogeneity}} = 0.30$) (Aune et al. 2011).

There is a biological plausibility of the protective effect of whole grains consumption on risk of colorectal cancer. They are rich in dietary fiber that may act by increasing stool bulk and decreasing transit time and so the carcinogens contact with the colorectal mucosa (Lipkin et al. 1999). Fiber can bind secondary bile acids that have tumor-promoting capacities in animal experiments (Nagengast et al. 1995). Otherwise bacterial fermentation of fibre results in the production of short chain fatty acids, which may have protective effects against colorectal cancer (Slavin 2000). Recently, a new Mediterranean Diet pyramid has been proposed, dividing the whole grains that are placed at the base from the refined grains that are placed at the top (D'Alessandro & De Pergola 2014).

Olive oil

It was the only added fat in the Mediterranean Diet of the early 1960s. A systematic review of several studies conducted in southern Europe reported that the pooled RRs between extreme levels of olive oil consumption were 0.30–0.40 for upper aero-digestive tract cancers and a meta-analysis of several studies conducted in southern Europe showed a summary RR of breast cancer of 0.62 (95% CI; 0.44, 0.88) for the highest versus the lowest level of olive oil consumption (Pelucchi et al. 2011).

A contemporary meta-analysis of 19 observational studies involving 13,800 cancer patients and 23,340 controls reported that people in the group of highest olive oil consumption had a lower odds of having any type of cancer (logOR: −0.41; 95% CI, −0.53, −0.29), a lower odds of developing breast cancer (logOR: −0.45; 95% CI, −0.78, −0.12) and of developing a cancer of the digestive system (logOR: −0.36; 95% CI, −0.50, −0.21) compared with the group with the lowest intake. Heterogeneity of the effect-size measures was observed in studies performed in the Mediterranean region, whereas no heterogeneity was observed in non-Mediterranean regions (Psaltopoulou et al. 2011).

Otherwise, the olive oil intake was not associated with cancer mortality in the EPIC-Spain cohort (Buckland et al. 2012a).

The olive oil consumption in 62,284 postmenopausal women recruited from Mediterranean countries of the EPIC study (Spain, Italy and Greece) was not associated with the risk of breast cancer in multivariate analysis. However, there was a suggestion of a potentially beneficial effect in estrogen and progesterone receptor-negative tumors whereas no association was found between the intake of olive oil and estrogen and progesterone receptor-positive tumors (Buckland et al. 2012b).

Plausible protective mechanisms of anticancer properties of olive oil are linked to oleic acid, a MUFA that can regulate cancer-related oncogenes such as HER2 at various cancer cell lines, including breast, ovarian and stomach (Colomer & Menéndez 2006) and to phenolic compounds that characterize the extra virgin olive oil. The latter induce apoptotic cell deaths at various cancer cell lines (Cárdeno et al. 2013; Escrich et al. 2014), have anti-inflammatory effects and reduce DNA oxidative damage that is considered to be a crucial step in human carcinogenesis (Cárdeno et al. 2013; Cicerale et al. 2009). A limitation of the studies cited above (Buckland et al. 2012a,b) is that no information about specific types of olive oil such as extra virgin olive oil was available in data analysis.

Fruit and vegetables

Since the 1980s there was a growing scientific interest about the possibility that fruit and vegetables may reduce cancer risk. The Continuous Update Project, aimed at updating the work of 2007 WCRF/AIRC, has recently judged the role of non-starchy vegetables and fruits (containing carotenoids, beta-carotene or vitamin C) in decreasing the risk of mouth, pharynx, larynx, oesophagus and stomach and the role of fruits (containing carotenoids) in decreasing the risk of lung cancer as "probable" (www.wcrf.org 2015). On the other hand, the Continuous Update Project has reassessed the role of dietary fiber in colorectal cancer etiology from "probable" to "convincing" (Norat et al. 2014).

A higher consumption of fruit and vegetables was not substantially associated with the risk of cancer mortality in a recent meta-analysis of prospective cohort studies (Wang et al. 2014).

In a recent editorial, Key suggests, on the basis of available evidence, that increasing intake of fruits and vegetables is less important than obesity and alcohol consumption in reducing cancer rates in well-nourished people (Key 2011).

Plausible biological mechanisms for protective effects of fruit and vegetables on cancer risk are linked to vitamins, trace minerals, dietary fiber and other biologically active compounds that they contain. These phytochemicals have antioxidant activity and influence phase II conjugating enzymes detoxifying intermediate mutagens that can damage DNA, RNA, proteins and lipids (Lampe 1999).

Legumes

Broad beans, chickpeas, peas, lentils, pinto beans, black-eyed beans, white beans and white lupine beans are legumes usually used in the Mediterranean Diet (Kalogeropoulos et al. 2010). They are high in fiber, contain proteins and are low glycemic index foods (Curran 2012). Saponins, tannins, protease inhibitors and phytic acid found in pulses possess antioxidant and anti-carcinogenic effects, indicating that pulses may have significant anti-cancer effects (Mudryj et al. 2014). Phenolic compounds in pulses can have anti-cancer activity with several mechanisms of action, including modulation of detoxifying enzymes, induction of cell cycle arrest and apoptosis (Campos-Vega et al. 2010).

There is "limited" evidence that legumes, including soya and soya products, can reduce the risk of stomach and prostate cancers (WCRF/AIRC 2007).

Fish

The intake of fish was not very high in the cohorts of Greece and Nicotera (Southern Italy) of the Seven Countries Study. Fidanza reported that 1.6–2.0% of the daily caloric intake came from fish in Nicotera (Fidanza et al. 2004) whereas Kromhout reported an intake of 18 g/day in Crete and of 60 g/day in Corfu (Kromhout et al. 1989).

Fish consumption has been shown to have a protective effect for several cancers mainly those of the digestive tract in a series of case-control studies (Fernández et al. 2006). In large cohort studies no association with breast cancer (Engeset et al. 2006) and an inverse significant association with high intake of fish and colorectal cancer (Norat et al. 2005) were found. A systematic review of 106 prospective cohort and case-control studies investigated the relationship between the intake of fish and incidence of prostate, breast or colorectal cancers, but the results were unsubstantial (Sala-Vila & Calder 2011). However, concerning the n-3 long-chain (n-3 LC)-PUFAs from which the protective effects of fish intake depend, the study underlines some factors that may mask potential protective associations with fish consumption. Among these, a not clear definition of the type of fish that can be more or less rich in n-3 LC-PUFAs (fatty fish versus lean fish), the cutoff for the highest level of intake that may be not necessarily coherent with a protective effect in cancer prevention, the type of storage and cooking that can alter the n-3 LC-PUFAs content of fish is studied. Improved measures of dietary exposure or biomarkers to correlate self-report, were considered important for a better definition of the relationship between fish intake and these types of cancers (Sala-Vila & Calder 2011).

However, a following meta-analysis of cohort and case-control studies reported that fish consumption decreased the risk of colorectal cancer by 12% (summary OR: 0.88; 95% CI: 0.80, 0.95) with heterogeneity among case-control studies but not among cohort studies (Wu et al. 2012). A further systematic review of case-control and cohort studies reported no relation among fish intake and prostate cancer incidence (Lovegrove et al. 2015), and a meta-analysis of 21 cohort studies reported that marine n-3 LC PUFAs were associated with 15% reduction of risk of breast cancer (summary RR: 0.85; 95% CI: 0.76, 0.96; $I^2 = 67\%$ for highest versus lowest category) if they were measured as dietary intake and with a similar entity if they were measured as tissue biomarkers (summary RR: 0.86; 95% CI: 0.71, 1.03; $I^2 = 8\%$). Dose-response analysis indicated that the risk of breast

cancer was reduced by 5% per 0.1 g/day increment of marine n-3 LC-PUFAs (summary RR: 0.95, 95% CI: 0.95, 0.90; I^2 = 52%). No significant association was observed between fish intake and breast cancer risk (Zheng et al. 2013).

Several molecular mechanisms through which n-3 LC-PUFAs may modify the carcinogenic process have been proposed. Among these are, a suppression of arachidonic-acid derived eicosanoid biosynthesis that are linked to inflammation and carcinogenesis; influence on transcription factor activity (perixosome proliferator-activated receptors, nuclear transcription factor kB), gene expression and signal transduction pathways implied in cell growth and differentiation; improvement of insulin sensitivity (Larsson et al. 2004); inhibition of production of many important tumor angiogenic mediators (Spencer et al. 2009).

Red wine

Ethanol is classified by the International Agency for Research on Cancer (IARC) as a human carcinogen (WHO/IARC 2010) and the positive relationship between alcoholic drinks and some cancers (e.g. mouth, pharynx, larynx, oesophagus, colorectal in men, breast in pre- and post-menopause) was considered "convincing" by the experts of WCRF/AIRC and the evidence did not show any safe limit of intake (WCRF/AIRC 2007). The Continuous Update Project confirmed these conclusions and considered the role of alcohol in increasing the risk of liver cancer "convincing" and in increasing the risk of colorectum cancer "convincing" in man and "probable" in women (www.wcrf.org 2015). Reactive metabolites of alcohol, such as acetaldehyde, may be carcinogenic (WCRF/AIRC 2007).

In the Mediterranean Diet of the early 1960s, the use of moderate intake of red wine during the meals was habitual (Fidanza et al. 2004). Red wine contains a wide range of polyphenols that are a complex mixture of flavonoids (anthocyanins and flavan-3-ols) and non-flavonoids (resveratrol, cinnamates and gallic acid) (Arranz et al. 2012). White wine contains polyphenols in low amounts. Among the non-flavonoids, resveratrol can act as a chemo-preventive agent *in vitro*. Tumor initiation, promotion and progression are affected by resveratrol via multiple pathways (Kraft et al. 2009). In a controlled clinical trial on pre-menopausal women, red wine consumption in daily moderate amounts was associated with changes in serum hormones consistent with an aromatase inhibition preventing the conversion of androgens to estrogen (Shufelt et al. 2012) and this association was not observed in white wine consumption. In the Minnesota Breast Cancer Cohort Study, red, and not white, wine intake had an inverse association with percent breast density (a risk factor for breast cancer) after adjustment for other sources of alcohol (Vachon et al. 2000).

No relationship was evident between moderate red wine consumption and the risk of colorectal (Chao, Haque, Caan, et al. 2010) or prostate cancer (Chao, Haque, Van Den Eeden, et al. 2010) in cohort studies.

Clinical studies based on specific and accurate biomarkers of alcohol (ethylglucoronide or ethylene glycol) or wine consumption (polyphenolic metabolites) in urine, should be useful to better understand the relationship between wine consumption and cancer (Arranz et al. 2012).

Nuts

Only few studies have evaluated the relationship between nuts consumption and cancer risk. In addition, some of the study groups together nuts, pulses, legumes, and seeds making it difficult to evaluate a real effect of nuts (Papanastasopoulos & Stebbing 2013). The protective effect of nuts and seeds seems to be evident against digestive neoplasms. In 478,040 subjects from the EPIC cohort there was a 31% colon cancer risk reduction at the highest consumption of nuts and seeds (average intake 16 g/day) versus the lowest consumption (HR: 0.69; 95% CI: 0.50, 0.95; p for trend = 0.04) in women (Jenab et al. 2004). In a prospective study from Taiwan in 23,943 subjects, the consumption of ≥2 peanut products/week was associated with a 58% reduction of colorectal risk in women (RR: 0.42; 95% CI: 0.21, 0.84; p for trend = 0.01) in comparison with a lower consumption of 0–1 peanut products/week. No significant association was found in men (Yeh et al. 2006). A recent meta-analysis reported that the highest intake of nuts significantly decreased the risk of cancer deaths compared with the lowest category (summary RR: 0.86; 95% CI: 0.75, 0.98; I^2 = 16%), but no dose-effect was detected (Grosso et al. 2015).

Evidence of a protective effect of nuts against cancer risk came from *in vitro* studies and the colorectum is an organ in which the effect of nuts is biologically plausible (González & Salas-Salvadó 2006). Bioactive compounds in nuts such as ellagic acid, anacardic acid, genistein, resveratrol and phytic acid have been found to affect several cellular processes involved in cell survival, apoptosis, cell proliferation, cell invasion and angiogenesis (Falasca et al. 2014).

Herbs and spices

The use of several herbs and spices was very common in the traditional Mediterranean Diet of Nicotera in

the early 1960s. Garlic, onions, oregano, rosemary, chilli pepper and parsley were used to season dishes (Alberti 2006). Estimating herbs' and spices' intakes is very problematic because they are generally consumed in conjunction with other foods and in trace amounts (Kaefer & Milner 2008). Many of the phytochemicals in herbs and spices are potent biological agents that can influence cancer risk (Craig 1999; Kaefer & Milner 2011). Garlic retards cancer inhibiting the formation of nitrosamines that are potent carcinogens, and can also inhibit the formation of DNA adducts (Craig 1999; Kaefer & Milner 2011). A low mortality from stomach cancer was reported in some countries in relation to high intake of garlic or onions (Craig 1999). In a recent analysis of eight case-control studies, the use of garlic and onions was moderately and inversely associated with the risk of head and neck cancers (Galeone et al. 2015). Sulfides in garlic and onions and phthalides in umbelliferous herbs such as parsley inhibit tumor formation by stimulating glutathione transferase, a detoxifying phase II enzyme (Craig 1999). Basil promotes phase II enzymes and allows the conjugation and elimination of some carcinogens (Kaefer & Milner 2011). Rosemary extracts have been found to inhibit the proliferation of various human cancer cell lines, including lung, prostate, liver and breast (Kaefer & Milner 2011). Terpenoids in the Labiate family to which basil, fennel, mint, rosemary, oregano, sage and thyme belong to and Umbellifereae family to which anise, fennel, and parsley belong to were shown to suppress tumor growth by inhibiting HMG-CoA reductase in the cholesterol synthesis. Tumor cells synthesize and accumulate cholesterol faster than normal cells (Craig 1999).

Non-Mediterranean foods

Refined cereal grains

The intake of refined grains (bread, pasta, or rice) was significantly associated with an increased risk of upper digestive tract, stomach, colorectal, breast and thyroid cancers in an integrated series of case-control studies conducted in northern Italy (Chatenoud et al. 1999). More recently, the association of bread and pasta consumption with breast (in women) and colorectal (in men and women) cancers was assessed using data from two Italian case-control studies (Augustin et al. 2013). No significant associations were found in men for either bread or pasta. In women bread consumption was significantly and positively associated with breast and colorectal cancer risk; pasta consumption was positively and significantly associated with colorectal cancer risk (Augustin et al. 2013). Biological plausible mechanisms relatedly involve the glycemic index and estrogen metabolism. Carbohydrate foods with high glycemic index such as bread and rice (Atkinson et al. 2008) increase the postprandial blood glucose and insulin levels more than the same amount of carbohydrate foods with low glycemic index values. Higher glycemic index diets could stimulate insulin-like growth factor-I (IGF-I) receptors or reduce IGF-binding protein that results in mitogenic and anti-apoptotic effects on mammary and colorectal cell lines. IGF-I may also negatively regulate the synthesis of sex-hormone-binding globulin, increasing estrogen bioavailability and activating estrogen receptors. In turn, estrogen acts as a cell-proliferating hormone for mammalian cells (Augustin et al. 2013).

A meta-analysis of observational studies showed that high glycemic index and glycemic load diets can moderately increase the risk of the hormone-related (breast, endometrium, ovary, and prostate) and digestive-tract (esophagus, stomach, colorectal, liver, and pancreas) cancers. However the associations were not significant with the exception of that between dietary glycemic index and colorectal cancer (Turati et al. 2015).

Red and processed meat

In 2011 the Continuous Update Project confirmed the 2007 WCRF/AIRC report that there are convincing evidence that red and processed meat increase the risk of colorectum cancer (www.wcrf.org 2015). In a meta-analysis of prospective studies every 100 g/day of red meat increased the risk of colorectal cancer by 17% (summary RR: 1.17; 95% CI: 1.05, 1.31) and every 50 g/day of processed meat by 18% (summary RR:1.18; 95% CI: 1.10–1.28) (Chan et al. 2011).

Putative mechanisms by which red and processed meat contribute to cancer risk are linked to: 1. heme iron that is supposed to rise lipid peroxidation giving rise to DNA etheno-adducts, 2. heterocyclic amine formed at high temperature with important mutagenic and genotoxic action, 3. polycyclic aromatic hydrocarbons formed by strong heating of meat and fat with significant role in mutagenesis and carcinogenesis and 4. sodium nitrite (used as color fixer or as a preservative in meat) which forms with secondary amines from proteins, carcinogenic nitrosamines (Mosby et al. 2012). Processed fish also is a risk factor for colorectal cancer (Mosby et al. 2012).

Poultry

There is "limited" evidence for the relationship between poultry intake and cancer (WCRF/AIRC

2007). White meat (and poultry in particular) is considered moderately protective or neutral on cancer risk (Marangoni et al. 2015). However processing of poultry meat can result in the same risk factors that processing red meat (Mosby et al. 2012).

Milk and dairy foods

Milk and dairy foods are a heterogeneous group of foods and this makes evaluation of their association with cancer risk very difficult. In the traditional Mediterranean Diet of the early 1960s, they were whole milk, yogurt and cheese in small amounts.

Two recent meta-analysis of observational studies have recently confirmed the judgment of 2007 WCRF/AIRC report (WCRF/AIRC 2007) of an existing protective effect of milk for colon cancer. The summary RR for high versus low milk intake on colon cancer risk was 0.78 (95% CI: 0.67, 0.92) in a meta-analysis of 60 observational studies. Milk intake was not associated with rectal cancer risk (Huncharek et al. 2009). In a meta-analysis of cohort studies, the summary RR for a 200 g/day increase in the milk intake was 0.88 (95% CI: 0.79, 0.97; $I^2 = 44\%$; $p_{\text{heterogeneity}} = 0.11$) for colon cancer. The inverse association between milk intake (200 g/day) and rectum cancer was not statistically significant (Aune et al. 2012).

Among the plausible biological mechanisms of the protective effect of dairy towards colon cancer risk are the high calcium content which may bind pro-inflammatory secondary bile acids and ionized fatty acids and thus reduce their proliferative effects in the colonic epithelium. Calcium can influence multiple intracellular pathways involved in differentiation in normal cells and in apoptosis in transformed cells [revised in: (Aune et al. 2012)].

Eggs

A recent meta-analysis of prospective observational studies found that consuming $\geq$5 eggs/week was significantly associated with an increased risk of breast cancer compared with no egg consumption with the summary RR of 1.04 (95% CI: 1.01, 1.07) for consuming 5 eggs/week, and 1.09 (95% CI: 1.03, 1.15) for consuming about 9 eggs/week. The study provides only "limited" evidence to support a positive association between egg intake and risk of ovarian and fatal prostate cancer (Keum et al. 2015). One meta-analysis of observational studies reported a modest positive dose-response association of egg consumption and development of gastrointestinal neoplasm, in particular colon cancer (summary OR: 1.29; 95% CI: 1.14, 1.46; $p_{\text{heterogeneity}} < 0.22$) (Tse & Eslick 2014). One meta-analysis of observational studies reported no evidence of a significant influence of egg consumption on prostate cancer incidence and mortality (Xie & He 2012).

Biological plausibility of a possible role of egg consumption in the cancer risk involves: 1. an increase in secondary bile acids dietary fat-dependent, that damages colonic lumen epithelial cells and in turn promotes the proliferation of the colorectal epithelium and tumor formation; 2. a stimulation of cholecystokinin secretion induced by egg yolk, that increases the colonic exposure to carcinogenic bile acids; 3. high content in choline that is necessary in membrane production of growing cancer cells [revised in: (Tse & Eslick 2014)].

Pathogenetic mechanism in cancer development and Mediterranean Diet

There is emerging evidence of a strong association between obesity and an increased risk of cancer (Prieto-Hontoria et al. 2011) and the most significant diet-related risk factor for cancer development is obesity (Mosby et al. 2012). Obesity is associated with some cancer types: oesophageal adenocarcinoma, postmenopausal breast, endometrial, colorectal, kidney and prostate. The number of cancer cases caused by obesity is estimated to be 20%. Several mechanisms link excess body weight to cancer and four main systems have been identified as potential producer of cancer in obesity: insulin, IGF-I, sex steroids hormones and adipokines (De Pergola & Silvestris 2013; Simone et al. 2016). Hyperinsulinaemia characterizes the state of insulin resistance which is frequently seen in obesity. Insulin up-regulates hepatic production of IGF-I and both act as growth factors able to promote cancer cell proliferation and to decrease apoptosis (Yakar et al. 2005). In women obesity is associated with an increased serum concentration of estradiol and decreased serum concentration of testosterone. The increased levels of estradiol depend on a peripheral conversion of androgens to estradiol by an increased aromatase activity in the adipose tissue. Estrogens have proliferative effects on epithelial tissue of both breast and endometrium (Prieto-Hontoria et al. 2011). Obesity is associated to systemic low-grade inflammation that has an important role in the pathogenesis of insulin resistance, atherosclerosis and cancer. Tumor necrosis factor-α, interleukin-6, plasminogen activator inhibitor-1, visfatin and leptin have a role in cancer development (van Kruijsdijk et al. 2009; Prieto-Hontoria et al. 2011).

There is some evidence of a possible role of the Mediterranean Diet in preventing overweight/obesity because of the high fiber content, the low energy density of the components and increased postprandial fat oxidation of MUFAs of olive oil (Buckland et al. 2008). There is some evidence from meta-analysis of intervention trials that adherence to Mediterranean Diet significantly increases adiponectin levels and reduces high-sensitive C reactive protein, interleukin-6 levels, and intracellular adhesion molecule-1 in comparison with control diets (Schwingshackl & Hoffmann 2014b). Vegetable- and fruit-based or "healthy" patterns tended to be inversely associated with biomarkers of inflammation in a systematic review (Barbaresko et al. 2013).

Conclusions

Overall, there is epidemiological evidence that components of plant based food diet have a protective role in cancer prevention. With regards to the role of the Mediterranean Diet in cancer prevention, we think that the protective role of the Mediterranean Diet against cancer has not definitely been established. The adherence to the Mediterranean Diet in prospective studies included in the meta-analyses (Sofi et al. 2014; Schwingshackl & Hoffmann 2014a, 2015) evaluating the relationship between this dietary pattern and cancer risk, was established, with exception of one study (Menotti et al. 2012), through a priori indexes. Two aspects should be considered. First, the pattern defined as the Mediterranean Diet has some qualitative and/or quantitative divergences from the traditional Mediterranean Diet of the early 1960s. Whole grain cereals, extra virgin olive oil and red wine characterize this dietary pattern beyond other plant based food diets. Just a few studies considered only whole grains in the category of cereals (Fung et al. 2006; Mitrou et al. 2007; Tognon et al. 2012; George et al. 2014; Lopez-Garcia et al. 2014; Reedy et al. 2014; Harmon et al. 2015; Vormund et al. 2015), a few studies considered olive oil (Buckland et al. 2010; Buckland et al. 2011; Agnoli et al. 2013; Buckland et al. 2013) whereas in the studies carried out in non-Mediterranean countries MUFAs that can derive from meat were considered (Hoffman & Gerber 2013) with exception of one (Bamia et al. 2013). In all studies with exception of two (Tognon et al. 2012; Vormund et al. 2015) alcohol was considered instead of wine. With regards to the quantitative divergences, the use of median as a cutoff to score the intakes does not necessarily meet the true quantitative composition of the Mediterranean Diet (D'Alessandro & De Pergola 2015). Further epidemiological studies need to take into account a universal definition of the Mediterranean Diet, preferably that of the early 1960s, to evaluate the role of this dietary pattern in cancer prevention. The use of biomarkers could improve dietary exposure established through self-reporting. The plasma alkylresorcinols, urinary hydroxytyrosol and polyphenolic metabolites could be useful as biomarkers of whole grain wheat (Kyrø et al. 2014), extra virgin olive oil (Estruch et al. 2013) and, red wine intake (Arranz et al. 2012), respectively. However, a healthy diet, the control of obesity and the decrease in smoking constitute a well-established target in cancer prevention in Europe (Giacosa et al. 2012).

Disclosure statement

The authors declare no conflict of interest.

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World Cancer Research Fund

EAT WELL DURING CANCER

Helping you to cope with common side-effects of cancer and cancer treatment

Who is this booklet for?

This booklet is for people living with cancer and those having cancer treatment, who want to know more about how to cope with the common side-effects, but also want to follow as healthy a diet and lifestyle as possible.

This is a general guide and is not suitable for people who are eating very little, have lost a lot of weight unintentionally or are receiving palliative care, as they will need specialist information and advice.

If you have completed your treatment or feel able to eat normally, our website **wcrf-uk.org** has lots of useful information about healthy eating and being active that may be more suitable for you.

If you follow a special diet for another medical condition, such as heart disease, diabetes or renal failure, or have a colostomy or ileostomy, this booklet may not be suitable for you. Talk to your doctor or dietitian about safe changes to make to your diet.

If you are really struggling with eating and you're not already seeing a dietitian, then ask your doctor to refer you to one.

About us

World Cancer Research Fund is the leading UK charity dedicated to the prevention of cancer. Our mission is to champion the latest and most authoritative scientific research from around the world on cancer prevention and survival through diet, weight and physical activity so that we can help people make informed lifestyle choices to protect themselves against cancer.

The cornerstone of our research programme is our Continuous Update Project (CUP). It's the world's largest source of scientific research on cancer prevention and survival through diet, weight and physical activity. A panel of world-renowned independent experts review the scientific research to develop Cancer Prevention Recommendations based on the best evidence. Find out more: **wcrf-uk.org/our-research**

This booklet was written with the support of dietitians from the British Dietetic Association (BDA)'s Oncology Specialist Group to ensure the information is based on the most up-to-date scientific evidence and practical expert advice, and it has been endorsed by the BDA. Our nutritionists have also created recipes to help you put this advice into practice.

Document endorsed by BDA The Association of UK Dietitians

Dear reader,

Thank you for choosing this World Cancer Research Fund booklet. If you're living with cancer or having cancer treatment, you might not be able to eat and drink what you are used to, or as much. This can be difficult, especially if you've always enjoyed your food and now find mealtimes challenging.

On top of the symptoms caused by cancer itself, treatments such as chemotherapy, radiotherapy and surgery can cause side-effects that make it more difficult to eat normally and absorb what you need from food.

We wrote this booklet to help you cope with the common side-effects while also eating the most nutritious and healthy foods possible and keeping active.

Eating well generally means eating a diet rich in wholegrains, vegetables, fruit and pulses, with lean meat, fish and lower fat dairy. It also means limiting foods and drinks that are high in added sugar, salt or saturated fat such as butter, cream and cheese. This includes highly processed foods and drinks such as biscuits, crisps, fast foods, ice cream and sugar-sweetened drinks (like cola).

In line with our Cancer Prevention Recommendations, we also advise limiting the amount of red meat you eat, and avoiding processed meats (like ham and bacon) and alcoholic drinks.

If you're having problems eating, or if you're losing weight, you may need to make some changes to your diet for a while. Everyone is different, so not every piece of advice in this booklet will help everyone, but try experimenting to see what is helpful for you.

It is important to let your doctor or health professional know about any symptoms or side-effects you're experiencing. Alongside advice from your doctor, we hope the tips and recipes in this booklet will help you get the balance right between coping with the side-effects you're experiencing, feeling better in yourself, and eating well and enjoying your food.

I hope you find this booklet helpful.

Kind regards,

Dr Kate Allen
Executive Director,
Science and Public Affairs

Contents

Specialised recipes

Common questions answered

Do I need to follow a ‘fad’ diet?

‘Fad’ diets (diets that are very restrictive, include few foods or focus on unusual combinations of foods) that claim to help you fight cancer can seem very appealing and get a lot of media attention. However, there’s no scientific evidence that following any type of diet can cure cancer or replace cancer treatments. Also, following a ‘fad’ diet while you’re having treatment can have risks, such as not providing your body with all the nutrients it needs.

If I follow the recommendations in this booklet, do I still need to take my medication?

There is no scientific evidence that changes to your diet or lifestyle can cure cancer, so it is important to continue taking prescribed medication as instructed by your doctor. If you feel you no longer need a certain medication, do make sure you discuss this with your doctor before you stop taking it.

As with all medication, it is important to ask your doctor or a pharmacist, or to read the patient information, to check if it interacts with any foods or drinks.

What about supplements and natural remedies?

We advise people to get all their nutrients from their food and drink, where possible. If you aren’t able to eat as normal, your doctor or dietitian may prescribe supplements for you. It is important that you take these as suggested. However, if you are considering taking other supplements or homeopathic, natural or herbal remedies that haven’t been prescribed for you, it is important to discuss this with your doctor before you start taking them to check they are safe for you.

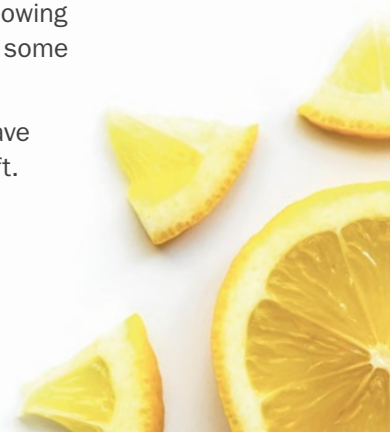
Loss of appetite

There are lots of reasons why you might lose your appetite when you have cancer. It could be the cancer itself, your treatment, or other side-effects like tiredness, feeling sick or taste changes that are making you feel less hungry. Feeling anxious can also play a part – worrying about your health can make it hard to think about food.

What can help?

Mealtimes

- Everyone is different, so try experimenting to see what you can tolerate. For some people, big meals might seem overwhelming, so try to eat little and often. You could try having five or six small meals or snacks a day, served on small plates as this may be less off-putting. If you find it difficult to eat this often, you could try swapping snacks for high-calorie (high-energy) drinks, like smoothies (**see pages 38–39 for our filling fruit smoothie recipe**).
- If you find drinks make you feel full, it might be best to avoid drinking at mealtimes to allow you to eat as much as possible.
- Try to make meal and snack times as relaxing as possible. This will depend on what works for you – perhaps a quiet room with no distraction makes you feel more comfortable, or you may prefer to have friends and family around you, or have some music on.
- It's best to try to sit upright while you're eating and to take your time with your meal – chewing and swallowing slowly. If you feel sick or full, you could try getting some fresh air and eating again later.
- Every little counts – be positive about what you have managed to eat rather than focusing on what's left.
- Make your food look as appealing as possible, for example by adding a garnish of herbs or wedge of lemon.



Choosing what to eat

- It can be hard to know what you actually want to eat when you don't have an appetite, so keep a variety of ready-to-eat snacks to hand (**see page 15 for some snack ideas**) or ask your family to prepare meals or foods that you liked in the past – these can be portioned up into small meals and frozen so they are quickly available when you feel like trying them.
- Eat what you fancy, when you fancy, and try to eat a little more when your appetite is at its best.
- Some people find strong flavours can help stimulate their appetite, such as foods and drinks that are spicy, sweet or bitter (such as chicory, black coffee and tonic water).

Looking after yourself

- If you smoke, try to cut down as much as possible, as smoking can reduce your appetite. Ask your doctor for support if you want to stop smoking.
- Gentle exercise, like going for a walk, could help to increase your appetite. **See pages 46–47 for more about the benefits of keeping active.**
- It can help to talk to family and friends about your change in appetite and let them know what will help you, whether that's being able to graze on food throughout the day or having quiet mealtimes.
- If you're feeling anxious or worried, try to get some support from your doctor, another health professional or someone else you trust. **See pages 49–50 for helpful contacts.**

If you're losing weight

Loss of appetite can mean that you start to lose weight. If this is happening to you, you can also **try the tips in the weight loss section on pages 10–15.**

If you've tried to make changes but keep losing weight or cannot put any weight back on, ask your dietitian or doctor for more support. They may be able to prescribe medication to help increase your appetite, as well as high-energy drinks (liquid food supplements) to add extra calories to what you are able to eat.

Weight loss

Losing weight is a common side-effect of cancer. Cancer itself can cause changes to your appetite (see pages 8–9) or to the way your body uses nutrients from food and drink. Cancer treatments can also make it harder for your body to absorb what it needs from your food, and make it more difficult for you to eat in the first place – all of which can lead to weight loss.

What can help?

The best way to slow down or stop weight loss is to make sure each mouthful you eat or drink gives you as many calories (energy) as possible.

When you have cancer, you may also lose muscle, so it's also important to choose high-protein foods that can help your body build muscle and repair tissue.

This section looks at how you can do this in the healthiest way possible by opting for foods that are not only high in calories and protein, but are also beneficial for long-term health.

Please note, if your weight loss is rapid or severe, you should prioritise slowing down or stopping weight loss. This may mean eating high-calorie foods that aren't generally considered to be healthy if they're what appeal to you or are all you can tolerate.

Which foods to choose?

Foods that are higher in fat have more calories per mouthful. However, it is still best to opt for foods containing healthier fats, such as oily fish, seeds and avocados. It is also best to avoid foods high in added sugar and salt, highly processed foods, such as biscuits, chocolate, fast foods like burgers, chips and fried chicken, and sugar-sweetened drinks like cola.

Guidance on...

Dairy foods

Dairy foods, such as milk, plain yoghurt and cheese, are a good source of calories, protein and important micronutrients like calcium, meaning they can contribute to a healthy diet. However, some dairy foods are particularly high in saturated fats, so to keep your intake of these less healthy fats down, it's best to avoid butter, cream and ice cream. Swapping butter for oil or oil-based spreads would help to reduce the amount of saturated fat you're eating while still keeping the amount of calories high.

It is important to note that if you are still losing weight, calories and protein intake should be your priority, so you may want to include some of these higher fat dairy foods in your diet for the time being.

Red and processed meat

Red meat, such as beef, pork and lamb, is a good source of protein and important micronutrients like iron so can contribute to a healthy diet. However, our research has shown that eating processed meat or too much red meat increases cancer risk. For this reason, we recommend eating moderate amounts of red meat and avoiding processed meat, such as ham, bacon, salami and hot dogs.

Fruit and veg

Fruit and vegetables are an important source of vitamins and minerals, which are good for our overall health. However, they tend to be quite low in calories and high in fibre, which means they can fill us up on few calories. It is important that you keep eating fruit and vegetables, but try to opt for higher calorie ones such as sweet potatoes, squashes, root vegetables (such as parsnips), avocados, peas, sweetcorn, bananas and dried fruit.

Have a look at our shopping list for the foods that we recommend >>

Your shopping list

Food	High-calorie	High-protein
Red meat, eg beef, pork, lamb – eat in moderation	✓	✓
Poultry, eg chicken, turkey		✓
Meat alternatives, eg tofu, soya		✓
Non-oily fish, eg cod, haddock		✓
Oily fish, eg salmon, mackerel	✓	✓
Eggs		✓
Pulses (beans and lentils)		✓
Houmous and tahini	✓	✓
Nuts, seeds and nut butter	✓	✓
Full-fat and evaporated milk	✓	✓
Skimmed and semi-skimmed milk		✓
Skimmed milk powder		✓
Soya milk		✓
Greek and natural yoghurt		✓
Cottage cheese		✓
Creme fraiche	✓	
Full-fat cheese	✓	✓
Avocado	✓	
Vegetable oils for cooking or salad dressings	✓	
Vegetable oil-based spreads	✓	

‘Boosting’ your food

Here are a few ideas for increasing the calories and protein in your everyday food.

If you’re having...

Puddings or breakfast cereals

Add...

- Nuts or seeds
- Dried fruit
- Banana
- Whole milk or fortified milk (see next page)
- Greek or natural yoghurt

If you’re having...

Sandwiches, toast or crackers

Add...

- A thick layer of cream cheese, cottage cheese, nut butter or houmous
- A filling of sliced avocado and tuna or chicken

If you’re having...

Vegetables, mashed potatoes, beans or sauces

Add...

- Oil
- Whole milk or fortified milk (see next page)
- Oil-based spread
- Cheese
- Egg (hard-boiled or added when making savoury sauces)

If you’re having...

Salads

Add...

- Avocado slices, nuts, seeds and pulses
- Oil-based dressings
- Oily fish, cooked lean meat or poultry
- Houmous
- New potatoes
- Hard-boiled egg
- A serving of bread (ideally wholemeal) with oil-based spread

If you’re having...

Casseroles, meat dishes or soups

Add...

- Lentils or beans
- Rice, noodles or pasta (ideally brown or wholewheat)
- More lean meat, fish or meat alternatives like tofu
- More oil when cooking
- Greek yoghurt or creme fraiche before serving
- A serving of bread (ideally wholemeal) or potatoes with oil-based spread

Mealtimes

- Try to eat little and often – you don't have to stick to three meals a day. You could have a snack or small meal every two hours or so, including one before bed.
- Keep high-calorie snacks and easy-to-prepare foods to hand at home and when you're out.
- Instead of steaming or baking your food, you could also increase the calories by cooking with oil, such as olive or rapeseed oil.
- To get as many calories as you can from every mouthful, try to avoid clear soups, eating lots of fruit and vegetables or having a large drink just before or during mealtimes. These can fill you up without giving you many calories.

High-calorie drinks

- **Fortified milk:** add 2–4 tablespoons of skimmed milk powder to a pint (570ml) of whole milk. Keep it in the fridge for up to two days and use in hot and cold drinks, smoothies, on cereal and for cooking. This adds calories and protein without adding much volume.
- **Smoothies:** make a smoothie by blending together fortified milk, yoghurt, fresh fruit – like banana, mango or berries – nuts, nut butter, seeds or avocado (**see pages 38–39 for our filling fruit smoothie recipe**).

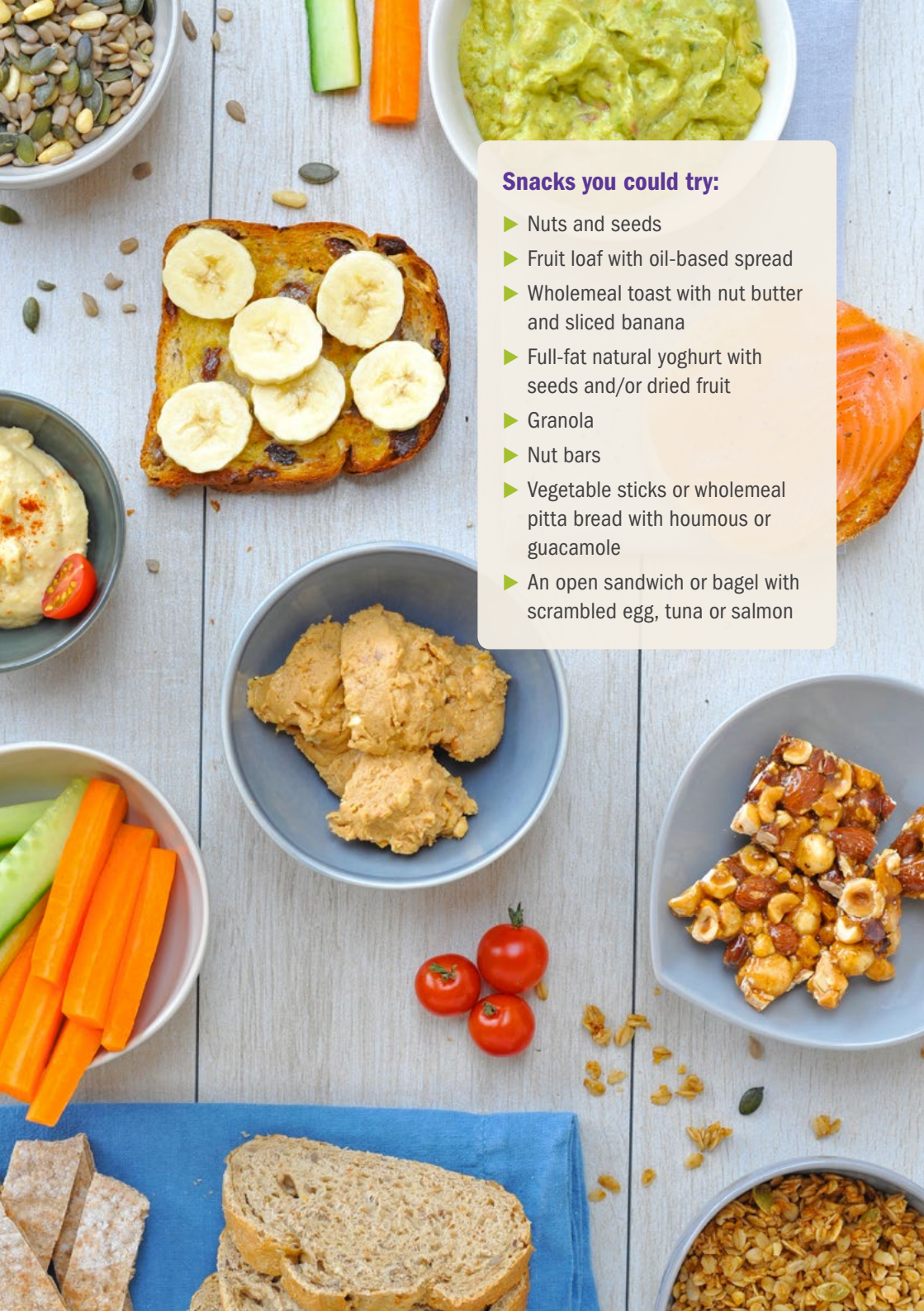
Keep track of your weight. If you've made changes to your diet but keep losing weight or cannot put on any weight, ask your dietitian or doctor for more support.

Keeping active

Even if you're losing weight, it's a good idea to keep active.

Being active, especially doing strengthening exercises, could help stimulate muscle growth and help prevent you losing muscle and strength.

See pages 46–47 for more information and ideas for keeping active.



Snacks you could try:

- Nuts and seeds
- Fruit loaf with oil-based spread
- Wholemeal toast with nut butter and sliced banana
- Full-fat natural yoghurt with seeds and/or dried fruit
- Granola
- Nut bars
- Vegetable sticks or wholemeal pitta bread with houmous or guacamole
- An open sandwich or bagel with scrambled egg, tuna or salmon


High in
calories and
protein

Salmon with a nut and seed crust

Ingredients

2 salmon fillets

2 medium potatoes, suitable for mashing, peeled and cubed

2 tbsp whole milk

1 tbsp rapeseed oil

160g frozen peas, cooked as instructed

Nut and seed crust

30g chopped hazelnuts

30g oats

10g pumpkin seeds

4 tsp rapeseed oil

Small handful fresh coriander

½ lime, zest only

Freshly ground black pepper, to taste

Method

1. Preheat the oven to 180°C/Fan 160°C.
2. Add all the ingredients for the crust to a blender, and pulse for about a minute.
3. Place the salmon, skin-side-down, on a foil-lined baking tray. Spoon half the crust on top of each fillet and gently pat it down.
4. Place the salmon in the oven and bake for 20 minutes.
5. While the salmon cooks, put the potatoes in a large saucepan and cover with cold water. Place over a high heat and bring to boil. Reduce the heat, cover and allow to simmer for about 15 minutes until cooked.
6. Remove the potatoes from the heat and drain carefully. Add the milk and oil, then mash until smooth.
7. Transfer the cooked salmon, mashed potato and cooked peas onto two serving plates and serve.

Nutrition information (per portion)

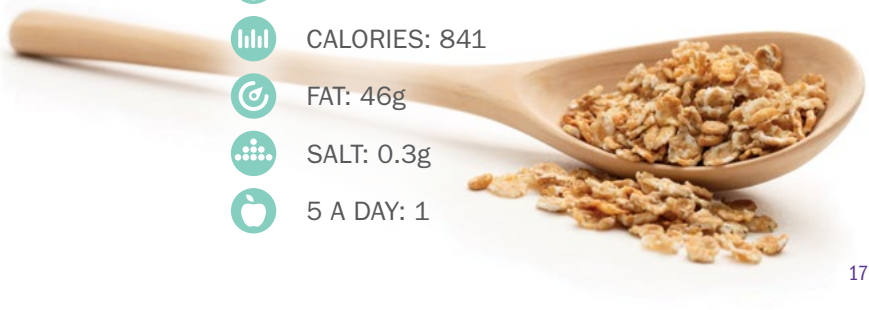
SERVES: 2

CALORIES: 841

FAT: 46g

SALT: 0.3g

5 A DAY: 1




High in calories and protein

Chicken fajitas

Ingredients

1 tbsp rapeseed oil

1 medium onion, sliced

1 pepper, deseeded and sliced

480g chicken breast, diced

4 wholemeal wraps

2 tbsp natural yoghurt

Guacamole

1–2 tsp lime juice

1 ripe avocado, peeled and diced

Small handful fresh coriander, finely chopped (optional)

Seasoning

1 level tbsp cornflour

½–1 tsp cayenne pepper

½ tsp cumin

½ tsp garlic granules

¼–½ tsp smoked paprika

¼ tsp cinnamon

Method

1. Add the lime juice and avocado to a bowl, then mash thoroughly. Stir in the coriander, cover and refrigerate until needed.
2. Warm the oil in a large, non-stick frying pan over a medium heat. Add the onion and pepper; and cook for 3–4 minutes until they start to soften but not brown.
3. Add the chicken to the pan and cook for a further 4–5 minutes until the chicken is browned on all sides.
4. Mix together all the seasoning ingredients, and sprinkle over the chicken and vegetables – you may not need to use all of the seasoning, but the rest will keep for another time. Stir for 2 minutes to cook the spices and ensure everything is coated in them.
5. Warm the wraps in the oven (about 100°C) for about 2–3 minutes (optional). Place each wrap on a plate and spoon a quarter of the chicken and vegetable mix onto each, followed by a quarter of the guacamole and half a tablespoon of yoghurt. Fold the wrap and enjoy.

Nutrition information (per portion)

SERVES: 4

CALORIES: 495

FAT: 18.5g

SALT: 1.2g

5 A DAY: 1.5

Banana and peanut butter flapjacks

Ingredients

- 3 ripe bananas
- 200g oats
- 50g dried fruit, chopped into small pieces if necessary
- 60g seeds
- 2 tbsp smooth peanut butter (ideally a brand that contains no added salt and sugar)
- 2 tbsp sunflower spread
- 1 tbsp honey
- 1 tsp cinnamon

Method

1. Preheat the oven to 180°C/Fan 160°C. Line a baking tray with baking parchment.
2. In a large bowl, mash the bananas into a smooth paste. Add the oats, dried fruit and seeds; and mix thoroughly.
3. Warm the peanut butter, sunflower spread, honey and cinnamon in a saucepan over a low heat for about 2 minutes or until the spread has melted, stirring continuously.
4. Pour the melted spread mixture over the banana and oats; and mix thoroughly.
5. Transfer the mixture into the lined baking tray and spread to an even thickness.
6. Bake in the oven for 35–40 minutes until cooked through and golden brown.
7. Carefully turn out onto a cooling rack and allow to cool before cutting into 12 equal-sized squares.
8. Serve or store in a sealed container – eat within 5 days. Alternatively, freeze and use within 3 months.

TIP: Peanut butter can be swapped for other types of nut butter, such as almond butter, if preferred.

Nutrition information (per flapjack)

- MAKES: 12
- CALORIES: 169
- FAT: 7.1g
- SALT: 0.1g
- 5 A DAY: less than 0.5


High in
calories and
protein

Diarrhoea

Diarrhoea (frequent, loose or watery stools) can be a side-effect of cancer treatments such as chemotherapy, radiotherapy, targeted therapies and surgery. Infections and some medications, such as antibiotics, can also cause diarrhoea.

What can help?

If you have diarrhoea, it is important to seek advice from your doctor, as there are many causes that require different types of treatment. If you're prescribed medication to help with diarrhoea, it's important you take it as directed.

If you continue to have diarrhoea after your treatment has finished or beyond the time you were told you might experience problems, do seek further advice from your doctor.

Replacing lost fluids

Regardless of the cause, diarrhoea can make you dehydrated, so aim to have plenty of hot and cold drinks to replace any fluids you might be losing. As a guide, aim to drink at least two litres (3.5 pints) a day – this is at least 10–14 glasses. As well as sipping on water throughout the day, you could try drinking:

- **Sugar-free fruit cordials and squashes, and diluted fruit juice**
- **Clear soups, Oxo or Bovril**
- **Unsweetened coffee, tea, herbal tea and fruit tea**
- **Milk* or milk alternatives,** such as soya, rice, almond or hazelnut milk

* Some people may find milk can make their diarrhoea worse. If this is the case for you, you could try lactose-free milk or milk alternatives.

Keep eating

Try not to restrict the amount you're eating, to make sure you don't miss out on important nutrients. It may be best to eat little and often, rather than have three main meals, to avoid large amounts passing through your bowel. You could also try chewing and swallowing your food slowly. If you find that diarrhoea is disturbing your sleep, try to avoid eating close to bedtime.

Foods to avoid

Some people find certain foods can make their diarrhoea worse, so you might want to consider whether these foods affect you. If they do, try avoiding them or reducing the amount you eat:

- **Greasy, fatty and fried foods**
- **Caffeine**, such as in tea, coffee, cola and chocolate
- **Spicy foods**
- **Alcohol**
- **Nuts and seeds**

Can a low-fibre diet help?

Until recently, people with diarrhoea were advised to reduce the amount of fibre in their diet. However, for many causes of diarrhoea, including chemotherapy and radiotherapy, there is actually no evidence that this will have any benefit. This is good news as it means that people can often continue to eat a normal, healthy, balanced diet containing adequate amounts of fibre-containing foods, such as vegetables, fruit, pulses (like beans and lentils) and wholegrain foods.

If you have really bad diarrhoea, speak to your doctor or a pharmacist, as you might need to replace lost salts with salty foods or a rehydrating solution such as Dioralyte.

Constipation

Constipation (not being able to pass stools regularly) can often be very uncomfortable and may make you feel full and sick.

It can be caused by:

- **Some cancer treatments** such as chemotherapy, biological therapy and surgery to the stomach or bowel
- **Anti-sickness and pain medications**
- **Not eating enough fibre**
- **Not drinking enough fluid**
- **Not doing enough physical activity**

The advice below may not be appropriate if constipation is caused by a tumour obstructing the stomach or bowel. If you are concerned, speak to your doctor or dietitian before making any changes to your diet.

What can help?

Eating a high-fibre diet

Fibre helps to keep our bowel movements regular. Eating more high-fibre food may help to relieve constipation. Have a look at our shopping list for high-fibre food ideas.

Drinking enough fluid

It's important to drink plenty of fluid, particularly when eating more fibre, as not drinking enough can make constipation worse. As a guide, aim to drink at least two litres (3.5 pints) a day – this is at least 10–14 glasses. However, if you aren't able to eat as much as you usually would, it may be best to discuss how much you should drink with your doctor or dietitian.

Some people find having a warm drink when they wake up helps get their bowel moving.

Your shopping list – high-fibre foods

- Wholegrain, granary or wholemeal bread
- Fruit and veg (with edible skin or peel on)
- Oats
- Wholegrain breakfast cereal
- Brown rice and pasta
- Dried fruit
- Nuts and seeds, like linseeds
- Pulses, like beans, peas and lentils
- Rye, digestive or bran biscuits and crackers

Wind

Passing wind is a normal bodily function – on average, people pass wind about 15–25 times a day. If you find you're passing wind more often than usual, this may be caused by:

- **Pelvic radiotherapy**
- **Bowel surgery**
- **Constipation**
- **Certain medications**
- **Your cancer stopping you from digesting and absorbing your food properly (malabsorption)**

Keeping active

Keep as active as possible and, if you can, avoid spending too much time sitting or lying down.

Some regular gentle exercise, such as going for a short walk every day, can help keep your bowel movements regular.

See pages 46–47 for more ideas about keeping active.

If things don't seem to be improving, see your doctor. They may be able to prescribe laxatives. If you have bowel cancer, speak to your dietitian or doctor for advice before making any changes to your diet, as a high-fibre diet can make symptoms worse.

What can help?

Foods to avoid

Some people find that these foods can cause wind so you might want to consider whether these foods affect you. If they do, try avoiding them or reducing the amount you eat:

- **Beans, cabbage, brussels sprouts, sweetcorn, cauliflower, onions**
- **Pickles**
- **Fizzy drinks**
- **Some artificial sweeteners, including mannitol, sorbitol and xylitol**

Other tips

- Try to eat little and often. Chewing your food well and sipping slowly may also help.
- You could try common remedies such as peppermint water, capsules or tea, fennel tea and baby's gripewater.
- Gentle exercise could help to get your bowel moving. **See pages 46–47 for more ideas about keeping active.** The advice on managing constipation might also help (**see previous page**).

Tell your doctor if passing wind becomes painful or if symptoms don't get better.


High in
fibre

Minestrone soup

Ingredients

- 100g wholewheat spaghetti
- Spray oil
- 1 medium onion
- 2 courgettes, diced
- 2 large carrots, washed and diced
- 1 x 400g can chopped tomatoes
- 1 reduced-salt vegetable stock cube, dissolved into 500ml hot water
- ½ tsp dried, mixed herbs
- Freshly ground black pepper, to taste
- 1 x 410g can cannellini beans, drained
- 100g kale or savoy cabbage, finely shredded

Method

1. Cook the pasta as instructed, until al dente (cooked but still has a bite). Drain, rinse in cold water and set aside until needed.
2. Coat a large non-stick saucepan in spray oil and place over a medium heat to warm. Add the onion and cook for 3–4 minutes until it begins to soften. Then add the courgette and carrot; and cook for a further 3 minutes, stirring continuously.
3. Add the remaining ingredients and bring to the boil. Reduce the temperature, cover and simmer gently for 10 minutes.
4. Add the pasta and continue cooking for 2–3 minutes or until the vegetables are tender. Remove from the heat and serve.

TIP: If you're losing weight or struggling to maintain your weight, you can add calories to this dish by using more oil when cooking the vegetables and by stirring in creme fraiche before serving.

Nutrition information (per portion)

- SERVES: 4
- CALORIES: 196
- FAT: 3.1g
- SALT: 0.9g
- 5 A DAY: 4.5

Vegetable paella

Ingredients

- 2 tbsp rapeseed oil
- 1 tsp cumin seeds
- 1 medium onion, diced
- 2 cloves garlic, crushed
- 240g brown basmati rice
- 1 x 400g can chickpeas, drained
- 1 reduced-salt vegetable stock cube, dissolved into 750ml hot water
- ¼ tsp saffron threads (alternatively, use ¼ tsp turmeric or smoked paprika)
- 300g green veg, such as green beans, peas, broad beans, asparagus or courgette, trimmed and cut into bite-sized pieces
- 1 red pepper, cut into thin strips
- ½ lemon, cut into 4 wedges

Method

1. Warm the oil in a large frying pan or paella dish over a medium heat. Then add the cumin seeds to flavour the oil. Add the onion and garlic and stir for about 1 minute.
2. Add the rice and chickpeas to the pan, and then add the stock and saffron (or turmeric or paprika). Mix thoroughly. Make sure all the rice and chickpeas are covered with stock.
3. Bring to the boil, and then reduce the heat and cover. Simmer for 30–35 minutes, stirring occasionally. Add more water if required.
4. Add the green vegetables and cook for a further 5 minutes.
5. Add the pepper, mix thoroughly, replace the cover and cook for a further 3–4 minutes, or until all the liquid has been absorbed and the rice is tender.
6. Serve with a wedge of lemon.

TIP: For extra protein, add diced lean meat or poultry, or prawns to this dish. If using raw meat or prawns, add with the green vegetables. If they're already cooked, add at step 5.

Nutrition information (per portion)

- SERVES: 4
- CALORIES: 396
- FAT: 9g
- SALT: 0.3g
- 5 A DAY: 2.5


High in
fibre


High in fibre

Wholemeal bread and butter pudding

Ingredients

- 20g sunflower spread, or similar
- 6 small slices wholemeal bread (from a 400g loaf), lightly toasted
- 75g dried fruit, chopped if necessary
- 1 large egg and 1 large egg yolk
- 2 tsp caster sugar
- 400ml skimmed milk
- 1 tsp vanilla extract (no alcohol)
- ½ tsp ground cinnamon
- Fresh nutmeg, grated (or ¼ tsp ground nutmeg)

Method

1. Preheat the oven to 170°C/Fan 150°C.
2. Spread a thin layer of sunflower spread on one side of each slice of bread and then cut each slice in half. Arrange layers of bread, spread side up, and dried fruit in an ovenproof dish.
3. To make the custard, lightly beat the egg and egg yolk in a mixing bowl, then add the sugar, milk and vanilla extract.
4. Transfer the custard into a saucepan and set over a medium heat. Stir continuously until the custard starts to thicken – it should just coat the back of your spoon. Then stir in the cinnamon and nutmeg. Pour the custard over the bread and dried fruit, and leave to soak for 5–10 minutes.
5. Bake in the oven for about 30 minutes until the edges of the bread are golden and the custard starts to set.
6. Serve immediately or allow to cool before covering and refrigerating – eat within 3 days.

TIP: To add calories, use fortified whole milk (see page 14) and add 1 tablespoon of flaked almonds with the dried fruit.

Nutrition information (per portion)

SERVES: 4
CALORIES: 256
FAT: 6.9g
SALT: 0.7g
5 A DAY: 0

Nausea (feeling sick)

Feeling and being sick can be a symptom of cancer or a side-effect of treatments such as chemotherapy, radiotherapy, biological therapies or hormone therapies. Some medications such as pain medication and bisphosphonates (medication that slows down or prevents bone damage) can also cause sickness, as can the other side-effects of cancer treatment such as constipation. It is important to discuss this with your doctor as they can prescribe anti-sickness medication, which should be taken as prescribed and preferably before meals to ensure that it's working when you eat.

What can help?

Choosing what to eat

- Everyone is different, so try experimenting to see what you can tolerate.
- Small, light meals or snacks, eaten often, might be easier than large meals. If possible, avoid having an empty stomach as this can make you feel sick too. Try nibbling on dry foods, like toast or crackers, especially first thing in the morning.
- Some people find they only want bland food, such as potato, rice and pasta, whereas others prefer salty things like Marmite, soup, salty crackers, popcorn or nuts. Start with foods you can tolerate and gradually build up to a more varied diet if you start feeling better.
- Some people find that food or drink containing ginger or peppermint can help settle their stomach.
- If the smell of cooking makes you feel sick, opt for cold foods and snacks, or frozen food that you can reheat quickly (make sure it's cooked properly). It's fine to use convenience foods, cans or packets if that's easier. You could also ask friends or family to cook meals while you're in another room. If they cook you something but you don't manage to eat it, you could put it in the fridge or freezer for later.

Drinking enough fluid

It is important to avoid becoming dehydrated, especially if you've actually been sick. Ice cold, fizzy drinks, such as sparkling mineral water or soda water, might help. Some people also find that milk helps to settle their stomach. It's best to sip your drinks slowly and to have drinks before or after your meals, rather than while you're eating.

Foods to avoid

These foods and drinks can make sickness worse, so you might want to see if avoiding them helps:

- **Greasy, fatty and fried foods**
- **Spicy food**
- **Caffeinated drinks**, such as tea, coffee and cola
- **Alcohol**

Mealtimes

- If strong smells make you feel nauseous, try eating in a room where there's lots of fresh air and away from the smell of cooking or other strong smells such as flowers.
- Anxiety can make nausea worse, so try to make yourself as comfortable and relaxed as possible. Wearing loose clothing might also help.
- Try to sit upright while you're eating and for a while after – if possible, don't lie down for two hours after eating. Also, try to avoid doing anything too active straight after eating.

If you have experienced severe vomiting and can't keep any food or drink down, tell your doctor. They can prescribe anti-sickness medication for you.

Mouth problems

You might experience mouth problems, particularly during and after chemotherapy, radiotherapy to the head and neck, or if you have cancer of the mouth or throat. The good news is that these problems are often temporary.

Some of the most common mouth problems are:

- **Soreness and ulcers in your mouth and throat**
- **Mouth infections,** such as thrush
- **Dry mouth and lack of saliva**
- **Tooth problems and bleeding gums**
- **Difficulty swallowing and chewing**
- **Bad breath**
- **Thick, sticky saliva**

What can help?

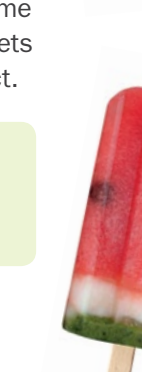
Looking after your mouth, teeth and dentures

- Try to keep your mouth as clean as possible. This should help your mouth feel more fresh and comfortable, and may improve the taste of food.
- Remember to brush your teeth regularly. If your mouth and gums are sore, it may be best to use a soft-bristled toothbrush.
- Visit your dentist or oral hygienist regularly to make sure that there aren't any problems with your teeth or gums.
- Use an alcohol-free mouthwash. Your doctor may also prescribe a special mouthwash.
- If you wear dentures, clean them regularly and try leaving them out of your mouth for as long as possible, to stop them irritating your gums.

Soothing your mouth

If you have a dry mouth, sucking on sugar-free boiled sweets or chewing sugar-free gum can help stimulate saliva and keep your mouth moist. It is important to note that excessive consumption of some sweeteners used in sugar-free sweets and gum can have a laxative effect.

If you smoke, try to give up as smoking irritates the mouth and slows down healing.



If you have a sore mouth, cold foods and drinks, such as fruit juice-based ice lollies, yoghurt and frozen yoghurt, smoothies, sugar-free jellies, gazpacho (**see our recipe on pages 36–37**) and crushed ice can be very soothing.

Sipping drinks throughout the day will keep your mouth feeling fresh and help prevent you from becoming dehydrated.

Avoiding certain foods

While you have a sore mouth, try to avoid any foods or drinks that might irritate your mouth or that are hard to swallow, such as:

- **Rough, dry, crunchy or very chewy foods** like hard breakfast cereals, crusty bread, nuts, raw vegetables, dry biscuits and tough meat.
- **Very hot foods and drinks**.
- **Sharp-tasting, salty and spicy foods** like pickles, vinegar, garlic, raw onion and chilli.
- **Food that sticks to the roof of your mouth** like pastry or peanut butter.
- **Acidic foods and drinks** like citrus fruits or pineapple.
- **Alcohol**, especially spirits and wine.

If you've lost weight, use our tips and recipes on pages 10–21 to help you get the most out of every mouthful.

Choosing softer foods

Adapt your favourite foods to make them as soft and moist as possible. This will make them easier to chew and swallow, and less likely to irritate your mouth. Try these tips:

- Add extra sauce and gravy to your meals.
- Slow-cook lean meat and vegetables in casseroles and stews to make them soft.
- If you're having a pudding, serve it with custard or yoghurt.
- For breakfast, have porridge, yoghurt with stewed fruit or mashed banana, soft cereals – such as Weetabix – with lots of milk, or a smoothie (**see pages 38–39 for our filling fruit smoothie recipe**).
- For lunch, try cutting the crusts off your sandwiches or have smooth soups.

Mashing, blending or liquidising your food can make it easier to eat – for example, blending casseroles or curries to make thick soups, pureeing vegetables and fruit, and mashing potatoes.

Tell your doctor if you're experiencing any mouth problems. They may be able to prescribe pain medication, special mouthwashes or gels.


Soothing
for sore
mouths

Gazpacho

Ingredients

- 2 x 400g can plum tomatoes
- 1–2 cloves garlic, crushed
- 1 medium onion, diced
- 1 green pepper, deseeded and diced
- 1 red pepper, deseeded and diced
- 1 medium cucumber, peeled and diced
- ¼ tsp cumin
- 6 tbsp extra virgin olive oil
- 100g wholemeal bread, toasted, soaked in water for 5 minutes
- 1–2 tbsp red wine vinegar or sherry vinegar
- Freshly ground black pepper, to taste

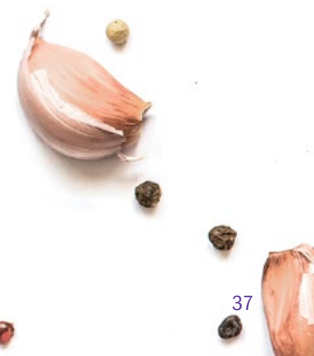
Method

1. Place the tomatoes, garlic, onion, peppers, cucumber, cumin and olive oil in a food processor and blend.
2. Squeeze as much water as possible out of the bread, tear into small pieces and add to the mixture in the food processor. Blend vigorously until smooth.
3. Add the vinegar and pepper.
4. Pass the soup through a sieve to remove any seeds (optional). Cover and refrigerate. Serve when chilled.

TIP: To increase the amount of protein and calories in the gazpacho, add a 400g can (drained) of chickpeas or beans, such as butter beans or cannellini beans, at step 1.

Nutrition information (per portion)

- SERVES: 6
- CALORIES: 215
- FAT: 13.4g
- SALT: 0.2g
- 5 A DAY: 2



Filling fruit smoothie

Ingredients

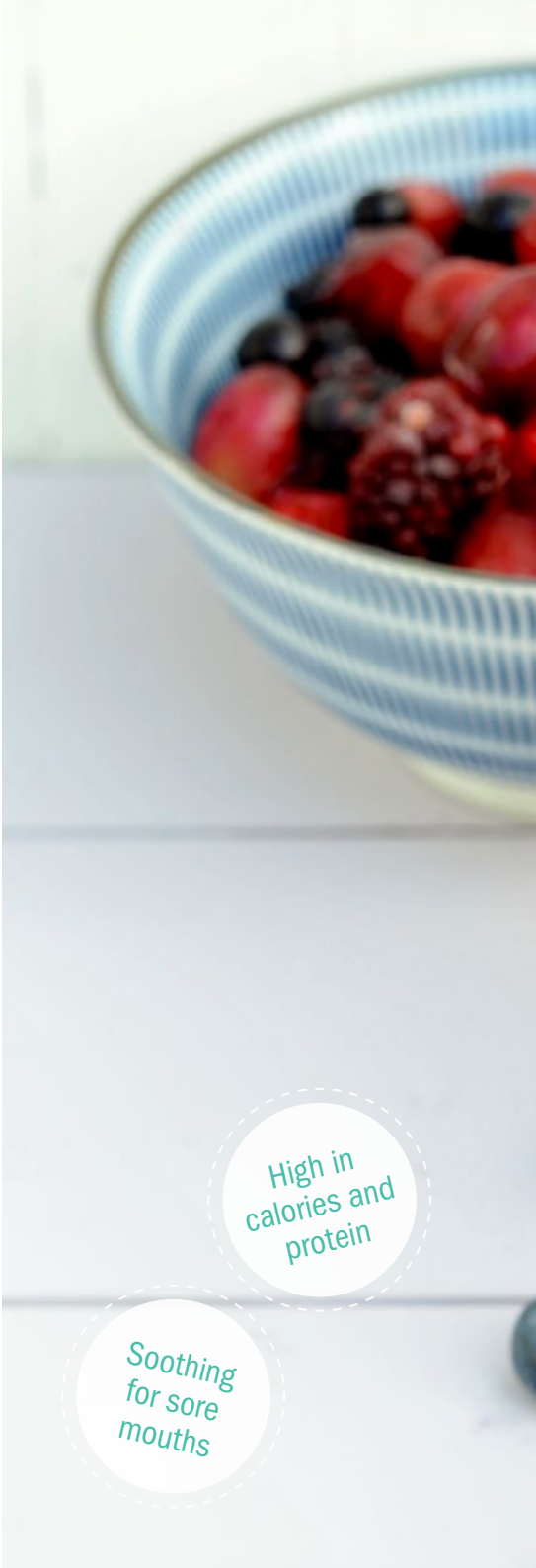
- 100ml whole milk
- 1 level tbsp skimmed milk powder
- 1 tbsp natural yoghurt
- Ice cubes (optional)
- 1 heaped tsp peanut butter (ideally a brand that contains no added salt and sugar)
- 1 medium banana
- 160g frozen berries
- 40g oats
- 15g seeds

Method

Place all the ingredients in a blender, and blend until smooth. Pour into two glasses and serve.

Nutrition information (per portion)

- SERVES: 2
- CALORIES: 312
- FAT: 12.2g
- SALT: 0.2g
- 5 A DAY: 1.5



Taste changes

Cancer treatments such as chemotherapy, radiotherapy and certain medications can affect the way foods and drinks taste, as can cancer itself. Food may taste unpleasant, bland or particularly metallic, sweet or salty. The good news is that taste changes are often temporary.

What can help?

Taking care of your mouth

Having a dry mouth can affect your sense of taste so try to drink plenty of fluids and keep your mouth and tongue clean by brushing regularly – using a soft-bristled toothbrush may be more comfortable. You may also want to see your dentist or oral hygienist to make sure that there aren't any problems with your teeth or gums.

Choosing your food

- It might help to eat what you enjoy and ignore what you don't fancy for the time being. If a food you normally like tastes unpleasant, you could always try it again another time as your taste may change. Some people find that cold food tastes more pleasant than hot food.
- If your food tastes metallic, it might help to avoid canned food and drink and to avoid cooking with metal pots and pans. You could also try using plastic cutlery and adding grated carrot, cinnamon or a little honey to your food to help sweeten it.

Adding flavour

Serving food with strong-flavoured condiments, such as pickles, mustard, vinegar, salad dressings or lemon juice can help make them more appealing, as could adding more flavour to your cooking by using herbs, spices, seasoning and marinades. You could:

- Roast lean meat with strong herbs like rosemary, thyme and mint.
- Cook minced or diced lean meat with garlic, ginger or even cinnamon and nutmeg.
- Cook chicken and turkey with garlic, tarragon, basil, lemon juice or chilli.
- Cook fish with fennel, dill, pepper, lime, parsley or coriander.
- Spice up dishes with strong-flavoured vegetables, such as celery, onions, leeks and tomatoes.
- Sweeten desserts and breakfast foods such as porridge with a sprinkle of cinnamon or nutmeg.

If you also have a sore mouth, it's probably best to avoid too much spice or spicy foods (**see pages 34–35 for tips to soothe a sore mouth**).

Choosing fresh tastes

Try sharp, fresh-tasting foods like lemon, and drinks such as bitter lemon. These may help stimulate your taste buds, increase the flow of saliva and get rid of any unpleasant tastes in your mouth. However, certain citrus fruits, particularly grapefruit, can affect the way some medications work so check with your doctor or pharmacist first.

Trying different textures

Experiment with different textures to see if they make things tastier – for example, you might prefer toasted bread and crackers to soft bread and potatoes. To add extra crunch, try sprinkling seeds or dried onion over savoury dishes, or chopped nuts over desserts. However, avoid doing this if your mouth is very dry or sore.

Reducing your risk of infection

Your immune system helps protect your body from infection and disease but it may not work as well as normal during and after cancer treatment. Treatments such as chemotherapy aim to stop cancer cells from growing and dividing but they also affect normal cells, such as white blood cells, which are part of your immune system. If you have a low number of white blood cells, you're at a higher risk of infection or food poisoning.

If you have blood cancer, you are more likely to be 'neutropenic' (which means your white blood cell levels are very low) so you may have to be even more careful to avoid infection or food poisoning and will need specific advice from your doctor or dietitian (**also see pages 49–50 for details of who to contact for more advice**).

The good news is that immune cells recover when treatment stops. You can also take some simple steps to protect your immune system and prevent infection.

How can I protect my immune system?

While you're having cancer treatment, eating well and keeping active can help keep your immune system working at its best.

The best way to support your immune system is by making sure your body gets all the nutrients it needs from a balanced and varied diet rather than focusing on specific foods. As well as eating plenty of vegetables and fruit, try to eat enough calories (energy) and protein, especially if you've lost weight (**see pages 10–15 for more advice**).

How can I prevent infection?

Following good food hygiene

Practising good food hygiene is very important to protect you from food poisoning. Make sure you:

- **Wash your hands with soap and warm water before and after preparing, cooking and eating food.**
- **Clean all worktops and chopping boards before and after cooking.**
- **Check food is in date (especially food that has a 'use by' date) and doesn't have any visible mould on it.**
- **Try to avoid buying foods that are kept unpackaged, especially foods that you won't cook before eating, such as bread and baked goods from bakeries, sandwich fillings in cafes and deli foods.**
- **Keep raw meat and fish away from ready-to-eat foods, such as bread, salad and fruit, and prepare them using different chopping boards and utensils.**
- **Store raw meat in a clean, sealed container on the bottom shelf of the fridge.**
- **Wash fruit and vegetables thoroughly under cold running water before eating.**
- **Check that food is heated through before you eat it.**
- **Store food at the correct temperature and make sure you defrost and reheat foods safely.**

Foods to avoid

While you're having cancer treatment, it's also important to be careful with certain foods that are more likely to contain harmful bacteria. It's normally best to avoid paté, raw or undercooked seafood and fish, and cheeses made from unpasteurised milk, such as brie and blue-veined cheeses. Speak to your doctor or dietitian for more specific advice.

Fatigue (extreme tiredness)

Fatigue is common in people who have cancer – and it's more than the usual feeling of tiredness. You may feel very tired or exhausted most or all of the time. Fatigue can have a big impact on your everyday life, making you feel both physically and mentally drained, and leaving you with little energy or motivation.

A combination of different factors could cause fatigue:

- **The effect of cancer and cancer treatments on your body**
- **Problems with eating and drinking**
- **Low levels of red blood cells (anaemia)**
- **Other symptoms and side-effects,** such as pain or breathlessness
- **Side-effects of some medication**
- **Sleeping difficulties**
- **Anxiety and depression**

If your fatigue is a side-effect of being anaemic (having low levels of red blood cells), your doctor will prescribe you with medication that will help. If you are concerned, speak to your doctor.

Living with fatigue can be difficult, but there are ways to help manage and improve it.

What can help?

Being as active as possible

Being active may be the last thing you feel like doing if you're tired, but there's lots of research to show that doing some light to moderate physical activity every day can help improve fatigue and make you feel more energised.

Keeping active can also increase your appetite and generally boost your wellbeing.

See pages 46–47 for more ideas about keeping active.

Mealtimes

It's quite common to feel too tired to prepare or cook any meals. Here are some ideas for dealing with this:

- Friends and family are often keen to help – maybe they could prepare some meals for you to freeze or do a weekly shop for you?
- Supermarkets stock lots of healthy convenience and ready-made meals and snacks that don't need much preparation that you could try.
- If you're too tired to go out, why not try online shopping?
- There are companies that can deliver good quality frozen or cooked meals, or meal preparation kits to your door – ask a dietitian if they can recommend one in your area.

Eating and drinking well

- Eating well and keeping a healthy weight can help you to keep up your strength and improve your energy levels.
- If possible, choose foods that release energy over a longer period of time, such as potatoes with skin on and wholegrain foods (wholemeal bread, brown rice and wholewheat pasta, and unsweetened, wholegrain breakfast cereals). Sugary foods may give you a quick boost but won't give you energy for very long.
- Being dehydrated can make you feel tired, so try to drink plenty of liquid such as water, milk, sugar-free squash, diluted juice or herbal tea. Aim to drink at least 1.2 litres (2.1 pints) a day – this is at least six to eight glasses.
- If you've also lost weight, **you could follow our advice on pages 10–15 to help increase your calorie and protein intake**.
- If you have mouth problems, **see pages 34–35 for tips and advice on how to make food more palatable**.

Keeping active

Alongside eating well, it is important to keep active when you have cancer. Making time for physical activity can have many benefits.

What are the benefits of keeping active?

There's growing evidence that people who are active before and after a cancer diagnosis have a better chance of survival. On top of this, physical activity can:

- **Help reduce fatigue**
- **Boost your immune system**
- **Keep your heart and lungs healthy**
- **Reduce the risk of other diseases**, such as heart disease, Type 2 diabetes and high blood pressure
- **Help reduce anxiety and depression, and improve your mood**
- **Improve your muscle strength and reduce muscle loss**. This is especially important if you're losing weight as a result of cancer or cancer treatment
- **Help with some of the other side-effects mentioned elsewhere in this booklet**, such as easing constipation by helping to move food through your bowel

Is it safe for me to exercise?

Generally it's safe and beneficial for people with cancer to exercise. However, it's best to start slowly and build up if you aren't used to exercising regularly. You may also want to let your doctor or nurse know before you start being more active as they might be able to signpost you towards a qualified exercise specialist who can give you individual advice and support.

Weight gain

Some cancer treatments, such as hormone therapy for breast or prostate cancer, may cause weight gain, while some medicines, such as steroids, may increase your appetite, meaning you want to eat more than usual. Being physically active, together with eating a healthy diet, can help you stay a healthy weight.

How can I get more active?

It can be tricky to know where to start, so here are some tips:

- Start at a level that's right for you – this will probably depend on how much exercise you've done in the past, what stage you're at with your cancer and treatment, and how well you're feeling.
- It can be especially hard if you are feeling tired, but even doing a small amount of activity is better than nothing. Exercising with a friend or relative can also help to make it more enjoyable.
- Ideally you should aim to do 150 minutes of moderate intensity exercise a week. This includes activities such as brisk walking or swimming.

  This might sound like a lot if you haven't exercised for a long time, so try to start small and set yourself achievable goals. You could begin with a five to 10-minute walk, two or three times a week. As this starts to feel easier, you can build up the amount you do.

What sort of activity should I do?

A good way to get more active is by walking. Going for a walk every day gets you out into the fresh air and will make sure you aren't completely inactive.

It sounds obvious, but try to pick activities you enjoy. It doesn't have to be a sport or exercising in the gym – it could be swimming, gardening or dancing.

Try some strength exercises too. These will help stimulate muscle growth and help prevent you losing muscle and strength. Strength exercises can be done using free weights (eg dumbbells or even water bottles or cans of food), weight machines or resistance bands. You can also do exercises that use your own body weight, such as squats or press-ups. Everyday activities like carrying shopping can also help.

Other advice you might find helpful

World Cancer Research Fund's main focus is to help people reduce their risk of developing cancer by following our Cancer Prevention Recommendations. As well as advice and tips on making healthy diet and lifestyle choices, we have lots of simple, healthy recipes that put our recommendations into practice.

We also provide advice to people who are living beyond a cancer diagnosis, to help them live long, healthy lives and to reduce their risk of developing cancer again. This advice can be found on our website.

Visit our website **wcrf-uk.org** or call us on **020 7343 4205** for more information.

Your feedback

We're always looking for ways to improve the information we provide.

If you have any comments or suggestions about any aspect of this booklet or our other health information, we would welcome your feedback. We'd also love to hear if any of the advice helped you, or if you have any tips you'd like to share with others. Any changes you suggest mean we can make our information better for other people. Email us at **resources@wcrf.org** with your feedback.

Advice from other organisations

General advice and support

Macmillan Cancer Support

Macmillan's Support Line is a free and confidential service, open Monday to Friday from 9am to 8pm. They can:

- Help with any questions about your treatment
- Provide information about financial support
- Give you details of support groups in your area
- Just be there for you to talk to

Call free on **0808 808 0000** or visit their website **macmillan.org.uk/talktous**

Advanced cancer care

Marie Curie

Marie Curie provides care, both at home and in their hospices, and support for people with terminal cancer. Their helpline is open Monday to Friday from 8am to 6pm, and Saturday from 11am to 5pm.

Call free on **0800 090 2309** or visit their website **mariecurie.org.uk**

Blood cancer and the neutropenic diet

Bloodwise

The charity Bloodwise, with the support of the British Dietetic Association's Oncology Specialist Group, has produced a patient booklet called **Eating Well with Neutropenia**, which is full of safe dietary advice for people who are neutropenic (**see page 42**), to help them avoid infection. You can download a free copy from **bloodwise.org.uk**

Advice on specific cancer types

There are many cancer charities in the UK, so knowing which advice you can trust can be difficult. A simple way of telling if an organisation is giving evidence-based and trustworthy advice is to look out for the **Information Standard** logo (see back cover). This logo can only be used by organisations that have shown that they have a robust procedure for producing their health information, and use the most up-to-date evidence.

Find a dietitian

British Dietetic Association

To find a registered dietitian in your area, call **0121 200 8080** or visit **bda.uk.com**

Support for carers

Carers UK

Caring for a loved one who is going through cancer can be incredibly difficult and isolating, so it is important to seek support if you need it. Carers UK offers support to carers, and can help to put you in contact with local support groups. Their helplines are open Monday to Friday from 9am to 4pm.

Call free on **0808 808 7777** (or on **028 9043 9843** if you're in Northern Ireland) or visit their website **carersuk.org**

Disclaimer

This booklet has been written with specialist oncology dietitians, and should be safe to follow for most adults who have cancer, with the exception of those excluded in the 'Who is this booklet for?' section on page 3. However, where the advice in this booklet differs from the advice given to you by your doctor or dietitian, it is always best to follow their advice as it will be specific to your needs. It should also be noted that many of the specific suggestions in this booklet, while safe, will not help everyone. They are suggestions that other people have found helpful, that you might also want to try.

For references used in this booklet or to request the information in large print, please contact us.

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REVIEW ARTICLE

Association of Obesity-Related Metabolic Disruptions With Cancer Risk and Outcome

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ABSTRACT

Over the past 40 years, the prevalence of obesity has increased epidemically worldwide, which raises significant concerns regarding public health and the associated economic burden. Obesity is a major risk factor for several conditions including cardiovascular disease and type 2 diabetes, and recent evidence suggests that obesity negatively affects cancer risk and outcome. The relationship between obesity and cancer is complex and involves multiple factors both at the systemic and cellular level. Indeed, disruptions in insulin metabolism, adipokines, inflammation, and sex hormones all contribute to the adverse effects of obesity in cancer development and progression. The focus of this review will be the impact of these systemic obesity-related factors on cancer biology, incidence, and outcome. Potential therapeutic interventions and current clinical trials targeting obesity and its associated factors will also be discussed.

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INTRODUCTION

Obesity is an endemic health concern. The worldwide prevalence of obesity more than doubled between 1975 and 2014 (from 6.4% to 14.9% in women and from 3.2% to 10.8% in men),[1] and obesity is increasingly recognized as a potentially modifiable factor associated with poor prognosis in several cancers, such as those of the breast, colon, and endometrium.[2-4] Obesity is an altered physiologic state that is associated with insulin resistance (hyperinsulinemia and dysglycemia), and altered adipokines (higher leptin and lower adiponectin), sex hormones ([SHs] estrogens, androgens, and testosterone), and inflammation, all of which may impact cancer.[5] These alterations in physiology impact a number of biologic processes implicated in tumor development and progression. Indeed, numerous signaling pathways known to promote oncogenesis lie downstream of receptors that are sensitive to many of the systemic changes intrinsic to the obese state. This article will provide a review of the impact of obesity on tumor biology and intracellular signaling and will evaluate the most recent evidence supporting a role for obesity and associated disruptions in systemic physiology in the incidence and outcome of major cancers. In addition, the potential for treatment of obesity-associated cancers with targeted pharmacologic therapies will be discussed. Obesity-mediated changes in inflammatory markers, a process that also adversely affects cancer outcomes, will be addressed elsewhere in this Special Series issue.

INSULIN RESISTANCE AND CANCER DEVELOPMENT AND OUTCOME

Both insulin and glucose are elevated in the obesity-associated insulin resistance syndrome and have been implicated in cancer risk and prognosis. Fasting glucose was associated with increased risk of future cancer death (hazard ratio [HR], 1.25; 95% CI, 1.19 to 1.31) in an individual-patient meta-analysis of 820,900 patients enrolled onto 97 prospective studies.[6] Although this estimate includes associations of glucose with both incidence of and mortality from cancer, the clinical evidence in this case is observational, and a causal association cannot be assumed. Increases in systemic glucose levels may impact tumor development and progression by providing tumor cells with the required energy to maintain their rapid rates of cell division. Cancer cells are known to exhibit alterations in glucose metabolism, particularly their reliance on glycolysis as a means of energy production even in the presence of oxygen—a metabolic switch known as the Warburg effect.[7] Although glycolysis is a less efficient form of energy

production compared with mitochondrial oxidative phosphorylation, cancer cells surmount this shortcoming by upregulating glucose transporters,[8,9] thus increasing their glucose uptake and potentially increasing their sensitivity to the elevated systemic glucose levels found in obesity. Consequently, the hyperglycemia exhibited by obese patients may impact tumor growth by providing cancer cells with an abundance of fuel and enabling them to maintain their rapid proliferative rates.

Disruptions in insulin metabolism also adversely affect cancer risk and outcome. Elevated insulin or C-peptide (cleaved from proinsulin) is associated with increased risk of numerous cancers, including those of the breast and colon.[10,11] In addition to its classic effects on glucose metabolism, insulin can also act as a growth factor. Indeed, stimulation of the insulin receptor results in the activation of numerous growth-promoting and proliferative intracellular signaling pathways. The insulin receptor is a heterotetramer composed of two extracellular α subunits that are responsible for binding insulin and two transmembrane β subunits that exhibit tyrosine kinase activity.[12] Two isoforms (insulin receptor A [IR-A] and insulin receptor B [IR-B]) of the insulin receptor exist, which differ in inclusion (IR-B) or exclusion (IR-A) of a 12 amino–acid peptide located on the C-terminal end of the α subunit,[13] and display tissue-specific expression. The full-length IR-B receptor is found in insulin target tissues such as the muscle, liver, and adipose tissue, whereas the shortened IR-A isoform is expressed by embryonic tissues and regulates growth and proliferative signaling pathways required during fetal development.[14,15] Of note, IR-A can heterodimerize with the insulin-like growth factor receptor and transmit signals emanating from not only insulin, but also insulin-like growth factor 1.[16]

Signaling downstream of the insulin receptor is mediated by a variety of intracellular signaling proteins and adaptors that are recruited to several phosphotyrosines near the kinase domains of the β subunits that present after ligand binding (Fig 1). The most prevalent insulin receptor targets include the insulin receptor substrates 1 to 4, DOK4, DOK5, Shc, and Gab1, which are responsible for differentially stimulating signaling downstream of the receptor by interacting with a variety of effector proteins.[17] Among the myriad of signals regulated by the insulin receptor are two of the most prominent pathways implicated in human cancer, namely, the phosphatidylinositol 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) and Ras signaling networks. The p85 regulatory subunit of PI3K interacts with the phosphorylated insulin receptor substrate proteins leading to subsequent activation of Akt and mTOR signaling and increases in protein synthesis, cell growth, survival, and proliferation.[18] Likewise, the protein

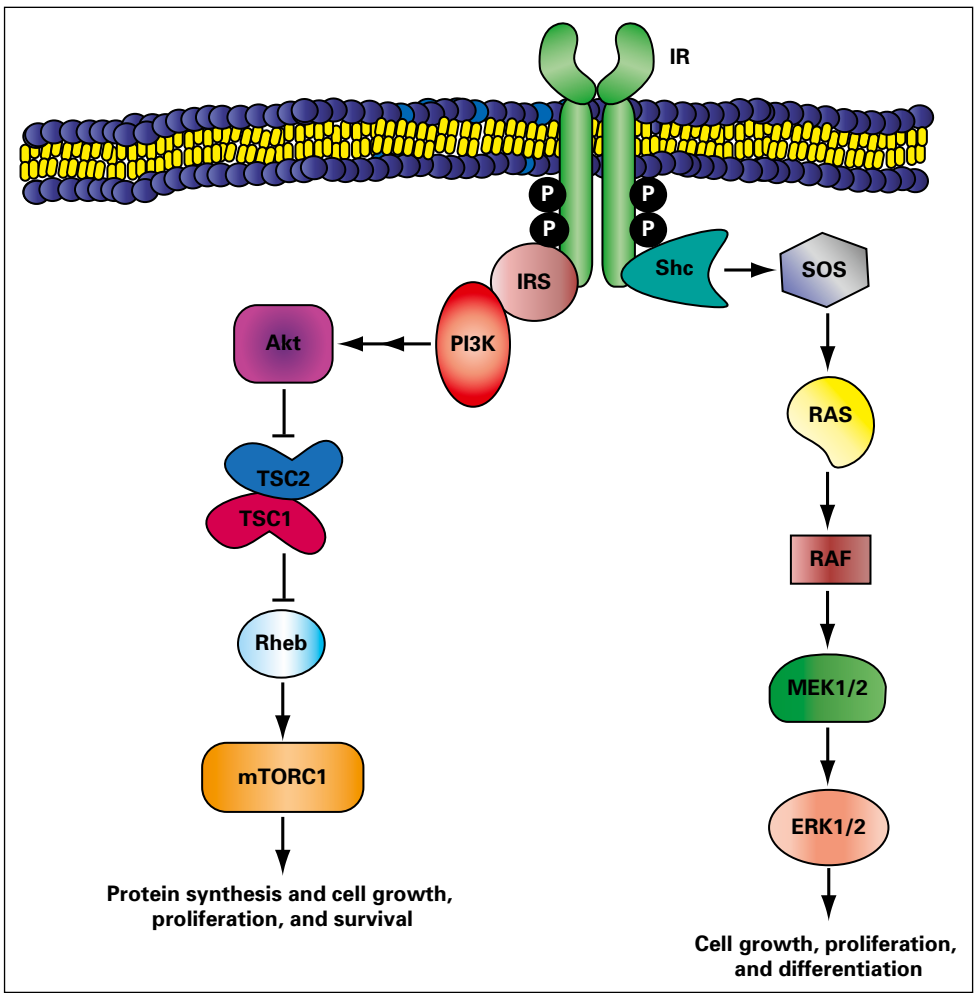

Fig 1. The insulin signaling pathway. Binding of insulin to its receptor (IR) leads to stimulation of its kinase activity and the phosphorylation of several tyrosine residues located on the β subunits of the receptor. A variety of intracellular signaling proteins and adaptors are recruited to these phosphotyrosines, including the IR substrates (IRSs) and Shc. These proteins are responsible for differentially regulating downstream signaling events by interacting with effector proteins such as phosphatidylinositol 3-kinase (PI3K) and son of sevenless (SOS). Activation of PI3K signaling leads to stimulation of Akt and mammalian target of rapamycin complex 1 (mTORC1) and subsequent increases in protein synthesis, cell proliferation, growth, and survival, whereas activation of SOS engages the RAS/MAPK signaling network and promotes cell proliferation and differentiation. ERK1/2, extracellular signal-regulated kinase 1/2; MEK1/2, mitogen-activated protein kinase kinase 1/2; P, phosphorylation; Rheb, Ras homolog enriched in brain; TSC, tuberous sclerosis complex.

complex Grb/SOS is recruited to the insulin receptor via interactions with the phosphorylated adaptor Shc, resulting in increased Ras signaling and stimulation of its downstream effectors MEK1/2 and ERK1/2, which regulate cell cycle entry, differentiation, and other mitogenic processes.[19] Remarkably, a wide spectrum of cultured cancer cell lines express high levels of the insulin receptor.[20,21] This is particularly evident in breast cancer (BC) cells in culture and is paralleled in tumor samples from patients with BC; more than 90% of BC tumor specimens in two different patient cohorts were found to express the insulin receptor.[22,23] Furthermore, insulin receptor expression and activation in tumors have been linked to poor survival in patients with BC.[24] Thus, in the context of obesity-associated hyperinsulinemia, the insulin receptor and its associated signaling pathways may play a significant role in mediating the adverse effects of obesity in breast and other cancers. Table 1 provides a summary of the association of insulin and glucose levels with outcomes of BC, colorectal cancer, and prostate cancer (PC).

BC

There is growing recognition that metabolic syndrome, also known as the insulin resistance syndrome, is linked to increased BC risk (relative risk, 1.47; 95% CI, 1.15 to 1.87)[39] and BC mortality (for women age $\geq$ 60 years: relative risk, 1.23; 95% CI, 1.04 to 1.45).[40] The biomarkers most consistently associated with BC prognosis are those related to insulin and glucose homeostasis. Some studies suggest a relationship between glucose, measured at the time of diagnosis, and BC outcomes. Recent work by our group demonstrated that fasting glucose correlated with distant disease–free survival (DDFS; HR, 1.88; 95% CI, 1.06 to 3.35 for the 87.5th *v* 12.5th percentile of glucose; $P < .034$) during the first 5 years after diagnosis in multivariable analyses.[25] In addition, high insulin levels and high homeostasis model assessment (a measure of insulin resistance) in nondiabetic patients with BC are associated with an elevated risk of recurrence.[25,26,29] Fasting insulin at the time of BC diagnosis is also correlated with DDFS and overall survival (OS; HR, 2.05; 95% CI, 1.16 to 3.62; and HR, 2.57; 95% CI, 1.18 to 5.59, respectively, for upper *v* lower quartile), and similar trends were observed for homeostasis model assessment and C-peptide.[25]

Colorectal Cancer

Altered insulin and glucose metabolism has also been linked to colorectal cancer. Higher fasting insulin and glucose levels correlated with the recurrence of colorectal adenomas in the Polyp Prevention Trial (odds ratio [OR], 1.56; 95% CI, 1.00 to 2.43; and OR, 1.49; 95% CI, 0.95 to 4.79, respectively), with glucose exhibiting the strongest association with advanced adenomas (OR, 2.43; 95% CI, 1.23 to 4.79).[41] Fasting blood sugar was also related to increased risk of polyp recurrence (fasting blood glucose $>$ *v* $\leq$ 126 mg/dL: OR, 2.04; 95% CI, 1.40 to 2.98), after adjustment for age, body mass index (BMI), and several adenoma characteristics.[41a] Higher C-peptide has been associated with increased risk of mortality in patients with nonmetastatic colorectal cancer (upper *v* lower quartile: HR, 1.87; 95% CI, 1.04 to 3.36).[42] The presence of the metabolic syndrome (characterized by metabolic disturbances including dysglycemia, hyperinsulinemia/diabetes, dyslipidemia, hypertension, and other attributes) has also been linked to a significantly lower rate of disease-free survival (absence *v* presence of the syndrome: HR, 0.733; 95% CI, 0.545 to 0.987) but not OS.[27] At the cellular level, phosphorylated insulin receptor staining was more frequent in adenomas than in adenocarcinomas, and disease-free survival in patients with colorectal cancer was better when tumors exhibited insulin receptor phosphorylation, possibly reflecting better tumor differentiation.[43]

PC

Emerging evidence also indicates that metabolic alterations can impact PC development and disease progression. Higher C-peptide, measured before PC diagnosis, was related to PC-specific mortality in men (upper *v* lower quartile: HR, 2.38; 95% CI, 1.31 to 4.30),[44] and fasting glucose was associated with an increased risk of recurrence in two separate studies (Macleod et al[28] study: HR, 1.54; 95% CI, 1.10 to 2.15; Wright et al[45] study: HR, 1.5; 95% CI 1.1 to 2.0). Furthermore, men with high-risk PC have been reported to have higher fasting insulin levels at diagnosis than those with medium- or low-risk disease.[46]

Table 1. Biomarkers and Outcomes of Breast, Colorectal, and Prostate Cancer

Biomarker and Cancer	Outcome
Metabolic marker	
Breast cancer	Higher insulin, glucose, and homeostasis model assessment at the time of diagnosis associated with worse outcomes (more recurrences and/or deaths)[25,26]
Colorectal cancer	Higher insulin and glucose and metabolic syndrome associated with worse outcome (recurrence)[27]
Prostate cancer	Higher glucose associated with worse outcome (recurrence)[28]
Adipokine	
Breast cancer	Higher adiponectin associated with better outcomes (fewer recurrences and/or deaths)[29,30]; higher leptin associated with worse outcomes (more recurrences and/or deaths) with long-term[25] but not short-term[30,31] follow-up; not associated with outcome of metastatic breast cancer[32]
Colorectal cancer	Higher adiponectin associated with lower risk of recurrence[33] and less advanced tumor grade but not stage[34] Higher leptin associated with higher stage and vascular invasion[35]; association with relapse or survival not known
Prostate cancer	Higher leptin not associated with future risk of lethal prostate cancer[36] and inconsistently associated with prostate cancer having adverse pathologic characteristics[36-38] Higher adiponectin associated with lower future risk of lethal prostate cancer[36] and inconsistently with prostate cancer having adverse pathologic characteristics[36,37]

ADIPOKINES AND OUTCOMES OF COMMON OBESITY-ASSOCIATED CANCERS

Alterations in adipokines, particularly leptin and adiponectin, are commonly observed in obese patients and may influence tumorigenesis. Adiponectin and leptin are produced by adipose tissue; however, their biosynthesis and secretion are affected differently in the context of obesity. Whereas leptin levels are elevated in the obese state, adiponectin secretion is reduced, resulting in disruptions in a variety of critical processes implicated in whole-body homeostasis, as well as cancer.[47]

The receptors for adiponectin, AdipoR1 and AdipoR2, and the receptor for leptin, Ob-R, are expressed by most cancer cells, but stimulation of these receptors by their respective ligands has opposing effects.[48,49] Binding of adiponectin to its receptors leads to the activation of intracellular pathways that negatively regulate proliferation, such as the adenosine monophosphate–activated protein kinase (AMPK) signaling network, and inhibition of those that stimulate cell division and growth, including PI3K/Akt/mTOR, JAK/STAT, and ERK1/2.[49,50] In contrast, stimulation of the leptin receptor causes activation of some of these same cellular networks, namely the PI3K and JAK/STAT pathways.[51] Thus, the imbalance in circulating levels of adiponectin and leptin observed in the obese state may lead to a series of changes in intracellular signaling networks favoring cancer cell proliferation and growth, with possible implications on the incidence and outcome of numerous obesity-related cancers (Table 1).

BC

A recent meta-analysis of 15 observational studies identified an inverse relationship between adiponectin and BC risk in both pre- and postmenopausal women (standardized risk ratio, 0.80; 95% CI, 0.63 to 1.01; and standardized risk ratio, 0.72; 95% CI, 0.30 to 1.72, respectively).[52] Conversely, higher levels of leptin seem to track with increased BC risk (eg, in one study, OR for upper *v* lower tertile was 1.98 [95% CI, 1.20 to 3.29]).[53] For example, we reported that serum leptin was not associated with DDFS or OS in BC at 6 years of follow-up (adjusting for tumor- and treatment-related factors) in a prospective study of 471 women.[25] However, with 12.1 years of follow-up, higher leptin correlated with worse DDFS and OS (upper *v* lower quartiles: HR, 1.52; 95% CI, 1.09 to 2.11 for DDFS; and HR, 1.71; 95% CI, 1.30 to 2.24 for OS). In a separate study involving 747 women, leptin did not influence the risk of relapse with short follow-up (5 years), whereas increased adiponectin[30] was associated with a reduced risk of recurrence in estrogen receptor (ER)– and progesterone receptor–negative (but not positive) patients. In another cohort study involving 527 women, higher adiponectin correlated with longer survival (greater than *v* less than median level: HR for death, 0.39; 95% CI, 0.15 to 0.95).[29] Given these observations and the disparate biologic effects of these factors, it has been suggested that the ratio of adiponectin to leptin should be used when evaluating BC risk.[54]

Colorectal Cancer

Adipokines are also implicated in the development and severity of colorectal cancer. A recent review and meta-analysis concluded that adiponectin demonstrated a protective effect (attenuated by BMI and waist circumference) on the risk of colorectal cancer, whereas no association was observed for leptin.[55] Adiponectin may impact colorectal cancer recurrence, because presurgical adiponectin levels were found to be lower in patients experiencing recurrences versus patients without recurrence.[33] Serum adiponectin levels were also inversely associated with tumor grade.[33,34] In contrast, patients with favorable tumor characteristics (eg lower stage, lymph node negativity) exhibited lower leptin levels compared with patients with unfavorable characteristics.[35]

PC

There is little evidence that adiponectin is related to risk of PC (overall or the aggressive subtypes).[36,37] Adiponectin has been inversely associated with PC stage in overweight or obese men, but not normal-weight men,[38] and with risk of future development of high-grade or lethal cancer.[36]

Leptin was not linked to overall PC risk in one study,[36] whereas in another study,[56] higher leptin was affiliated with higher grade PC or intraepithelial neoplasia (*v* controls) in men with normal (but not increased) prostate volume. Other studies indicate that leptin does not correlate with PC stage at diagnosis.[38]

REPRODUCTIVE HORMONES AND CANCER OUTCOME

SHs, including estrogens and androgens, have been associated with both BMI and the risk of BC.[57] Specifically, estrone (E1), estradiol (E2), and testosterone have been significantly associated with both BMI and BC risk.[58,59] In contrast, SH influence on BC prognosis is less well established. Six independent studies reported that SH levels were associated with BC prognosis in women.[60-65] These studies were quite variable in terms of design, sample size, time of SH sample collection in relation to BC diagnosis, study end points, and, in some cases, even clinical findings. Among the six studies, five measured outcome from BC diagnosis and ranged in size from 92[60] to 774 patients,[61] with follow-up between 5,434 person-years[61] and 14.5 years.[65] The one nested cohort study comprised 4,600 women who were observed for 19 years.[64] All except one study[62] measured E2, three studies measured testosterone,[62,64,65] and one study measured E1, E2, and estrone sulfate.[60] The studies are summarized in Table 2.

For testosterone, one study described a statistically significant association between high ($\geq$ 0.40 ng/mL) testosterone and lower BC event-free survival (HR, 1.77; 95% CI, 1.06 to 2.96),[62] whereas a second study with a similar number of outcome events found no association.[65] Even less concordance was seen for estrogen. Two studies found higher E2 levels associated with worse prognosis[60,63] for ER-negative disease[63]; Lønning et al[60] reported that the disease-free interval correlated negatively with E2 ($P < .05$) and estrone sulfate ($P = .025$) levels, and Kim et al[63] reported that metastases were more likely to occur in women with ER-negative cancers who had high E2 levels (HR, 3.32; 95% CI, 1.05 to 10.51; $P = .04$). Findings were quite different in other reports. One study found that women with high E1 levels and with ER-negative disease had lower BC-specific mortality,[65] whereas another study found a trend for improved prognosis in premenopausal women with high E2,[61] and a third demonstrated no relationship between death from BC

Table 2. Studies of Sex Hormones and BC Outcome

Study	Sample	Steroids Measured	Follow-Up	Outcomes	Results
Lønning et al,[60] 1996	92 postmenopausal women with relapsed BC	E1, E2, E1S; samples obtained at relapse	Not stated	DFI; length of DFI in the subgroup of patients in whom this extended > 2 years (DFI)	E1S ($P < .025$) and E1S/E1 ratio ($P < .005$) correlated negatively with DFI; E1S ($P < .025$) and E2 ($P < .05$) and E2/E1 and E1S/E1 ratios ($P < .05$) correlated negatively with DFS
Holmberg et al,[61] 2001	774 pre- and postmenopausal women with BC	E2; samples obtained 1-2 days preoperatively	5,435 person-years	BC-specific mortality	Only premenopausal women with E2 > 500 pmol/L had tendency for improved prognosis (HR, 0.7; NS)
Micheli et al,[62] 2007	194 postmenopausal women with T1-2N0M0 BC	Testosterone; samples obtained 3 months after surgery	14 years	Event-free survival (any cancer)	High testosterone (≥ 0.40 ng/mL) was associated with lower BC event-free survival (adjusted HR, 2.05; 95% CI, 1.28 to 3.27); high testosterone was also associated with higher risk of BC events, relapse, and second primary cancer (adjusted HR, 1.77; 95% CI, 1.06 to 2.96)
Kim et al,[63] 2013	313 postmenopausal women with BC	E2, FSH; samples obtained within 3 months before surgery	52 months	Metastasis-free survival	In ER-negative tumors, high E2 level was associated with worse metastasis-free survival (HR, 3.32; 95% CI, 1.05 to 10.51; $P = .04$)
Benn et al,[64] 2015	4,716 women in the Copenhagen City Heart Study	E2 (measured in 4,600 patients); testosterone (measured in 4,716 patients); samples obtained at baseline study entry	19 years	Ischemic heart disease or death from any cause; 144 BC deaths were reported	Highest testosterone quintile (with middle as reference) had an increased risk of BC death (HR, 1.55; 95% CI, 0.81 to 2.97; $P = .06$); E2 was not associated with risk of BC death
Duggan et al,[65] 2016	358 postmenopausal women with stage I-III BC	E1, E2, testosterone, SHBG; samples obtained 30 months after diagnosis	14.5 years	BC-specific mortality and all-cause mortality	Log-transformed SHBG was associated with reduced BC-specific mortality (HR, 0.48; 95% CI, 0.26 to 0.89) and all-cause mortality (HR, 0.64; 95% CI, 0.43 to 0.97); E1 was associated with reduced BC-specific mortality for ER-negative tumors (HR, 0.16; 95% CI, 0.05 to 0.63); testosterone was not associated with BC-specific mortality

Abbreviations: BC, breast cancer; DFI, disease-free interval; DFS, disease-free survival; E1, estrone; E2, estradiol; E1S, estrone sulfate; ER, estrogen receptor; FSH, follicular-stimulating hormone; HR, hazard ratio; NS, not significant; SHBG; sex hormone binding globulin.

and E2 levels.[64] These mixed results for SH association with BC prognosis stand in contrast to the strong and consistent findings seen with regard to BC incidence where higher E1 and E2 levels were associated with higher incidence.

For other cancers, including endometrial cancer and PC, where incidence has been associated with SH levels, our review found no reports examining associations with outcome (recurrence or survival). In the Women's Health Initiative observational study cohort of 93,676 postmenopausal women, higher E2 levels were strongly associated with endometrial cancer incidence.[66] Surprisingly, low testosterone levels[67] and low bioavailable testosterone levels[68] have been significantly associated with higher PC risk.

THERAPEUTIC OPTIONS

The targeting of insulin and metabolic-related factors is of interest as a means of preventing cancer and improving prognosis. Although the association of these metabolic markers with cancer outcome was found in observational studies, and these findings are consistent with preclinical reports, these observations are subject to bias and confounding; thus, a causal relationship cannot be assumed. Consequently, evaluation of pharmacologic therapies and lifestyle interventions is warranted. Metformin, a biguanide used in the treatment of type 2 diabetes, has emerged as a potential anticancer agent. Multiple clinical and epidemiologic studies indicate that metformin use by patients with diabetes is associated with decreased cancer incidence and cancer-related mortality.[69-71] Preclinical studies focusing on a range of cancer types have characterized the direct effects of metformin, which involve activation of AMPK and reductions in mTOR signaling, protein synthesis, and cell proliferation.[72-74] After the success of metformin in vitro, in addition to strong retrospective clinical and epidemiologic data, numerous early-phase clinical trials were initiated to evaluate the efficacy of metformin in the treatment of various cancers including those of the breast, prostate, colon, and pancreas.[75-79] Many of these studies focused on assessing the direct, AMPK-mediated effects of metformin in tumor tissue but also revealed the importance of the insulin-mediated, indirect effects of the drug. Indeed, the ability of metformin to lower insulin proved to be an important aspect of its mechanism of action in patients with BC, in whom decreases in circulating insulin, reductions in tumor-specific expression of the insulin receptor, and reductions in the phosphorylation of Akt and ERK1/2 in tumors were observed.[22]

Larger phase II and III trials have also recently been launched to evaluate the effects of metformin on cancer outcomes. In BC, the National Cancer Institute of Canada Clinical Trials Group MA.32 study will assess the effectiveness of metformin versus placebo on invasive disease-free survival and other outcomes in 3,649 nondiabetic women.[80] Final results will not be available for

approximately 3 years; however, an assessment of the impact of 6 months of metformin administration on metabolic factors in the first 492 patients revealed beneficial effects on body weight, insulin, glucose, leptin, and C-reactive protein.[80] The Gynecologic Oncology Group and National Cancer Institute are conducting a randomized phase II/III trial examining the effects of chemotherapy (carboplatin and paclitaxel) plus metformin or placebo on progression-free survival and OS in patients with stage III or IV endometrial cancer.[81] In PC, the Metformin Active Surveillance Trial (MAST), a randomized, double-blind, placebo-controlled trial of metformin is enrolling men with low-risk PC to investigate whether metformin can reduce time to progression.[82] In addition to pharmacologic therapies targeting obesity and associated factors, numerous lifestyle interventions are also being investigated and are discussed in detail elsewhere in this Special Series issue.[83]

SUMMARY AND OUTLOOK

Alterations in adipokines (leptin and adiponectin), insulin metabolism (hyperinsulinemia and dysglycemia), inflammation, and SH (estrogens, androgens, and testosterone) are commonly observed in obese patients and may influence carcinogenesis. Numerous clinical studies have identified associations of these obesity-related factors with increased risk and poor outcome for several cancers, particularly those of the breast, colon, and prostate. However, future studies are needed to comprehensively characterize the effects of obesity on cancer initiation, development, and progression. In particular, clinical trials incorporating weight loss, either through lifestyle interventions or pharmacologic agents, are needed to determine whether the effects of obesity on cancer risk and outcomes can be reduced or reversed. Such focused research will aid in the development and implementation of effective therapeutic strategies targeting obesity for the treatment and prevention of cancer.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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Association of Obesity-Related Metabolic Disruptions With Cancer Risk and Outcome

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The rationale for a role for diet and nutrition in the prevention and treatment of cancer

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There is considerable evidence to support dietary recommendations for prevention of cancer as well as for patients undergoing or recovering from cancer treatment. We consider here implications from human, animal and in-vitro studies of the effects of dietary factors (macronutrients and micronutrients-phytochemicals) on cancer. An important epidemiology study, the China Project found a significant correlation between disease incidence and markers of animal product consumption. Evidence of the role of animal protein in the promotion of cancer also comes from animal studies. Food restriction has been shown in human and animal studies to slow cancer progression. Phytochemicals from whole plant foods are protective against oxidative stress, inhibit cell proliferation, induce cell-cycle arrest, and apoptosis, act as antiangiogenesis factors, and inhibit cyclooxygenase-2, which has been related to metastasis. Some mechanisms that mediate the effect of diet on cancer involve cell signaling through insulin factors and mammalian target of rapamycin, a nutrient sensing complex related to growth, altered gene expression through epigenetics, and the effects of microbial metabolites produced by the gut microbiota that is strongly influenced by dietary factors. The evidence accumulating for many years indicates that diet, what we eat every day, can affect disease. Besides preventing the development of cancer, this could also be harnessed to positively influence treatment outcomes as well as prevent recurrence. As research strategies developed for drug studies are not appropriate, it is important that new methodologies be developed to study these effects. *European Journal of Cancer Prevention* **00:000–000**

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Introduction

Advances are continually being made in imaging technology and applied to cancer diagnosis and monitoring. Coupled with the Precision Medicine Initiative, which aims to select therapies most appropriate for individual patients, these technologies offer the possibility of early diagnosis and improved survival. However, as exciting as these advances are, they do not address the fact that a large percentage of cancer occurrences are affected by extrinsic (lifestyle) factors (Wu *et al.*, 2016). In the case of cardiovascular disease (CVD), Dean Ornish *et al.* (1990) and others have established that diet and lifestyle changes can prevent progression and even reverse CVD. Ornish *et al.* (2005) have also focused on prostate cancer, showing that intensive lifestyle changes resulted in significant decreases in serum prostate-specific antigen compared with conventional prostate treatment. Fontana *et al.* (2006) report that a low-protein low-calorie diet and exercise training are associated with low plasma growth factors linked to an increased risk of cancer. Evidence from other studies suggests that the most important lifestyle factor is diet (Barnard *et al.*, 2003).

Much of the early work relating diet and cancer focused on the intake of macronutrients. Macronutrients consist of protein, carbohydrates (including fiber), and fats, and are necessary for energy and structure. In particular, fat intake has been the focus of a number of studies. Micronutrients (such as phytochemicals) make up the other dietary category and include vitamins, antioxidants, and other molecules that support metabolic function. Clues on the impact of macronutrients on cancer risk can be found in studies of individuals following vegetarian or vegan diets, which reduce or eliminate consumption of animal products (protein and fat). A recent meta-analysis of observational studies found that vegan diets conferred a significantly reduced risk of incidence from total cancer (Dinu *et al.*, 2016). In addition to a reduction in animal products, these diets most likely represent an increase in micronutrient consumption through increases in fruits and vegetables. Here, we consider the possibility that a diet limiting animal products, consuming a variety of

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whole plant foods (the main source of micronutrients), and practicing some form of limited fasting or time restricted eating can influence cancer development. Some of the mechanisms by which diet is believed to interact with cancer are discussed. Below, we present a brief summary of the evidence. A more extensive discussion and references can be found online in Supplementary Material (Supplemental digital content 1, *http://links.lww.com/EJCP/A195*).

Epidemiology and clinical studies related to macronutrients

Early epidemiology studies provide clues on the relationship between diet and disease. Post-war Japanese had age-adjusted mortality from common cancers 5–10-fold lower than in the USA at the same time (McCarty, 2014). Their diet was quasi-vegan, with 6% of total calories from animal products. Other studies exploring the differences in cancer rates between immigrants to the USA and rates in the native countries suggest diet and nutrition to be important elements.

A study carried out in China in the 1970s and the 1980s (The China Project) found associations between diet and cancer as well as other diseases (Campbell *et al.*, 1998). The factors that correlated with disease were plasma cholesterol and blood urea nitrogen; both correlate with intake of animal products (Campbell *et al.*, 1998).

In the 1970s and the 1980s, dietary fat and its relation to disease became a focus of nutrition committees in the USA. The link between fat intake and cancer risk was supported by the observation of a positive correlation of breast cancer with total fat intake across countries. However, the correlation only holds for animal fat intake (Carroll, 1975). Two studies exploring whether lower fat intake would be associated with a lower rate of breast cancer did not find a significant reduction (Willett *et al.*, 1992; Patterson *et al.*, 2003). A confounding factor is the dietary changes used to achieve the lower fat. Lower fat meats (e.g. skinless chicken breast) contain protein and significant levels of cholesterol, which can both be related to cancer. That cholesterol is a potential factor in breast cancer development is reviewed by Danilo and Frank (2012). An association of cholesterol and cholesterol esters with breast and other cancers is discussed in a review by Antalis and Buhman (2012).

The marked increase in breast cancer deaths in Japan, China, and Korea from 1975 to the present has occurred with the adoption of a 'western' lifestyle, particularly diets with a high intake of animal foods. Although this does not prove a cause–effect relationship, it is suggestive (Chlebowski, 2013).

Fiber, another macronutrient, was not found to be protective against cancer in a report published in 1982 by the National Academy of Sciences (USA); however, recent meta-analysis studies suggest positive effects of high fiber intake on colorectal cancer (CRC) (Aune *et al.*, 2011). The connection between diet and CRC is believed to be mediated by microbial metabolites and the composition of the gut microbiota is strongly influenced by dietary factors (Louis *et al.*, 2014). High meat intake has been associated with increased occurrences of CRC (Potter *et al.*, 1993).

Mechanisms (macronutrients)

Protein

Diet and cancer incidence has been explored through experimental research in rodents. Early work in the 1990s as well as a recent study showed a positive correlation between the percentage of calories allocated to protein and incidence of cancer in rat models (Youngman and Campbell, 1991; Fontana *et al.*, 2013). In both studies, replacing animal protein with plant protein inhibited tumor growth.

Mechanisms suggested to mediate the effect of diet on cancer include signaling pathways involving mammalian target of rapamycin (mTOR in complexes mTORC1 and mTORC2). mTOR responds to nutrients (glucose and amino acids), growth factors [e.g. insulin and insulin-like-growth factor (IGF-1)], energy, and stress promoting cell growth when activated and suppressing it when inhibited (low nutrient) (Zoncu *et al.*, 2011). Some amino acids, in particular leucine, high in dairy and meats, appear to act more strongly on mTORC1 signaling (Yan and Lamb, 2012). The differential effect of plant versus animal protein is likely because of their different amino acid profiles. Protein intake is an important regulator of circulating IGF-1, which is associated with increased risk of cancer. These systems evolved to promote survival under conditions of nutrient deprivation. The constant availability and intake of food today leads to chronic activation of mTOR and potentially to aberrant cell responses (Zoncu *et al.*, 2011).

Cholesterol

Cholesterol is a lipid important in the formation and stabilization of membranes. Abnormal cholesterol metabolism has been observed in experimental and human tumors (Tosi and Tugnoli, 2005; Antalis and Buhman, 2012). Antalis and Buhman (2012) reviewed epidemiology and mechanistic studies of the relationship among lipoproteins, cholesterol, and cancer.

Fiber

The positive effect of fiber intake on CRC could be associated with rapid intestinal transit times, leaving less time for carcinogens to act upon the mucosa. Another mechanism in reducing CRC risk is through the production of butyrate as a metabolite from non-digestible fiber (Roediger, 1980). Low amounts of butyrate are rapidly metabolized by the mitochondria of the colonocytes, leading to increased proliferation, whereas an

excess of butyrate accumulating in the nucleus acts through an epigenetic mechanism as a histone deacetylase inhibitor reducing proliferation and increasing apoptosis (Bultman, 2014).

Mechanisms (micronutrient intake)

The focus on macronutrients and their restriction, however, is only part of the story. Epidemiology studies have consistently shown that diets high in fruits and vegetables are associated with a decreased risk of diseases such as cancer and CVD (Willett, 1995). Phytochemicals, the bioactive compounds in food, are protective against oxidative stress from free radicals and restrict the development and progression of cancer by various mechanisms. Dietary components can also affect the expression of genes through epigenetics. The primary mechanisms are DNA methylation, histone modifications through acetylation, and deacetylation resulting in transcriptionally active or inactive chromatin, respectively, and RNA silencing through micro RNAs (Supic *et al.*, 2013). A recent review explores phytochemical effects on the epigenome (Zam and Khadour, 2017).

Some specific examples of phytochemicals with anticancer properties include inositol hexaphosphate (phytic acid or IP6) found in grains, nuts, seeds, and legumes, lignans, and isoflavones, phytoestrogens found in high concentrations in flaxseeds and many other foods (Buck *et al.*, 2010), and salicylates from plants and spices. Salicylic acid acts to inhibit the production of prostaglandins from catalysis of arachidonic acid by cyclooxygenase-2 (Duthie and Wood, 2011). Prostaglandins can act on the lymphatic vasculature, leading to increased metastasis (Karnezis *et al.*, 2012).

A review of the effect of dietary phytochemicals on angiogenesis in breast cancer was reported in 2012 [Reuben *et al.* (2012)]. However, angiogenesis is a component of all tumor growth, not just breast cancer. The catechins in tea, curcumin, ellagitannins, and soy isoflavones are some of the phytochemicals shown to inhibit proliferation through angiogenesis (Reuben *et al.*, 2012).

Berries, strawberries, and mushrooms are examples of whole foods with anticancer properties (Chen *et al.*, 2012). The common white button mushroom can significantly inhibit aromatase (the enzyme that converts androgens into estrogen) activity and could play an important role in the prevention of primary or recurrent breast cancer.

Mechanisms (food restriction)

Food restriction in general has been shown to be beneficial in the treatment of disease. Tumor metabolism has been a potential target since the discovery by Otto Warburg that many cancer cells show increased glucose uptake and fermentation to lactate even in the presence of oxygen (aerobic glycolysis). Animal studies of fasting protocols have shown positive results in prevention as well as treatment in cancer. Fasting, calorie restriction, and a carbohydrate-limited ketogenic diet have been used to slow cancer progression (Mukherjee *et al.*, 2002; Hursting *et al.*, 2003; Longo and Fontana, 2010). The mechanism for this is suggested to be a reduction in free IGF-1 (Raffaghello *et al.*, 2008). These positive effects of fasting in cancer treatment and prevention are aligned with the fact that obesity is linked to an increased incidence of cancer and worse outcomes in response to treatment (Khandekar *et al.*, 2011).

Exploring mechanisms of food restriction several studies have reported effects of fasting on the immune system (Cheng *et al.*, 2014; Dibiase *et al.*, 2016; Pietrocola *et al.*, 2016). A trial in healthy human individuals of a fasting mimicking diet resulted in reductions of blood glucose and IGF-1, both of which remained lower after resuming the normal diet (Brandhorst *et al.*, 2015).

A recent report suggests that restricting food consumption to certain hours of the day may be beneficial in preventing cancer recurrence (Marinac *et al.*, 2016). This suggests an alternative that may be easier to implement and more acceptable than traditional fasting.

Supplements versus whole foods

The β-carotene lung cancer studies present a cautionary tale in the use of supplements. Although observational studies indicated a lower risk associated with carotenoid foods, trials using β-carotene supplements showed no benefit and suggested an adverse outcome with more cancers in the supplement group (Albanes, 1999). A more recent review of antioxidant supplements found no evidence supporting primary or secondary prevention (Bjelakovic *et al.*, 2012). High folate intake through supplements may also be problematic as supplementation can promote progression of existing cancers (Kim, 2007). On the positive side, a meta-analysis found a reduction in all-cause mortality with vitamin D_3 (Chowdhury *et al.*, 2014). A supplement that may be of benefit is S-adenosylmethionine, an endogenous universal methyl donor reported to block skeletal metastasis in an animal model (Shukeir *et al.*, 2015). Although there is evidence that some supplements may be of benefit, their use should be approached with caution, particularly the intake of excessive amounts of single nutrients. The combination of phytochemicals in food has a synergistic effect so that the combined activity is greater than any one (Liu, 2004).

Conclusion

A previous review and a recent meta-analysis conclude that vegetarian/vegan diets are a useful strategy for reducing the risk of cancer (Lanou and Svenson, 2010; Dinu *et al.*, 2016). In this review, we have brought together data from many different types of studies relating to diet and cancer. In some cases, the epidemiology and intervention studies presented conflicting

results because of the focus on fat without controlling for animal products. Also, those studies carried out in Western countries generally did not have the range of fat or protein intake to observe differences observed in countries with a lower consumption of animal products. The evidence points to a diet with minimal animal products. This reduces both animal protein and cholesterol, and leaves room for a greater consumption of the micronutrients from fruits and vegetables. A common misperception is that adequate protein can only be obtained from animal sources. Dietary analyses of more than 70 000 participants from the Adventist Health Study 2 found that even those who ate animal protein less than once a month consumed 72.3 g/day, more than the recommended dietary allowance of 45–56 g/day (Rizzo *et al.*, 2013). The most important aspect of diet with respect to cancer (and health) is the phytonutrient content. Diet in addition to some food restriction (or just extended fasting between meals) can potentially decrease the risk of cancer as well as increase the effectiveness of treatment and decrease recurrence.

Some foods that appear to be particularly beneficial include fruit, especially berries, a variety of vegetables (especially greens), legumes (beans – black, lentils, etc.), nuts and seeds (especially flaxseed), mushrooms, onions, garlic, spices (e.g. turmeric and other Indian spices), herbs, and green tea.

Experimental protocols to test the effect of diet on disease are more difficult to design than those used for drugs. These difficulties are discussed in an article in Nature on the use of diet to alleviate symptoms of multiple sclerosis (Gupta, 2016). Nevertheless, the evidence that has been accumulating for many years indicates that diet can affect disease. It is therefore important that research strategies to study these effects be developed.

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Conflicts of interest

There are no conflicts of interest.

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Ministero della Salute

DIREZIONE GENERALE per L'IGIENE E LA SICUREZZA
DEGLI ALIMENTI E LA NUTRIZIONE

LINEE DI INDIRIZZO SUI PERCORSI NUTRIZIONALI NEI PAZIENTI ONCOLOGICI

Approvato in sede di Conferenza Stato Regioni, con Accordo (Rep. Atti n. 224/CSR) del 14/12/2017

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INDICE

1. RAZIONALE

Le neoplasie rappresentano la seconda causa di morte a livello mondiale. Nonostante lo sviluppo scientifico il carico mondiale dei tumori è in continuo aumento e si ritiene possa raddoppiare entro il 2030.

Le alterazioni dello stato nutrizionale sono altamente prevalenti nei malati oncologici e la malnutrizione per difetto è considerata "malattia nella malattia", con cui si stima convivano 33 milioni di persone in Europa (con patologie croniche e oncologiche), con un costo sociale di circa 120 miliardi di euro.

La scarsa attenzione per lo stato nutrizionale in corso di terapie oncologiche, ampiamente documentata nella letteratura internazionale, determina gravi conseguenze non solo sulla qualità della vita dei pazienti, ma anche sulla loro capacità di aderire ai diversi trattamenti proposti, con una conseguente peggior prognosi. Del resto, anche quando lo stato di malnutrizione viene riconosciuto, spesso non vengono attuate in maniera adeguata le necessarie misure correttive. Appare pertanto essenziale che la valutazione nutrizionale costituisca un elemento imprescindibile nell'approccio al paziente affetto da patologia oncologica, già nel corso della prima visita.

Queste osservazioni hanno evidenti implicazioni di politica sanitaria perché la programmazione e l'organizzazione dei servizi dovrebbero garantire un "**percorso nutrizionale del paziente oncologico"**, anche attraverso protocolli di collaborazione con i pediatri di libera scelta, con i medici di medicina generale e attraverso i contatti con gli specialisti del settore, per assicurare la continuità della cura indipendentemente dalla differenziazione e dall'organizzazione dei servizi a livello territoriale.

La consapevolezza della prevalenza e delle conseguenze negative della malnutrizione nel malato oncologico è ancora molto scarsa sia tra gli operatori sanitari sia tra i pazienti, ma un corretto e consapevole impiego delle conoscenze e delle tecniche relative ad un'adeguata nutrizione clinica in questi pazienti avrebbe una ricaduta positiva, con un favorevole impatto sugli esiti e sulla qualità di vita di questi pazienti e sulla spesa sanitaria.

Attualmente, in Italia, la gestione nutrizionale del paziente oncologico è molto variabile da regione a regione e non sempre la presa in carico e il supporto nutrizionale risultano appropriati. Per questo si rende opportuno un documento di indirizzo nazionale che proponga precisi standard di appropriatezza degli interventi nutrizionali nei pazienti oncologici, con l'individuazione di "Percorsi di Nutrizione Clinica nella gestione del malato oncologico e del soggetto che ha superato la malattia" sia in ospedale che sul territorio, prevedendo "modelli organizzativi che integrino le attività a livello ospedaliero, ambulatoriale e domiciliare.

Per questo il Ministero della salute ha ritenuto opportuno realizzare un documento nel quale si affrontano gli aspetti relativi agli screening e alla valutazione nutrizionale (che devono entrare a far parte della valutazione multidimensionale del malato oncologico, durante tutto il percorso terapeutico, "attivo" e "palliativo"), nonché alla presa in carico nutrizionale del malato oncologico (che va effettuata al momento della diagnosi di malattia e proseguita successivamente, nel cosiddetto "percorso parallelo metabolico-nutrizionale per il malato oncologico").

2. OBIETTIVI

Il presente documento, che tiene conto anche della Carta dei Diritti del Paziente Oncologico all'appropriato e tempestivo supporto nutrizionale, sottoscritta nel 2017 da AIOM, FAVO e SINPE, e del contributo delle Società Scientifiche ADI, SINUC e SISA e di Associazioni di pazienti, ha l'obiettivo di:

- definire lo screening nutrizionale (validato per la valutazione dello stato nutrizionale) e i bisogni specifici in ambito nutrizione alla diagnosi, durante il percorso terapeutico, al follow-up e per la prevenzione terziaria;
- presentare il Modello per la risposta organizzativa attraverso un percorso integrato che permetta lo svolgimento di un programma nutrizionale personalizzato e associato al trattamento oncologico sin dal primo accesso ai servizi (Ospedale e Day Surgery, Reti territoriali, Continuità assistenziale MMG e PLS, Assistenza domiciliare, Nutrizione artificiale), applicando anche approcci innovativi (quali i rapporti tra terapia oncologica e microbioma);
- descrivere la formazione ed informazione agli operatori sanitari.

Gli effetti attesi di queste raccomandazioni sono quelli di ridurre le complicanze mediche, conseguenti alla malnutrizione e di facilitare il recupero dello stato nutrizionale e della salute fisica, che costituiscono tappe essenziali nel processo di guarigione.

3. INTRODUZIONE

L'estrema variabilità dell'incidenza della malnutrizione nei pazienti oncologici depone per una grande variabilità nella percezione clinica di questo rilevante problema assistenziale. Infatti, a seconda del contesto sanitario e degli strumenti utilizzati per valutare la malnutrizione si osserva una prevalenza della malnutrizione compresa tra il 25% e il 70% in diversi Paesi europei ed extra-UE.

I pazienti oncologici risultano essere quelli che presentano più frequentemente problemi nutrizionali, anche in fasi di malattia estremamente precoci, come subito dopo un intervento chirurgico attuato con intento radicale e, quindi, in assenza di metastasi.

Tra i pazienti neoplastici che perdono peso corporeo, il 20-30% muore per le conseguenze dirette ed indirette della malnutrizione. Frequenza e gravità della perdita di peso variano a seconda del tipo di tumore: l'80% dei pazienti con neoplasia del tratto gastrointestinale superiore ed il 60% di quelli con neoplasia polmonare presentano perdita di peso già al momento della diagnosi. Inoltre, perdita di peso si verifica nel 72% delle neoplasie pancreatiche, nel 69% delle neoplasie esofagee, nel 67% delle neoplasie gastriche, nel 57% dei tumori del distretto testa-collo, nel 34% delle neoplasie del colon retto, nel 31% dei casi di linfoma non-Hodgkin.

La presenza di neoplasia può avere significative conseguenze negative sullo stato nutrizionale del paziente e la malnutrizione per difetto quali-quantitativa, che frequentemente si instaura, non può e non deve essere considerata un effetto collaterale non prevenibile e non trattabile.

Nel paziente oncologico la malnutrizione, nelle sue diverse forme, ha un impatto negativo sulla prognosi, sulla risposta e tolleranza ai trattamenti e sulla qualità di vita. La malnutrizione per difetto è un vero e proprio predittore indipendente di aumentata morbidità e mortalità e la perdita di peso corporeo e di massa muscolare inducono un maggiore rischio di tossicità da chemioterapia.

In altri casi, le terapie antitumorali e la chemioterapia possono determinare un aumento di peso e la malnutrizione per eccesso, con conseguente sovrappeso e/o obesità un evento frequente nelle donne

trattate per neoplasia mammaria, rappresentando un fattore di rischio per sindrome metabolica e per recidiva di malattia.

Gli interventi di nutrizione clinica devono quindi essere attuati per evitare, in tali tipologie di pazienti oncologici, che sovrappeso e obesità intervengano come fattori prognostici negativi.

In tutte le diverse situazioni, l'intervento nutrizionale deve essere tempestivo e costituire, sin dal primo contatto del paziente con le strutture sanitarie, una parte integrante del percorso diagnostico-terapeutico-assistenziale che costituisce l'insieme delle cure oncologiche, ed essere personalizzato, dinamico e sempre finalizzato a prevenire e correggere la malnutrizione.

Inoltre, considerata la patogenesi multifattoriale della malnutrizione (per difetto e per eccesso) nel malato oncologico, è indispensabile considerare le cure nutrizionali come parte integrante di un percorso che abbia come presupposto un approccio multimodale e multiprofessionale.

All'interno di questo percorso devono essere prese in considerazione, accanto al monitoraggio dello stato metabolico-nutrizionale, tutte le opzioni terapeutiche potenzialmente attuabili (counseling nutrizionale, integrazione nutrizionale orale, nutrizione enterale, nutrizione parenterale), rispettando un continuum terapeutico che prenda di volta in volta in considerazione la dinamica delle esigenze del malato oncologico. E' infine importante prevedere modalità per il monitoraggio e l'eventuale trattamento nutrizionale anche nei soggetti che hanno superato la malattia per la prevenzione a medio e a lungo termine delle complicanze legate alla malattia oncologica ed ai suoi trattamenti.

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4. SCREENING NUTRIZIONALE DEL PAZIENTE ONCOLOGICO

1- I fattori che incidono sullo sviluppo della malnutrizione e sul calo ponderale, spesso associato alla patologia neoplastica, possono essere plurimi e riconducibili sia alla stessa patologia oncologica (malassorbimento o localizzazione della neoplasia, o metastasi), sia all'intervento terapeutico (chemioterapia, radioterapia, chirurgia), che può comportare frequentemente sintomatologia contraddistinta da anoressia, nausea, vomito, disfagia, odinofagia e che può determinare un'alterazione dello stato di nutrizione o un peggioramento dello stesso. Anche i fattori psicologici possono incidere in maniera negativa sull'assunzione di cibo peggiorando l'eventuale stato di malnutrizione del paziente.

Le rilevanti finalità dello screening dello stato nutrizionale e della terapia nutrizionale specifica nel paziente oncologico sono quindi mirate a:

1. prevenire e trattare la nutrizione sin dalla fase iniziale della diagnosi di patologia oncologica;
2. ottimizzare e consolidare gli effetti della terapia antitumorale;
3. limitare gli effetti collaterali della terapia antitumorale;
4. migliorare la qualità di vita
5. prevenire complicanze delle terapie antitumorali e chemioterapiche quali obesità, sovrappeso e sindrome metabolica.

E' quindi necessario che ogni paziente venga valutato nei diversi momenti del percorso diagnostico e terapeutico per cercare di ottimizzare lo stato di nutrizione e per migliorare la qualità della vita del paziente, già compromessa dalla patologia oncologica. Quindi la valutazione dello stato nutrizionale deve essere effettuata in tutti i pazienti al momento della diagnosi e ripetuta ad intervalli predefiniti. Anche nel caso di un'iniziale assenza di modificazioni dello stato nutrizionale, è importante un attento controllo poiché tale condizione può essere modificata dagli effetti secondari delle terapie e/o

dall'evoluzione della malattia. Una valutazione tempestiva dello stato nutrizionale è necessaria per evidenziare le alterazioni in fase precoce, con lo scopo di aumentare l'efficacia degli interventi terapeutici.

La valutazione dello stato nutrizionale è un processo multimodale, basato sulla storia clinica, sui trattamenti effettuati ed in corso, sulla presenza di sintomi non controllati, sulla rilevazione dei parametri antropometrici e su test di laboratorio.

Mediante un corretto screening nutrizionale si possono identificare, in maniera rapida, quei pazienti con caratteristiche comunemente associate a problematiche nutrizionali, che devono essere sottoposti ad una valutazione globale dello stato nutrizionale.

2 - Già alla diagnosi di patologia tumorale i pazienti potrebbero presentare uno stato di malnutrizione provocato dalla patologia e sarà quindi fondamentale un'attenta valutazione. Lo screening nutrizionale si avvale di test e questionari relativamente semplici, veloci e facili da usare, compilabili dai pazienti e dai familiari. La valutazione dello stato di nutrizione dovrà essere anche stimata mediante un'attenta analisi clinica dei pazienti.

3 - Sulla base dello screening effettuato e dell'eventuale evidenza di malnutrizione, sarà necessario un intervento correttivo: la terapia nutrizionale potrà essere di tipo preventivo se il paziente inizia ad essere seguito dalla diagnosi oppure di supporto se riscontrato in corso di terapie, quali nelle chemio- e/o radioterapie a elevata tossicità intestinale, oppure nelle neoplasie delle prime vie digestive. Lo scopo dell'intervento nutrizionale mirerà a minimizzare o evitare la compromissione dello stato di nutrizione durante la terapia per migliorare lo stato fisico del paziente e migliorare l'efficacia della terapia.

Il follow up, con la valutazione dello stato nutrizionale, dovrà essere effettuato ogni qual volta ci si renda conto di alterazioni del peso significative del paziente: in particolare, il peso corporeo e il conseguente calcolo dell'indice di massa corporea (IMC), e eventualmente l'anamnesi alimentare,

permetteranno di valutare eventuali iniziali segnali di malnutrizione del paziente affetto da patologia oncologica.

Primo accesso ai servizi (Accesso in Ospedale, pre-ospedalizzazione chirurgica, valutazione inter-disciplinare nei Gruppi di Lavoro per patologia)

I Pazienti oncologici, indipendentemente dallo stadio della propria malattia (iniziale o avanzata), debbono ricevere una immediata valutazione dello stato nutrizionale come parte integrante e non eludibile del percorso diagnostico. Infatti, l'incidenza di uno stato di malnutrizione (anche inapparente ad una valutazione superficiale), di sarcopenia o di pre-cachessia può complicare un eventuale intervento chirurgico o rendere impossibile il mantenimento di un adeguato trattamento medico preoperatorio.

- **ANAMNESI e VALUTAZIONE ALIMENTARE**

  Il Patient Generated-Subjective Global Assessment (PG-SGA), il Subjective Global Assessment (SGA), il Malnutrition Screening Tool (MST) e il Mini Nutritional Assessment (MNA®) sono stati validati in ambito oncologico e hanno mostrato una sensibilità del 100% e una specificità del 92%, con un valore predittivo positivo pari a 0.8. La valutazione dei possibili motivi di un'alimentazione inadeguata (es. micosi cavo orale, nausea) sono fondamentali per determinare eventuali carenze di macronutrienti e micronutrienti ed intraprendere un intervento terapeutico mirato.

- **VALUTAZIONE CLINICO/ANTROPOMETRICA**

  Determinazione di a) peso attuale e perdita di peso nei precedenti 6 mesi, 3 mesi e nell'ultimo mese; b) altezza e circonferenza vita del paziente c) valutazione delle pliche cutanee (tricipitale, bicipitale, sottoscapolare e soprailiaca); d) performance status; e) presenza di mucositi, xerostomia, disfagia, nausea cronica, sensazione di precoce sazietà, stipsi, dolore ed

altri sintomi in grado di influenzare lo stato nutrizionale; e) Subjective Global Assessment of Nutritional Status (SGA, disponibile in molti siti, tra cui www.frontlinelabs.com);

In particolare il peso dovrà essere valutato alla prima visita e ad ogni visita di controllo per valutare in maniera precisa e puntuale eventuali variazioni con conseguente modifica dell'IMC. Variazioni significative permetteranno di iniziare una terapia nutrizionale mirata al ripristino proteico-energetico e/o idrosalino.

- **BIOIMPEDENZIOMETRIA (BIA)**

La BIA può essere utile per valutare la composizione corporea (massa adiposa e massa magra) e lo stato d'idratazione del soggetto, ma anche il metabolismo basale per stimare e effettuare un'analisi dell'introito calorico del paziente oncologico e che permetterà di strutturare interventi nutrizionali specifici mirati. Inoltre, alcuni studi recenti suggeriscono come dati ottenuti con questa tecnica diagnostica permettano di predire la risposta dei pazienti per la loro qualità di vita e anche per la mortalità.

- **ASSORBIMETRIA A RAGGI X A DOPPIA ENERGIA (DEXA)**

Nel caso in cui la/il paziente affetto da terapia oncologica debba effettuare terapia anti-ormonale (es inibitori aromatasi) sarà opportuno valutare mediante DEXA lo stato della composizione corporea in quanto tale valutazione permetterà non solo di caratterizzare e quantificare massa magra, massa adiposa, ma anche di valutare la densità minerale ossea. La valutazione della composizione corporea del paziente oncologico permette di affrontare eventuali interventi mirati per ottimizzare le diverse componenti come nel possibile caso di perdita di densità minerale ossea, ma anche per monitorizzare le specifiche terapie utilizzate.

- **Durante il percorso terapeutico per valutare lo stato nutrizionale ci si può avvalere di indici soggettivi ed oggettivi alcuni dei quali già utilizzati per lo screening dello stato di nutrizione alla diagnosi.**

A - Indici soggettivi. Per valutare la percezione del paziente riguardo al proprio stato nutrizionale, possono essere impiegati strumenti di valutazione multidimensionali oppure specifici.

Strumenti multidimensionali. Si tratta di strumenti di valutazione che prendono in esame più fattori. Tutti contengono specifici riferimenti all'appetito ed alle abitudini alimentari. I più comunemente usati nella pratica clinica sono: l'Edmonton Symptom Assessment Sistem (ESAS), il Memorial Symptom Assessment Scale, il Rotterdam Symptom Checklist e il Therapy Impact Questionnaire. Essi permettono di valutare non solo la percezione del paziente relativa alla nutrizione, ma anche la presenza e la gravità di altri sintomi che potrebbero contribuire a modificare le abitudini alimentari. Aspetto rilevante è la valutazione delle interazioni fra paziente e familiari, specie per quanto riguarda l'importanza attribuita all'alimentazione. Non è infrequente, infatti, il riscontro di famiglie all'interno delle quali l'alimentazione diventa argomento centrale di discussione, al punto da indurre nel paziente e nei familiari uno stato di ansia e depressione. Frequentemente viene usata la scala ESAS, costituita da dieci scale numeriche, con punteggi da 0 a 10, mirati a valutare la percezione di dolore, astenia, nausea, depressione, ansia, appetito, sensazione generale di benessere, dispnea, sonnolenza e qualità di vita (Tabella 109.2).

Per valutare le interazioni fra paziente e familiari, è preferibile utilizzare la Palliative Outcome Scale (POS) che, in 12 punti, valuta il dolore, il controllo dei sintomi, i bisogni del paziente e della sua famiglia, la comunicazione e l'informazione (Tabella 109.3).

Strumenti specifici. Uno strumento specifico per la valutazione dello stato nutrizionale è il Functional Assessment of Anorexia/Cachexia Treatment (FAACT, disponibile dal sito www.facit.org) o il Mini Nutritional Assessment.

B - Indici oggettivi. Una misura oggettiva dello stato nutrizionale può essere ottenuta mediante valutazioni di tipo clinico/antropometrico, biochimico e strumentale.

Valutazione clinico/antropometrica. Consiste in determinazioni già effettuate alla prima visita: a) peso attuale e perdita di peso nei precedenti 6 mesi, 3 mesi e nell'ultimo mese; b) presenza di mucositi, xerostomia, disfagia, nausea cronica, sensazione di precoce sazietà, stipsi, dolore ed altri sintomi in grado di influenzare lo stato nutrizionale; c) performance status; d) valutazione delle pliche cutanee; e) Subjective Global Assessment of Nutritional Status (SGA, disponibile in molti siti, tra cui www.frontlinelabs.com); f) valutazione dell'apporto calorico mediante diario alimentare compilato dal paziente. L'uso del diario alimentare potrebbe essere però sconsigliabile poiché vissuto, dalla maggioranza dei pazienti, come un controllo da parte dei sanitari, che spesso solleva nel contesto familiare discussioni e contrasti, portando l'alimentazione del paziente ad argomento centrale di ogni conversazione, con una conseguente accentuazione dell'ansia del paziente ed uno scadimento della sua qualità di vita. Le valutazioni clinico-antropometriche hanno il vantaggio di essere applicabili a tutti i pazienti, di essere ripetibili e poco costose.

Valutazione biochimica. Gli esami di laboratorio più comunemente impiegati nella valutazione dello stato nutrizionale sono: linfociti totali, proteine totali, albumina, rapporto albumina/globulina, pre-albumina, proteina legante il retinolo (RBP), transferrina, rapporto creatinina/altezza, transtiretina (TTR) e il Prognostic Inflammatory and Nutritional Index (PINI), basato sulla valutazione combinata di indici di alterazioni dello stato proteico e di infiammazione. Il PINI è calcolato come rapporto del prodotto tra l'alfa-1-glicoproteina acida (α1-AG) e la proteina C reattiva (PCR) ed il prodotto di albumina e prealbumina; un valore di PINI ≤ 1 è considerato normale. Il PINI fornisce informazioni utili da un punto di vista prognostico in pazienti in fase avanzata e consente di prevedere il rischio di tossicità ematologica alla chemioterapia. Si tratta, pertanto, di valutazioni dello stato nutrizionale riferite principalmente all'assetto proteico. Scopo del loro impiego è l'identificazione di alterazioni dello stato nutrizionale precocemente rispetto alla comparsa di alterazioni clinico-antropometriche. L'unico limite è relativo ai costi. In assenza di studi di farmaco-economia dedicati, si può però presumere che un'identificazione precoce di alterazioni dello stato nutrizionale consenta un

miglioramento delle condizioni generali del paziente e della sua qualità di vita, riducendo le complicanze legate alla malattia e ai trattamenti, nonché i ricoveri in ambiente ospedaliero.

Valutazione strumentale. Come già menzionato in precedenza, gli esami strumentali più comunemente usati sono: BIA e DEXA. Si tratta di esami molto specifici che consentono di valutare in maniera differenziata massa magra e massa grassa e, nel caso della DEXA, anche la densità minerale ossea.
Un possibile limite è rappresentato dai costi e dalla difficoltà di esecuzione nel contesto della routine oncologica.

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5. LA RISPOSTA ORGANIZZATIVA ATTRAVERSO UN PERCORSO INTEGRATO PER UN PROGRAMMA NUTRIZIONALE PERSONALIZZATO E INTEGRATO AL TRATTAMENTO ONCOLOGICO

Un modello organizzativo capace di assicurare ai pazienti interventi nutrizionali adeguati, tempestivi, efficaci, efficienti e sicuri deve fondarsi su gruppi di lavoro interdisciplinari e multiprofessionali, in cui le diverse figure operino in stretta integrazione specialistica.

Tali percorsi assistenziali trovano il loro presupposto operativo in una maggiore presenza di alcune figure professionali attualmente carenti in molte realtà regionali, come medici esperti in nutrizione clinica, dietisti e psicologi, che dovranno trovare adeguato e specifico riconoscimento in una aggiornata organizzazione sanitaria regionale, non solo a livello ospedaliero, ma anche territoriale.

Pur demandando alle diverse strutture assistenziali regionali una puntuale organizzazione dei PDTA nutrizionali, è opportuno identificare alcune tappe comuni che dovranno essere prese in considerazione nella attuazione pratica di misure volte ad affrontare il tema della malnutrizione dei pazienti oncologici. Appare opportuno prevedere l'inserimento di PDTA nutrizionali all'interno dei PDTA delle diverse patologie, per evitare inutili e dispendiose duplicazioni di attività per uno stesso paziente. Infatti, è possibile, oltre che auspicabile, che la valutazione dello stato nutrizionale, la definizione del piano di intervento di prima linea, la misura dei risultati conseguiti (in termini di miglioramento dello stato nutrizionale), il follow-up e l'elaborazione di modalità di intervento di livello successivo siano presenti in parallelo con quelle della valutazione dell'evoluzione della patologia oncologica di base.

Come la Legge 38/2010 ha reso obbligatorio l'inserimento della rilevazione del dolore nella cartella clinica, sarebbe importante che, indipendentemente dallo stadio di malattia e del trattamento programmato, anche l'indicazione dello stato nutrizionale dei Pazienti oncologici, possa essere considerata necessaria, con l'impiego di strumenti di misura specifici e concordati con i medici nutrizionisti, oltre che la descrizione delle misure che sono state programmate per trattare le diverse

situazioni e la risposta terapeutica a questi interventi. Questo consentirebbe infatti di ridurre l'impatto di sofferenze evitabili e consentirebbe una migliore utilizzazione delle risorse del SSN.

Attraverso la stesura di PDTA specifici interaziendali si può ovviare all'eventuale (e, purtroppo, frequente) assenza di medici nutrizionisti in tutte le Aziende sanitarie ove vengono trattati i Pazienti oncologici.

Il PDTA interaziendale, partendo da una specifica valutazione, mediante strumenti di misura riproducibili, validati e condivisi, dello stato nutrizionale e delle necessità specifiche di ogni singolo Paziente può consentire un piano di intervento personalizzato, applicabile immediatamente in tutti i contesti assistenziali ospedalieri e, successivamente, anche al domicilio del Paziente, attraverso l'assistenza territoriale, con la collaborazione dei MdMG, dei PdLS e dei medici del territorio.

Primo accesso ai servizi ospedalieri (ambulatorio oncologico o chirurgico, pre-ospedalizzazione chirurgica, valutazione inter-disciplinare nei Gruppi di Lavoro per patologia).

Valutazione dello stato nutrizionale.

I pazienti oncologici, indipendentemente dallo stadio della propria malattia (iniziale o avanzata), debbono ricevere una immediata valutazione dello stato nutrizionale come parte integrante e non eludibile del percorso diagnostico. La valutazione dello stato nutrizionale, basata sulla stretta interazione tra medici esperti in nutrizione clinica e gli specialisti coinvolti nel trattamento della singola patologia neoplastica, che avranno condiviso gli strumenti di misura più idonei per ciascuna patologia oncologica, dovrà essere riportata in maniera esplicita nella documentazione clinica, spiegata esaurientemente al paziente e ai suoi familiari e comunicata al Medico di medicina generale (MdMG). Attenzione dovrà essere posta nella valutazione della necessità di un eventuale sostegno psicologico del paziente e della sua famiglia.

Anche se spesso i pazienti e i loro familiari interpretano in modo positivo un aumento di peso in corso di patologia oncologica, non dobbiamo dimenticare che anche condizioni di sovrappeso o obesità

possono influenzare negativamente l'evoluzione della patologia oncologica, attraverso una minore efficacia di alcune terapie (basti pensare alla correlazione negativa tra inibitori della aromatasi e la sopravvivenza nelle pazienti obese operate per cancro della mammella) o la limitazione ad alcuni trattamenti medici. Anche questo disordine nutrizionale, spesso difficile da affrontare, dovrà essere oggetto di specifica valutazione interdisciplinare.

Definizione del piano nutrizionale

Riconosciuto e misurato l'eventuale deficit nutrizionale, stabilite le cause della malnutrizione e le eventuali patologie concomitanti che possano incidere negativamente, si dovrà stilare, attraverso modalità condivise, uno specifico piano di intervento, definendo il tipo di supporto nutrizionale necessario, le modalità di somministrazione, gli eventuali altri specialisti da coinvolgere, gli accertamenti ancora necessari, i presidi da utilizzare per il sostegno nutrizionale ed i tempi di verifica del conseguimento dei risultati programmati, in modo da definire la tempistica delle successive rivalutazioni clinico-strumentali e di laboratorio, oltre che l'identificazione delle terapie volte a controllare le eventuali patologie concomitanti.

In caso di sovrappeso con significative ripercussioni sulle possibilità terapeutiche, si dovranno istituire i necessari correttivi.

Programmazione del follow-up specifico.

Al termine della prima visita, si dovranno identificare le modalità ed i tempi di verifica dei risultati dell'intervento nutrizionale collegialmente proposto, condividendo il percorso con il MdMG e, in caso di necessità, con il responsabile dell'assistenza domiciliare per le necessarie integrazioni operative a livello domiciliare o attraverso strutture territoriali competenti.

Obiettivo misurabile di questa prima fase di intervento è rappresentato dalla riduzione della perdita di peso e della sarcopenia, dal miglioramento della qualità della vita e dalle variazioni delle condizioni cliniche prima dell'intervento primario (medico, chirurgico o radioterapico) sulla malattia oncologica.

Reti territoriali

Costituiscono un anello di congiunzione imprescindibile tra quanto deciso a livello ospedaliero e quanto dovrà essere attuato in periodi spesso molto lunghi presso il domicilio del Paziente.

La creazione di Reti territoriali per la nutrizione clinica (eventualmente all'interno di percorsi assistenziali già disponibili a livello regionale) consente di migliorare l'accesso, promuovere l'attivazione e l'integrazione con le reti già esistenti della terapia del dolore e delle cure palliative, garantendo ai pazienti risposte assistenziali su base regionale e in modo uniforme su tutto il territorio nazionale, con una migliore utilizzazione delle risorse economiche disponibili.

La definizione dei compiti delle diverse figure professionali coinvolte, insieme al MdMG e al Pediatra di libera scelta (PdLS), in questo processo di integrazione terapeutica rende evidente l'elevato livello di complessità organizzativa:

- Il medico nutrizionista definisce il piano nutrizionale e ne cura l'aggiornamento continuo attraverso la verifica periodica delle condizioni del paziente
- Il personale dietista collabora alla stesura del piano nutrizionale, valuta lo stato nutrizionale del paziente e ne controlla l'aderenza al programma individualizzato
- L'infermiere territoriale completa l'addestramento delle persone addette alla cura del paziente, iniziato in ambito ospedaliero, attua la terapia nutrizionale prescritta secondo protocolli validati, gestisce i presidi, controlla gli accessi enterali e parenterali, previene le complicanze locali, controllandone il trattamento, compila la cartella infermieristica e cura la tenuta della cartella clinica a livello domiciliare.
- Il Farmacista collabora con il medico nutrizionista alla definizione delle formule nutrizionali personalizzate, fornisce le miscele, i presidi e le attrezzature necessarie e svolge una funzione di farmacovigilanza.
- Potrà essere necessaria la presenza di psicologi o altre figure professionali idonee, capaci di collaborare a superare le grandi difficoltà di questa specifica area assistenziale.

In alcune Regioni sono previste figure professionali con il compito di coordinare, pianificare e seguire il percorso di deospedalizzazione sulla base del piano terapeutico redatto congiuntamente al momento della dimissione del paziente dalla struttura ospedaliera e condiviso con il MdMG e/o PdLS. Questi professionisti, che rappresentano il punto di collegamento tra la struttura ospedaliera, il territorio, il MdMG o il PdLS e coloro che sono coinvolti nell'assistenza domiciliare, dovranno prendere in carico il paziente e i suoi familiari già durante il ricovero ospedaliero, riducendo il rischio di pericolose carenze assistenziali tra le dimissioni e la reale presa in carico del paziente da parte del territorio. Inoltre, dovranno verificare, in accordo con i medici curanti, l'adesione al piano assistenziale nutrizionale e l'emergenza di eventuali ulteriori necessità. In assenza di queste figure di raccordo sarà elevato il rischio di una non ottimale assistenza con il conseguente rientro del paziente in ospedale, spesso attraverso un difficile percorso attraverso il pronto soccorso.

Nella organizzazione delle reti per la nutrizione dovranno essere identificati i criteri per la somministrazione del supporto nutrizionale a domicilio del paziente, distinguendo tra le diverse modalità disponibili nelle diverse situazioni cliniche, attraverso il riconoscimento delle necessità nutrizionali e di supporto del paziente, la verifica dei presupposti alla deospedalizzazione e della stabilità del quadro clinico, la presenza di adeguate condizioni sociali e ambientali, l'idoneità della persona addetta alla cura del paziente e la sua formazione, l'acquisizione del consenso informato, la definizione del programma nutrizionale e delle modalità di monitoraggio del follow-up, l'identificazione di indicatori di risultato clinico e la loro registrazione su supporti informatici condivisi.

Rilevante la codificazione dei rapporti tra le diverse figure professionali coinvolte, in particolare a livello territoriale (MdMG o PdLS, personale del Distretto, uffici amministrativi della ASL), al fine di garantire il controllo del materiale da inoltrare al domicilio del paziente, i tempi di consegna e la gestione di eventuali urgenze.

Infine, dovranno essere disponibili modalità di collegamento tra il personale impegnato nell'assistenza quotidiana con il centro di riferimento nutrizionale e con gli specialisti di area, al fine di garantire un adeguato aggiornamento professionale di tutti i soggetti coinvolti.

Continuità assistenziale MdMG e PdLS Assistenza domiciliare e strutture ospedaliere.

Nel corso degli ultimi anni un numero sempre maggiore di evidenze scientifiche ha confermato l'utilità, in termini di qualità e quantità di vita, di un approccio integrato precoce ai sintomi del malato oncologico e onco-ematologico.

Le cure simultanee nel malato oncologico rappresentano un modello organizzativo mirato a garantire la sua presa in carico globale attraverso un'assistenza continua, integrata e progressiva fra terapie oncologiche e Cure di supporto quando l'obiettivo principale non sia la sopravvivenza del malato. Tale modello, nuovo paradigma di cura per i malati oncologici, è stato inserito già nel Piano oncologico nazionale 2010-12 come obiettivo prioritario per migliorare la qualità della vita di questi pazienti. Nel 2012 anche la Società Americana di Oncologia Clinica (ASCO) ha ribadito che il modello di cure simultanee è il miglior modello, oggi utilizzabile, per il malato oncologico poiché riesce a garantire sia un più corretto ricorso ai servizi socio sanitari sia un più appropriato uso dei farmaci anche di quelli ad alto costo. La principale finalità è ottimizzare la qualità della vita in ogni fase della malattia, attraverso una meticolosa attenzione ai bisogni fisici, funzionali, psicologici, spirituali e sociali del malato e della sua famiglia.

Le cure simultanee richiedono tuttavia un cambiamento culturale e organizzativo rilevante per condividere scopi, valori e programmazione a livello di unità operative, gruppi multidisciplinari, servizi oncologici ospedalieri e servizi territoriali, ivi compreso il MdMG e i PdLS.

Per essere più aderenti ai bisogni del malato oncologico, è necessario pertanto identificare modelli organizzativi innovativi in grado di rispondere tempestivamente ai suoi bisogni e soprattutto facilitare l'integrazione e l'interfaccia tra ospedale e servizi del territorio. In particolare bisogna anticipare

l'integrazione dei servizi dedicati alle cure del malato oncologico evitando la frammentazione degli interventi ed il ritardo nella presa in carico globale del paziente.

La costituzione di una rete nutrizionale (intesa come Rete regionale indipendente o inserita in quelle già disponibili) dovrà necessariamente prevedere una stretta interazione con i MdMG e con i PdLS, evitando di trasferire sull'assistenza territoriale percorsi assistenziali che prevedono una stretta integrazione tra competenze specialistiche diverse e collocate in strutture che, in alcune realtà regionali, possono essere geograficamente distanti.

Al fine di semplificare i percorsi di rete e garantire la continuità assistenziale, alcune Regioni si sono dotate di piattaforme informatizzate, accessibili da remoto da tutte le figure coinvolte nel percorso di governo clinico assistenziale e sociale.

Inoltre, estremamente promettente appare l'utilizzo di webcam per assicurare in ogni momento il contatto audio e video tra il paziente e il centro di riferimento. In questi casi, sarà possibile una comunicazione tra paziente e/o la persona che presta assistenza e la struttura assistenziale asincrona (via e-mail, internet, cellulare, sistemi di messaggistica automatizzati) o sincrona (via webcam, videoconferenza che coinvolge in tempo reale, con contatti faccia a faccia (immagine e voce) tramite apparecchiature (televisione, digitale fotocamera, videotelefono, ecc) per collegare la persona che presta aassistenza con uno o più pazienti contemporaneamente (es. ai fini della formazione, educazione, ecc).

In considerazione delle peculiari caratteristiche della assistenza nutrizionale, dovrà essere specificamente previsto un accordo con i MdMG e i PdLS che consenta loro di contribuire alla gestione dei loro pazienti, con garanzie di sostegno da parte della struttura ospedaliera di riferimento e della rete per la nutrizione in caso di comparsa di situazioni cliniche non più gestibili a domicilio.

Un precoce trattamento dei sintomi (tra cui la malnutrizione è uno dei principali) nei Pazienti oncologici si traduce in una drastica riduzione degli accessi in PS e degli accessi sotto-soglia (cioè, prima della data prevista per il ricovero successivo) e della degenza media nei Reparti (in quanto i

Pazienti sanno che sono disponibili specifici percorsi agevolati, qualora se ne manifestasse la necessità), con migliore utilizzazione delle risorse del SSN.

In questo contesto assistenziale il malato oncologico che necessita di un supporto nutrizionale dovrà proseguire attraverso le modalità assistenziali extra-ospedaliere descritte non solo il trattamento inizialmente condiviso con la struttura ospedaliera, ma anche una puntuale verifica dei risultati raggiunti e delle successive modalità di prosecuzione, modificazione o interruzione del supporto nutrizionale.

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6. FORMAZIONE SUGLI ASPETTI NUTRIZIONALI nel PAZIENTE ONCOLOGICO

Problematiche relative alla formazione dei professionisti in ambito sanitario

PRINCIPI GENERALI

Per far fronte alle problematiche relative allo stato nutrizionale del paziente oncologico è necessario che siano acquisite competenze trasversali nell'ambito delle professioni che, a diverso titolo, dovranno occuparsi di pazienti affetti da neoplasia.

E' opportuna, pertanto, una rivisitazione, in collaborazione con la Conferenza permanente dei Presidenti dei Corsi di laurea magistrale in Medicina e Chirurgia (http://presidenti-medicina.it/) , dei diversi curricula dei Corsi di Studio che a vario titolo formeranno professionisti che avranno a che fare con pazienti affetti da neoplasia perché gli aspetti nutrizionali diventino parte integrante della formazione.

Da una parte chi opera in ambito nutrizionale (medici con competenze/specializzazione in scienza dell'alimentazione e dietisti) deve acquisire, nel corso della formazione professionalizzante, competenze legate agli aspetti della malattia neoplastica (comprese le conseguenze e gli interventi che vengono messi in atto) che potranno influenzare lo stato di nutrizione o le modalità di esecuzione di un intervento nutrizionale. D'altro canto chi si occupa in maniera specifica della malattia neoplastica (oncologi, chirurghi, ematologi, radioterapisti) dovrà avere contezza delle conseguenze che la malattia neoplastica potrà avere sullo stato di nutrizione e della necessità di considerare questo come un target essenziale del percorso di cura.

La formazione in questo ambito dovrà mirare a far acquisire il "sapere" che comprende tutte quelle conoscenze necessarie alla valutazione ed alla interpretazione dei dati relativi allo stato di nutrizione

dei pazienti con neoplasia. Questo è, in linea di massima, caratterizzato da alterazioni del bilancio di energia e nutrienti (legati a difficoltà nell'introito alimentare per disfagia, mucositi, anoressia, stenosi; nell'assorbimento dei nutrienti ma anche all'eventuale aumento del fabbisogno energetico in una situazione di ipercatabolismo), della composizione corporea (es. frequente presenza di sarcopenia, variazioni – spesso riduzione, ma talvolta aumento- della massa grassa, variazioni dello stato di idratazione e della densità minerale ossea), delle funzioni biologiche e funzionali (assetto proteico e lipidemico, compenso glicemico, bilancio elettrolitico e idrico, autonomia e abilità nello svolgere le attività della vita quotidiana,…). E quindi, nell'ambito del "sapere" sarà necessario acquisire le conoscenze necessarie alla programmazione di un intervento nutrizionale in grado di ottimizzare gli apporti di energia e nutrienti, nel rispetto dei fabbisogni e delle condizioni cliniche, funzionali e metaboliche del paziente oncologico.

La formazione dovrà anche mirare a far acquisire la capacità di "saper fare" mettendo in condizione il professionista che prende in carico i pazienti con neoplasia, di utilizzare con competenza gli strumenti in grado di assicurare la precoce e ripetuta stima del rischio nutrizionale in tutti i pazienti, la valutazione approfondita dello stato di nutrizione (bilancio di energia e nutrienti, composizione e funzione corporea) in quei soggetti che presentano un rischio medio-elevato, la prescrizione di un intervento la cui intensità (dal counseling dietetico, alla dieta costruita ad personam, all'uso di oral nutritional supplement, alla nutrizione artificiale di supporto o totale) sarà funzione dello stato clinico-funzionale-nutrizionale e della prognosi. Inoltre, sarà anche necessario che il professionista sia in grado di valutare l'impatto che l'intervento nutrizionale può avere sulla qualità di vita del paziente onde evitare eventuali accanimenti terapeutici.

La formazione dovrà infine mirare a favorire l'abilità di lavorare in équipe, condividendo, nel rispetto dei ruoli, procedure e risultati. Tale formazione dovrà consentire al professionista di acquisire il "saper essere" necessario al confronto di idee, al rispetto di opinioni e posizioni diverse, alla conoscenza dei limiti del proprio intervento in un sistema complesso. L'intervento nutrizionale in ambito oncologico deve infatti integrarsi in una presa in carico multidimensionale del paziente. Lo stato di nutrizione e gli

aspetti più strettamente correlati alla neoplasia (deficit d'organo o conseguenze dell'intervento terapeutico) o alle conseguenze di questa (sul piano funzionale e psicologico) si debbono integrare nel definire gli aspetti complessivi bio-psico-sociali della malattia neoplastica. Non da ultimo sarà necessario essere formati a considerare gli aspetti etici di interventi che possono penalizzare la qualità di vita, aumentare rischi e prolungare sofferenze del paziente. Sarà infine necessario acquisire la capacità di "saper essere" nei confronti dei pazienti (e dei familiari di questi) che vivono la malattia neoplastica. Tutti questi aspetti vanno considerati nell'ambito di un lavoro di équipe in cui il paziente ed i suoi familiari, oltre che diverse figure professionali, con diverse competenze e sensibilità, siano coinvolti.

PRINCIPI ORDINAMENTALI ED APPLICAZIONE PRATICA NELLA PEDAGOGIA MEDICA E DELL'AREA SANITARIA

E' opportuna, pertanto, una rivisitazione dei diversi curricula dei Corsi di Studio che a vario titolo formeranno professionisti che avranno a che fare con pazienti affetti da neoplasia perché gli aspetti nutrizionali diventino parte integrante della formazione.

Ciò è ancor più vero perché in alcune indagini, condotte in diversi Paesi, solo una minoranza di medici ha potuto affermare di sentirsi adeguatamente formato per fornire una consulenza in ambito nutrizionale. In queste indagini emergeva il nesso tra i livelli delle conoscenze e quelli dell'istruzione ricevuta.

L'auspicio è che si operi nella revisione dei curricula dei diversi Corsi di Studio indicando tra i saperi minimi, che ogni studente deve aver acquisito alla fine del percorso di formazione, anche quelle informazioni necessarie a comprendere meglio le problematiche relative alla nutrizione clinica applicata ai pazienti affetti da neoplasia.

In particolare:

- Per il CLM a ciclo unico in Medicina e Chirurgia nelle Attività Elettive è possibile inserire dei CFU ad hoc, ma sarebbe auspicabile che i Corsi integrati esistenti di Oncologia e di medicina Interna prevedessero un focus specifico anche di poche ore che dia allo studente in formazione pre-laurea la conoscenza minima necessaria per sapere identificare la problematica
- In tutte le Scuole di specializzazione di Area Medica, in cui è possibile che lo specialista incontri un paziente oncologico inerente la specifica tipologia della scuola, la nutrizione del paziente oncologico va inserito fra gli obiettivi formativi, mentre nelle Scuole di Oncologia, Ematologia, Medicina Interna e Geriatria devono essere inserite specifiche attività professionalizzanti
- Per la Scuola di specializzazione in Scienza dell'alimentazione va sottolineato che quanto già previsto nell'ordinamento didattico (DM 68/15) va verificato come requisito necessario di accreditamento dando priorità all'acquisizione di specifiche competenze attraverso la collaborazione con la rete oncologica ospedaliera e territoriale che deve contribuire obbligatoriamente alla formazione degli specializzandi.
- Nelle Lauree delle professioni sanitarie particolare quella di Dietistica, ma anche quelle di Infermieristica ed Infermieristica Pediatrica la nutrizione del paziente oncologico va inserito fra gli obiettivi formativi e specifici tirocini devono essere svolti nei reparti oncologici.

PRINCIPI PER UNA FORMAZIONE ANDRAGOCICA E FORMAZIONE CONTINUA

Tutto quanto sopra è evidentemente valido sia per i giovani studenti in medicina e nelle Lauree delle professioni sanitarie sia per i giovani colleghi inseriti nei percorsi formativi delle Scuole di Specializzazione, ma non dobbiamo dimenticare che il medico ed il professionista sanitario, come pure lo specialista, hanno una vita professionale di decenni e per questo devono avere ulteriori occasioni di formazione volontaria ed obbligatoria.

Per la prima tipologia, cioè la formazione volontaria, tutti gli specialisti che affrontano il paziente oncologico dovrebbero avere a disposizione, da parte della formazione universitaria specialistica, Master Universitari e Corsi di Alta Formazione Universitari dedicati all'approfondimento degli aspetti nutrizionali del suddetto paziente. Sarebbe fondamentale richiedere ai singoli Atenei, o a Consorzi regionali degli stessi, di istituire uno o più corsi dedicati ai vari livelli di professionisti coinvolti, che qualifichino e riqualifichino sul tema il personale laureato e specialista.

Per la tipologia della formazione continua obbligatoria l'ambito della nutrizione del paziente oncologico dovrebbe rientrare, a pieno titolo, nell'ambito della formazione strategica per il SSN, cioè quella formazione per cui la CNFC potrebbe richiedere obbligatoriamente l'acquisizione di crediti ECM a chiunque operi attivamente nelle strutture oncologiche o sul territorio su questa tipologia di pazienti. Data l'evoluzione costante di questo ambito sarebbe opportuno un aggiornamento a scadenze predeterminate, attraverso corsi residenziali, o meglio e più semplicemente, corsi FAD. Per la predisposizione di tali corsi si potrebbe richiedere il contributo scientifico e formativo di un coordinamento delle Società Scientifiche del settore proprio al fine di avere una voce comune ed un adeguamento costante alle più attuali indicazioni.

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7. APPENDICE: LA NUTRIZIONE ARTIFICIALE

Nella pratica clinica può essere utile distinguere due differenti situazioni in base all'aspettativa di vita del paziente. In tutti i casi, il trattamento dell'anoressia e delle alterazioni dello stato nutrizionale, non può prescindere dal controllo dei sintomi correlati, che possono a loro volta peggiorare il quadro clinico, e dall'educazione alimentare del paziente e dei familiari.

Il trattamento farmacologico, scelto in base alle caratteristiche del paziente, si può avvalere dell'uso di progestinici e corticosteroidi, di cui è stata evidenziata l'efficacia, e/o di nuovi farmaci o farmaci riscoperti per uso diverso da quello consolidato e meritevoli di ulteriori studi.

La consulenza nutrizionale è finalizzata ad impostare una dieta personalizzata che tenga conto di: a) necessità di apporto calorico; b) gusti ed abitudini alimentari del paziente; c) presenza di sintomi e/o effetti collaterali in grado di modificare lo stato nutrizionale. Questo aspetto va considerato non solo nei pazienti in trattamento, ma anche in quelli in follow-up che, pur liberi da malattia, possono presentare esiti dei trattamenti in grado di alterare la nutrizione (enterite post-attinica, xerostomia, nausea cronica, solo per citarne alcuni); d) importanza attribuita all'alimentazione da parte del paziente e dei familiari; programma terapeutico della malattia di base. Importante è anche l'educazione nutrizionale del paziente e dei familiari. A tal fine possono essere fornite schede informative finalizzate ad istruire il paziente ed i familiari su come migliorare la nutrizione in funzione di specifici problemi. Le linee guida dell'American Society of Clinical Oncology o della Società Italiana di Oncologia Medica, recentemente pubblicate in accordo con la SINPE, raccomandano gli interventi di educazione alimentare come primo ed imprescindibile intervento nel supporto nutrizionale.

Gli integratori alimentari sono frequentemente impiegati nel trattamento dell'anoressia e del calo ponderale. Si tratta, in prevalenza, di integratori ipercalorici, iperproteici ed ipolipidici. Diversi studi e revisioni della letteratura hanno mostrato un effetto positivo di integratori contenenti EPA sull'appetito e sull'aumento del peso corporeo e della massa magra. Il ruolo della nutrizione artificiale nei pazienti

oncologici è ancora controverso. Da un punto di vista pratico, i problemi che si pongono di fronte ad un paziente con segni di malnutrizione e/o con incapacità ad alimentarsi sono:

a) è opportuno somministrare una nutrizione artificiale?

b) qual'è la via di somministrazione da preferire?

Riguardo al primo quesito, le indicazioni ad una nutrizione artificiale attualmente condivise sono: a) pazienti sottoposti a terapie con alte dosi e trapianto di midollo osseo. In questi pazienti la nutrizione artificiale ha mostrato di migliorare la risposta ai trattamenti e la sopravvivenza; b) pazienti in cui lo stato di malnutrizione può rendere impossibile la somministrazione della terapia. Questo si riscontra frequentemente nel caso di neoplasie del primo tratto dell'apparato digerente, come pure nei pazienti sottoposti a radioterapia per neoplasie della testa e del collo. Un precoce intervento nutrizionale consente di prevenire la perdita di peso, il peggioramento della qualità di vita e la disidratazione; c) pazienti in fase avanzata di malattia, in cui la prognosi sia tale da permettere di definire il rischio di morte per malnutrizione più elevato del rischio per morte di neoplasia. E' ampiamente dimostrata l'inefficacia della nutrizione artificiale nei pazienti al termine della vita, per quanto riguarda la sopravvivenza e la qualità di vita. Al contrario, può essere utile somministrare una nutrizione artificiale nei pazienti a prognosi più favorevole. Allo scopo di selezionare questi pazienti si possono usare scale appropriate, quali il Palliative Prognostic Score che, mediante la valutazione integrata di indicatori clinici e di laboratorio, consenta di definire le probabilità di sopravvivenza a 30 giorni. La nutrizione artificiale diventa utilizzabile per i pazienti con probabilità di sopravvivenza a 30 giorni superiore al 70% e con impossibilità ad assumere alimenti per via orale; nei pazienti con malattia più avanzata (possibilità di sopravvivenza a 30 giorni inferiore al 70%) può essere preferibile un semplice regime di idratazione. La nutrizione parenterale totale, nei pazienti con malattia in fase avanzata, è indicata solo se: 1) l'attesa di vita è primariamente condizionata dalla malnutrizione più che dalla malattia; 2) l'attesa di vita è superiore a 2 mesi; 3) le condizioni cliniche sono determinate dalla malnutrizione senza l'associazione di sintomi severi o non controllati; 4) il PS sec. Karnofky è maggiore di 50.

Relativamente alla via di somministrazione, nessuna differenza in termini di efficacia è stata dimostrata fra la parenterale e l'enterale. La somministrazione enterale, in presenza di un apparato gastro-enterico funzionante, è da preferire in quanto più semplice da somministrare anche a domicilio, con minori rischi di complicanze e anche meno costosa. Controindicazioni assolute sono: occlusione intestinale, diarrea grave e pancreatite acuta. In questi casi si può ricorrere ad una somministrazione per via parenterale.

Per quanto riguarda la ripartizione fra i vari componenti, non ci sono dimostrazioni circa la superiorità di un regime rispetto ad un altro relativamente al rapporto glucidi/lipidi ed all'impiego di aminoacidi a catena ramificata, a meno di concomitante presenza di altre comorbidità rilevanti dal punto di vista clinico.

Numerosi sono i farmaci utilizzabili come integrazione ad un corretto programma di sostegno nutrizionale, definito dallo specialista nutrizionista, in accordo con le diverse figure professionali impegnate nel percorso assistenziale del singolo paziente in ogni momento del percorso terapeutico.

8. APPROFONDIMENTO: IL MICROBIOMA

Prende il nome di Microbioma, l'insieme del patrimonio genetico e delle interazioni ambientali che vivono nel nostro organismo, soprattutto nell'apparato digerente ma anche sull'epidermide, nel cavo orale e in altri apparati. Nello specifico comparto intestinale, l'insieme dei microrganismi simbiontici che occupano l'intera lunghezza e larghezza del tratto, prende il nome di Microbiota intestinale il quale presenta una variabilità individuale, ospite specifica, determinata e suscettibile alle modificazioni esogene ed endogene come l'area geografica e lo stile di vita, e svolge molteplici funzioni che influenzano la fisiologia, i processi metabolici e, di conseguenza, lo stato di salute.

L'intestino umano, dunque, ospita centinaia di diverse specie di batteri, compresi anche funghi e virus e con un numero complessivo di cellule batteriche che supera l'intero ammontare di quelle che compongono il corpo umano, costituendo un vero e proprio ecosistema che svolge ruoli fisiologici importantissimi per la salute dell'ospite tanto da essere considerato un "organo microbico". E' per tale ragione che, negli ultimi anni, si sta approfondendo questo tema in campo biomedico; infatti, dopo l'ambizioso progetto del sequenziamento del genoma umano ora il via al sequenziamento del microbioma (Human Microbiome Project), ossia delle sequenze geniche delle popolazioni microbiche che colonizzano il tratto digerente, costituenti nel loro complesso il microbiota.

La composizione nelle singole specie microbiche varia tra individui diversi così che nello stesso individuo la comunità microbica tipica rappresenti un tratto distintivo e caratterizzante: a partire dalla nascita avvenuto il contatto con i genitori e con l'ambiente esterno e simultaneamente allo sviluppo del sistema immunologico intestinale; da questa specifica base individuale si susseguono varie modifiche nel corso delle diverse fasi della vita che possono tradursi anche nella possibilità dell'instaurarsi di particolari condizioni patologiche. Il Progetto Microbioma Umano, si prefigge anche l'obiettivo di

stabilire una certa stabilità funzionale atta a garantire un set base di reazioni biochimiche comuni all'interno della variabilità del microbioma.

La conoscenza circa le funzioni del microbioma porta con sé un cambiamento di prospettiva in cui l'analisi meta-genomica mostra come molti dei geni microbici codifichino per funzioni attualmente ancora sconosciute, aprendo uno scenario in cui lo stato di salute ed il benessere dell'organismo risultano programmate e regolate non solo attraverso le sequenze del DNA ma anche dalle variazioni epigenetiche che il microbioma attua sull'espressione dei stessi geni.

In nutrizione è importante descrivere ed approfondire il comparto di colonia batterica legato alla funzione digerente e dell'assorbimento. In ogni individuo troviamo da 1013 a 1014 microrganismi che contengono circa 100 volte più geni rispetto al nostro genoma. Studiando la loro distribuzione lungo l'apparato digerente notiamo come i microbi, soprattutto i batteri, occupino lo strato esterno del muco di rivestimento formando con esso, e con la mucosa, una vera e propria barriera difensiva.

Come riportato in letteratura, vi sono sempre più prove del fatto che i microrganismi intestinali ed i loro prodotti metabolici interagiscono con gli ormoni ivi prodotti, lo stato infiammatorio e pro- e infiammatorio e non ultima con la motilità stessa dell'intestino. La variazione della composizione del microbiota intestinale, può inoltre influenzare anche la spesa energetica, la sazietà e l'assunzione di cibo, influenzando quindi l'aumento o la perdita di peso corporeo.

Nota è anche la possibilità di interazioni tra i batteri del microbioma o loro particolari cataboliti ed il sistema immunitario e la mucosa intestinali. La flora batterica, infatti, ricopre anche importanti funzioni metaboliche, riducendo tossine alimentari e sostanze cancerogene, sintetizzando micronutrienti, fermentando sostanze alimentari altrimenti indigeribili, favorendo l'assorbimento in particolar modo di alcuni elettroliti e minerali e incidendo sulla crescita ed il differenziamento degli enterociti e colono citi attraverso la produzione di acidi grassi a catena corta.

In condizioni di normalità, la flora intestinale è prevalentemente aerobica nel tratto superiore e in maggioranza anaerobica nel tratto inferiore; gli studi dimostrano come questa differenza sia

fondamentale nel mantenimento delle corrette funzioni gastrointestinali, digestiva ed assorbitiva, e del sistema immunitario specifico dell'organo. E' stato, inoltre, ipotizzato che la flora intestinale specifica dell'individuo e con relativa efficienza metabolica specifica, possa presentare determinate caratteristiche di composizione della colonia batterica predisponenti all'obesità secondo meccanismi che influenzano l'energia ricavata dalla dieta.i La comunità microbiotica simbiotica, dunque, in salute o in condizioni di alterata funzionalità secondo un quadro che viene scientificamente definito di disbiosi, mostra pertanto una forte interazione con l'ambiente locale e le risposte sistemiche, svolgendo un ruolo fondamentale nella nutrizione, nell'immunità, nel metabolismo e nelle condizioni patologiche incluse i tumori.

Il cibo che consumiamo alimenta non solo noi ma anche la vasta e diversificata comunità microbica che risiede all'interno del nostro tratto gastrointestinale. In un processo di coevoluzione simbiotica, il microbiota intestinale è diventato essenziale per il mantenimento della salute. L'identificazione delle comunità microbiche associate alla cancerogenesi si profila, dunque, di cruciale importanza.

L'avvento della tecnologia di sequenziamento e della profilassi metabolica di nuova generazione hanno rivelato, in questi ultimi anni, la notevole complessità della diversità e della funzione microbica e che il microbiota nel suo essere produce un'ampia varietà di prodotti bioattivi che influenzano la salute. L'evoluzione delle tecniche molecolari indipendenti dalla coltura, ha permesso di identificare le principali specie batteriche in individui sani, le condizioni infiammatorie e di CRC (cancro del colon-retto), terzo più comune al mondo e causa di circa 500.000 decessi annui.

Alcuni recenti studi in proposito, hanno dimostrato le differenze nel microbiota intestinale tra pazienti con tumore del colon e individui sani, fornendo una migliore comprensione dell'interazione tra patologie e simbionti nello sviluppo del tumore. Non è stato individuato un singolo organismo causale in CRC, tuttavia, vi sono prove evidenti che la riduzione dei batteri protettivi, l'aumento di altri tra cui i membri del Fusobatterio, Bacteroides/Prevotella, e le variazioni legate all'età nel microbiota hanno un impatto sull'adenoma o sullo sviluppo del cancro. Sono stati individuati diversi modelli

composizionali di microbiota e le loro associazioni con stati di salute e di malattia; quello che è emerso da questa osservazione è una combinazione che vede un rapporto disturbato tra microbiota-ospite, definito *dysbiosis*, ora riconosciuto come la causa principale di una crescente lista di malattie, incluso il cancro del colon-retto (CRC).

Ci sono testimonianze, in vivo come in vitro, a supporto dell'evidenza che suggerisce come e quanto la dieta seleziona per la composizione della flora intestinale e in che modo diversi effetti promozionali e deleteri della dieta sono in realtà mediati dal microbiota. Ne è un esempio la scoperta della fattibilità della fibra alimentare come coadiuvante nella sintesi microbica del colon di metaboliti antiproliferativi e contro i carcinogeni, sottolineando la prerequisizione della modifica alimentare come misura fondamentale per frenare la pandemia del CRC.

Questo, come il consumo di carne rossa, sono solo alcuni esempi primari di come la nostra dieta, e la sua interazione con il microbioma, possa giocare un ruolo determinante in un percorso di riabilitazione anche delicato come quello oncologico.

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Cell Metabolism
Article

Low Protein Intake Is Associated with a Major Reduction in IGF-1, Cancer, and Overall Mortality in the 65 and Younger but Not Older Population

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SUMMARY

Mice and humans with growth hormone receptor/IGF-1 deficiencies display major reductions in age-related diseases. Because protein restriction reduces GHR-IGF-1 activity, we examined links between protein intake and mortality. Respondents aged 50–65 reporting high protein intake had a 75% increase in overall mortality and a 4-fold increase in cancer death risk during the following 18 years. These associations were either abolished or attenuated if the proteins were plant derived. Conversely, high protein intake was associated with reduced cancer and overall mortality in respondents over 65, but a 5-fold increase in diabetes mortality across all ages. Mouse studies confirmed the effect of high protein intake and GHR-IGF-1 signaling on the incidence and progression of breast and melanoma tumors, but also the detrimental effects of a low protein diet in the very old. These results suggest that low protein intake during middle age followed by moderate to high protein consumption in old adults may optimize healthspan and longevity.

INTRODUCTION

Caloric restriction (CR) without malnutrition has been consistently shown to increase longevity in a number of animal models, including yeast, *C. elegans*, and mice (Fontana et al., 2010). However, the effect of CR on the lifespan of nonhuman primates remains controversial and may be heavily influenced by dietary composition (Cava and Fontana, 2013; Colman et al., 2009; Fontana and Klein, 2007; Mattison et al., 2012; Mercken et al., 2013; Stein et al., 2012). The lifespan extension associated with CR in model organisms is believed to operate through its effects on growth hormone (GH) and GH receptor (GHR), leading to subsequent deficiencies in IGF-1 and insulin levels and signaling (Bartke et al., 2001; Bellush et al., 2000; Fontana et al., 2010; Hauck et al., 2002; Wei et al., 2009). The effect of the insulin/IGF-1 pathway on longevity was first described in *C. elegans* by showing that mutations in the insulin/IGF-1 receptor or in the downstream *age-1* gene caused a several-fold increase in lifespan (Johnson, 1990; Kenyon et al., 1993, Kenyon, 2010). Other studies revealed that mutations in orthologs of genes functioning in insulin/IGF-1 signaling, but also activated independently of insulin/IGF-1, including TOR-S6K and RAS-cAMP-PKA, promoted aging in multiple model organisms, thus providing evidence for the conserved regulation of aging by pro-growth nutrient signaling pathways (Fabrizio et al., 2001; Guarente and Kenyon, 2000; Kapahi and Zid, 2004; Kenyon, 2005, 2011; Longo, 1999; Tatar et al., 2001). Not surprisingly, in mice, growth hormone receptor deficiency (GHRD) or growth hormone deficiency (GHD), both of which display low levels of IGF-1 and insulin, cause the strongest lifespan extension but also reduction of age-related pathologies including cancer and insulin resistance/diabetes (Brown-Borg and Bartke, 2012; Brown-Borg et al., 1996; Masternak and Bartke, 2012).

Recently, we showed that humans with growth hormone receptor deficiency (GHRD), also exhibiting major deficiencies in serum IGF-1 and insulin levels, displayed no cancer mortality or diabetes. Despite having a higher prevalence of obesity, combined deaths from cardiac disease and stroke in this group were similar to those in their relatives (Guevara-Aguirre et al., 2011). Similar protection from cancer was also reported in a study that surveyed 230 GHRDs (Steuerman et al., 2011).

Protein restriction or restriction of particular amino acids, such as methionine and tryptophan, may explain part of the effects of calorie restriction and GHRD mutations on longevity and disease risk, since protein restriction is sufficient to reduce IGF-1 levels and can reduce cancer incidence or increase longevity in model organisms, independently of calorie intake (Ayala et al., 2007; Fontana et al., 2008, 2013; Gallinetti et al., 2013; Horáková et al., 1988; Hursting et al., 2007; Leto et al., 1976; Mair et al., 2005; Pamplona and Barja, 2006; Peng et al., 2012; Ross, 1961; Sanz et al., 2006; Smith et al., 1995; Youngman, 1993).

Here, we combined an epidemiological study of 6,381 US men and women aged 50 and above from NHANES III, the only nationally representative dietary survey in the United States, with mouse and cellular studies to understand the link between the level and source of proteins and amino acids, aging, diseases, and mortality.

RESULTS

Human Population

The study population included 6,381 adults ages 50 and over from NHANES III, a nationally representative, cross-sectional study. Our analytic sample had a mean age of 65 years and is representative of the United States population in ethnicity, education, and health characteristics (Table S1).

On average, subjects consumed 1,823 calories, of which the majority came from carbohydrates (51%), followed by fat (33%) and protein (16%), with most of it (11%) derived from animal protein. The percent of calorie intake from protein was used to categorize subjects into a high protein group (20% or more of calories from proteins), a moderate protein group (10%–19% of calories from proteins), and a low protein group (less than 10% of calories from proteins).

Mortality follow-up was available for all NHANES III participants through linkage with the National Death Index up until 2006 (DHHS, 2001). This provided the timing and cause of death. The follow-up period for mortality covered 83,308 total person-years over 18 years, with 40% overall mortality, 19% cardiovascular disease (CVD) mortality, 10% cancer mortality, and about 1% diabetes mortality.

Association between Protein and Mortality

Using Cox Proportional Hazard models, we found that high and moderate protein consumption were positively associated with diabetes-related mortality, but not associated with all-cause, CVD, or cancer mortality when subjects at all the ages above 50 were considered. Results showed that both the moderate and high protein intake groups had higher risks of diabetes mortality compared to participants in the low protein group. Although taken together these results indicate that moderate to high protein intake promotes diabetes mortality, larger studies are necessary to test this possibility further. An alternative explanation for the elevated diabetes mortality in the higher protein group is that, following a diabetes diagnosis, some individuals may switch to a diet comprised of higher protein, lower fat, and low carbohydrates. To test this, we examined the association between protein intake and diabetes mortality in participants who had no prevalence of diabetes at baseline (Table S7).

Among subjects with no diabetes at baseline, those in the high protein group had a 73-fold increase in risk (HR: 73.52; 95% CI: 4.47–1,209.70), while those in the moderate protein category had an almost 23-fold increase in the risk of diabetes mortality (HR: 22.93; 95% CI: 1.31–400.70). We underline that our hazard ratios and confidence intervals may be inflated due to our sample size and the extremely low incidence of diabetes mortality in the low protein group. Overall, there were only 21 diabetes deaths among persons without diabetes at baseline, only 1 of which was from the low protein group. Nevertheless, despite the small sample size, our results still show significant associations between increased protein intake and diabetes-related mortality.

To determine whether the association between protein and mortality differed for middle-aged and older adults, Cox proportional hazard models were rerun, testing for an interaction between protein consumption and age. Significant interactions were found for both all-cause and cancer mortality, indicating that the low protein diet was beneficial in mid-life; however, its benefits declined with age (Figure 1). Based on these results, we stratified the population into two age groups, those ages 50–65 (n = 3,039) and those ages 66+ (n = 3,342), and re-examined relationships between protein and cause-specific mortality. Among those ages 50–65, higher protein levels were linked to significantly increased risks of all-cause and cancer mortality (Table 1). In this age range, subjects in the high protein group had a 74% increase in their relative risk of all-cause mortality (HR: 1.74; 95% CI: 1.02–2.97) and were more than four times as likely to die of cancer (HR: 4.33; 95% CI: 1.96–9.56) when compared to those in the low protein group. None of these associations was significantly affected by controlling for percent calories from total fat or for percent calories from total carbohydrates. However, when the percent calories from animal protein was controlled for, the association between total protein and all-cause or cancer mortality was eliminated or significantly reduced, respectively, suggesting animal proteins are responsible for a significant portion of these relationships. When we controlled for the effect of plant-based protein, there was no change in the association between protein intake and mortality, indicating that high levels of animal proteins promote mortality and not that plant-based proteins have a protective effect (Table S5).

Compared to subjects reporting a low protein diet, subjects who consumed moderate levels of protein also had a 3-fold higher cancer mortality (HR: 3.06; 95% CI: 1.49–6.25), which was not accounted for by either percent calories from fat or percent calories from carbohydrates, but was marginally reduced when controlling for percent calories from animal protein (HR: 2.71; 95% CI: 1.24–5.91), although the size of the effect was not as large as for those in the high protein group. Taken together, these results indicate that respondents ages 50–65 consuming moderate to high levels of animal protein display a major increase in the risks for overall and cancer mortality; however, the risks may be somewhat decreased if protein does not come from an animal source. Similar results were obtained if the population 45–65 was considered, although few deaths occurred in the 45–50 group (data not shown).

In contrast to the findings above, among respondents who were 66 years of age and over at baseline, higher protein levels were associated with the opposite outcomes for overall and cancer mortality but a similar outcome for diabetes mortality

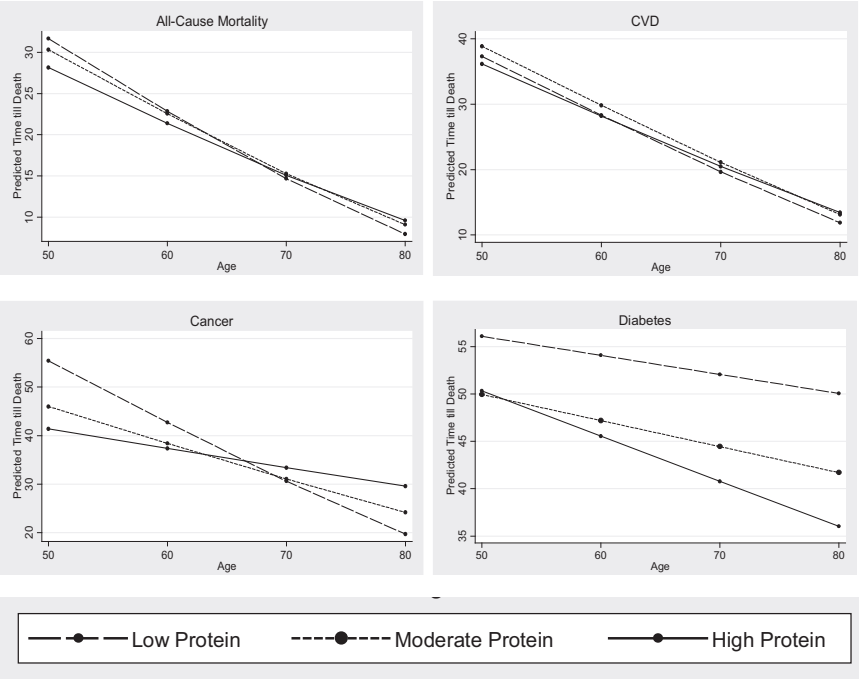

Figure 1. Association between Protein Intake and Mortality

Using Cox proportional hazard models, statistically significant ($p < 0.05$) interactions between age and protein group were found for all-cause and cancer mortality. Based on these models, predicted remaining life expectancy was calculated for each protein group by age at baseline. Overall, low protein appears to have a protective effect against all-cause and cancer mortality prior to age 66, at which point it becomes detrimental. No significant interactions were found for cardiovascular disease (CVD) and diabetes mortality.

(Table 1). When compared to those with low protein consumption, subjects who consumed high amounts of protein had a 28% reduction in all-cause mortality (HR: 0.72; 95% CI: 0.55–0.94), while subjects who consumed moderate amounts of protein displayed a 21% reduction in all-cause mortality (HR: 0.78; 95% CI: 0.62–0.99). Furthermore, this was not affected by percent calories from fat, from carbohydrates, or from animal protein. Subjects with high protein consumption also had a 60% reduction in cancer mortality (HR: 0.40; 95% CI: 0.23–0.71) compared to those with low protein diets, which was also not affected when controlling for other nutrient intake or protein source.

The Influence of IGF-1 on the Association between Protein and Mortality

Adjusted mean IGF-1 levels were positively associated with protein consumption for both age groups (Figure 2). Because IGF-1 was only available for a randomly selected subsample (n = 2,253), we re-examined the age-specific associations between protein and cause-specific mortality in this sample and found them to be similar to what was seen in the full sample, although with somewhat larger effect sizes (Table S3). Next we examined whether IGF-1 acted as a moderator or mediator in the association between protein and mortality. We found that while IGF-1 did not account for the association between protein consumption and mortality (Table S3), it was an important moderator of the association, as indicated by the statistically significant interactions between protein and IGF-1 level (Table S4).

From these models, predicted hazard ratios by IGF-1 and protein group were calculated (Figure S2). Results showed that for every 10 ng/ml increase in IGF-1, the mortality risk of cancer among subjects ages 50–65 increases for the high protein versus the low protein group by an additional 9% ($HR_{high\ protein\ x\ IGF\text{-}1}$: 1.09; 95% CI: 1.01–1.17). In contrast, among older subjects (66+ years), when comparing those in the low protein group, subjects with high or moderate protein diets had a reduced risk of CVD mortality if IGF-1 was also low; however, no benefits were found with increased IGF-1.

Protein Intake, IGF-1, and Cancer in Mice

To verify causation and understand the mechanism that may link proteins to cancer and overall mortality, we studied the effect of a range of protein intake (4%–18%) similar to that of subjects in the NHANES III study on the levels of circulating IGF-1, cancer incidence, and cancer progression in mice. Eighteen-week-old male C57BL/6 mice were fed continuously for 39 days with experimental, isocaloric diets designed to provide either a high (18%) or a low (4%–7%) amount of calories derived from protein, without imposing CR or causing malnutrition (Figures S1A and S1B).

To understand how the different levels of protein and IGF-1 may affect the ability of a newly formed tumor to survive and grow after 1 week on diets containing different protein levels, both groups were implanted subcutaneously with 20,000 syngeneic murine melanoma cells (B16). Tumor measurements began 15 days postimplantation and after 22 days on the diets, at which point incidence was found to be 100% for the high protein level (18%) group but 80% for the low protein level (4%) group (Figure 3A). At day 25, incidence rose to 90% in the low protein group and remained there until the end of the experiment (Figure 3A). From day 22 until the end of the experiment, tumor size was significantly smaller in the group consuming a lower amount of proteins, indicating a much slower tumor progression. At day 39 the mean tumor size was 78% larger in the high compared to the low protein group (day 36, $p = 0.0001$; day 39, $p < 0.0001$) (Figure 3B). To test the hypothesis that GHR signaling may be involved in this effect of protein levels, blood samples were obtained and analyzed at day 16 to determine the levels of IGF-1 and the IGF-1 inhibitory protein IGFBP-1 (Wolpin et al., 2007). Serum IGF-1 was 35% lower ($p = 0.0004$) in the low protein group when compared to animals fed the high protein diet (Figure 3C). Conversely, serum IGFBP-1 was 136% higher ($p = 0.003$) in the low protein group compared to the high protein group (Figure 3D).

To test further the hypothesis that the GHR-IGF-1 axis promotes cancer progression, we implanted subcutaneous

Table 1. Associations between Mortality and Protein Intake

	Hazard Ratio (95% CI)							
	Ages 50–65 (N = 3,039)				Ages 66+ (N = 3,342)			
	Model 1	Model 2	Model 3	Model 4	Model 1	Model 2	Model 3	Model 4
All-Cause Mortality								
Moderate protein (n = 4,798)	1.34 (0.81–2.22)	1.37 (0.82–2.27)	1.35 (0.80–2.29)	1.15 (0.67–1.96)	0.79 (0.62–0.99)	0.79 (0.62–0.99)	0.79 (0.62–0.99)	0.79 (0.61–1.01)
High protein (n = 1,146)	1.74 (1.02–2.97)	1.77 (1.03–3.03)	1.74 (0.99–3.05)	1.18 (0.60–2.31)	0.72 (0.55–0.94)	0.73 (0.56–0.95)	0.72 (0.55–0.94)	0.72 (0.50–1.02)
% kcal fat	–	0.99 (0.98–1.01)	–	–	–	1.00 (0.99–1.01)	–	–
% kcal carbs	–	–	1.00 (0.99–1.01)	–	–	–	1.00 (0.99–1.00)	–
% kcal animal protein	–	–	–	1.03 (1.00–1.06)	–	–	–	1.00 (0.98–1.02)
CVD Mortality								
Moderate protein (n = 4,798)	0.79 (0.40–1.54)	0.83 (0.43–1.60)	0.81 (0.41–1.62)	0.61 (0.29–1.29)	0.80 (0.57–1.12)	0.80 (0.57–1.12)	0.80 (0.57–1.12)	0.80 (0.56–1.14)
High protein (n = 1,146)	1.03 (0.51–2.09)	1.08 (0.54–2.15)	1.10 (0.52–2.31)	0.55 (0.19–1.62)	0.78 (0.54–1.14)	0.79 (0.54–1.15)	0.78 (0.53–1.15)	0.77 (0.48–1.25)
% kcal fat	–	0.99 (0.97–1.01)	–	–	–	1.00 (0.99–1.01)	–	–
% kcal carbs	–	–	1.00 (0.99–1.02)	–	–	–	1.00 (0.99–1.01)	–
% kcal animal protein	–	–	–	1.04 (0.99–1.11)	–	–	–	1.00 (0.98–1.02)
Cancer Mortality								
Moderate protein (n = 4,798)	3.06 (1.49–6.25)	3.13 (1.52–6.44)	3.56 (1.65–7.65)	2.71 (1.24–5.91)	0.67 (0.43–1.06)	0.67 (0.43–1.06)	0.67 (0.42–1.05)	0.66 (0.40–1.07)
High protein (n = 1,146)	4.33 (1.96–9.56)	4.42 (2.01–9.74)	4.98 (2.13–11.66)	3.19 (1.21–8.35)	0.40 (0.23–0.71)	0.41 (0.23–0.73)	0.39 (0.22–0.69)	0.38 (0.17–0.82)
% kcal fat	–	0.99 (0.98–1.01)	–	–	–	1.02 (1.01–1.03)	–	–
% kcal carbs	–	–	1.00 (0.98–1.01)	–	–	–	1.00 (0.99–1.01)	–
% kcal animal protein	–	–	–	1.02 (0.97–1.07)	–	–	–	1.00 (0.97–1.04)
Diabetes Mortality								
Moderate protein (n = 4,798)	3.43 (0.69–17.02)	3.36 (0.67–16.96)	3.41 (0.67–17.36)	2.99 (0.58–15.31)	5.38 (0.95–30.49)	5.05 (0.93–27.34)	4.93 (0.89–27.35)	6.20 (0.35–37.01)
High protein (n = 1,146)	3.93 (0.73–21.07)	3.88 (0.71–21.17)	3.90 (0.67–22.84)	2.77 (0.24–31.73)	10.64 (1.85–61.31)	10.42 (1.88–57.87)	9.07 (1.49–55.30)	15.16 (1.93–118.9)
% kcal fat	–	1.01 (0.97–1.05)	–	–	–	–	–	–
% kcal carbs	–	–	1.00 (0.96–1.04)	–	–	–	–	–
% kcal animal protein	–	–	–	1.02 (0.92–1.14)	–	–	–	–

Reference = low protein (n = 437 in both age groups). Model 1 (baseline model): Adjusted for age, sex, race/ethnicity, education, waist circumference, smoking, chronic conditions (diabetes, cancer, myocardial infarction), trying to lose weight in the last year, diet changed in the last year, reported intake representative of typical diet, and total calories. Model 2: Adjusted for covariates and % kcals from total fat. Model 3: Adjusted for covariates and % kcals from total carbohydrates. Model 4: Adjusted for covariates and % kcals from animal protein.

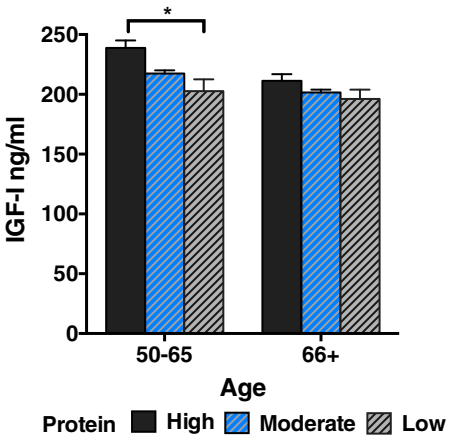

Figure 2. Serum IGF-1 Levels in Respondents 50–65 and 66+ Reporting Low, Moderate, or High Protein Intake

IGF-1 in respondents ages 50–65 is significantly lower among those with low protein intake when compared to high ($p = 0.004$). For those ages 66+ the difference between high and low intake becomes marginally significant ($p = 0.101$). The cohort for which IGF-1 levels were calculated includes 2,253 subjects. Of those ages 50–65 (n = 1,125), 89 were in the low protein category, 854 were in the moderate protein category, and 182 were in the high protein category. Of those ages 66+ (n = 1,128), 80 were in the low protein category, 867 were in the moderate protein category, and 181 were in the high protein category. Data points represent the mean ± SEM. *$p < 0.01$.

melanoma (B16) into GHR/IGF-1-deficient GHRKO mice and their respective age- and sex-matched littermate controls (18-week-old male C57BL/6 mice). Tumor measurements began 10 days postimplantation and continued until day 18. The data show that tumor progression is strongly inhibited in the GHRKO mice when compared to progression in the control group (Figures 3E and S1L; $p < 0.01$).

We also tested the effect of protein intake on breast cancer incidence and progression in a mouse model. Twelve-week-old female BALB/c mice were placed under the same dietary regimen as described for C57BL/6 mice, except that the mice had to be switched from a 4% to a 7% kcal from protein diet within the first week in order to prevent weight loss (Figures S1E and S1F). After a week of feeding on these diets, mice were implanted subcutaneously with 20,000 cells of syngeneic, murine breast cancer (4T1), and 15 days later animals were assessed for tumors. On day 18 postimplantation (day 25 on the diet), tumor incidence was 100% in the high protein (18%) group but only 70% in the low protein (7%) group. The incidence in the low protein group rose to 80% at day 39, where it remained until the end of the experiment (Figure 3F). A 45% smaller mean tumor size was also observed in the low protein group compared to the high protein group at the end of the experiment at day 53 ($p = 0.0038$) (Figure 3G). As for C57BL/6 mice, IGF-1 was measured after 16 days from the switch to different protein levels. In the low protein intake group, IGF-1 levels were reduced by 30% compared to those in the high level group ($p < 0.0001$) (Figure 3H). Additionally, a low protein intake also caused an IGFBP-1 increase of 84% ($p = 0.001$) (Figure 3I), similar to what was observed in the C57BL/6 genetic background (Figure 3D). Analogously, when soy protein intake was reduced from high levels to low levels, we observed a 30% decrease in IGF-1 ($p < 0.0001$) (Figure 3J) and a 140% increase in IGFBP-1 ($p < 0.0001$) (Figure 3K). Although there was a trend for an effect of substituting the same level of animal protein with plant protein on IGF-1 and IGFBP-1, the differences were not significant. These data suggest that lower protein intake may play a role in decreasing cancer incidence and/or progression in part by decreasing IGF-1 and increasing the IGF-1 inhibitor IGFBP-1. Additional studies on various types of animal- versus plant-based proteins are necessary to determine their effect on cancer, IGF-1, and IGFBP-1.

Cellular Studies

To test the hypothesis that there is a fundamental link between the level of amino acids and lifespan, the impact of the presence of specific concentrations of amino acids on yeast survival and mutation rate was assayed. A wild-type DBY746 *S. cerevisiae* strain was grown in the presence of half (0.5×), standard (1×), and double (2×) amino acid concentrations with all other nutrients maintained constant. Survival was measured at days 1, 3, 5, and 8. No survival differences were observed during days 1 and 3. At day 5, the two highest amino acid concentrations showed a trend for increased mortality, which resulted in a 10-fold decrease in surviving cells by day 8 (Figure 3L).

In order to assess the relationship between amino acids, aging, and age-dependent DNA damage, we used aging *S. cerevisiae* to measure spontaneous mutation rate (Madia et al., 2007). The mutation rate was 3- and 4-fold higher in 5-day-old but not young cells exposed to 1× and 2× amino acid levels, respectively, compared to cells exposed to a 0.5× amino acid concentration (Figure 3M). These results indicate that even in unicellular organisms, amino acids can promote cellular aging and age-dependent genomic instability.

To further discern the pathways involved in promoting age-dependent genomic instability, we measured the induction of stress responsive genes regulated by the Ras-PKA-Msn2/4 and Tor-Sch9-Gis1 pathways in the presence or absence of amino acids. For cells grown in control media containing only tryptophan (Trp), leucine (Leu), and histidine (His) (essential for growth in this strain), the presence of all amino acids in the media reduced the induction of stress resistance transcription factors Msn2/4 (STRE) and Gis1 (PDS), indicating that the addition of amino acids was sufficient to inhibit cellular protection (Figure 3N).

The Tor-Sch9 pathway extends longevity but also promotes DNA mutations (Madia et al., 2009; Wei et al., 2008). To determine whether Ras-cAMP-PKA signaling also regulates age-dependent genomic instability, we studied Ras2-deficient mutants. We confirmed that *ras2Δ* mutants are long-lived (Figure S1H) but also show that inactivation of Ras signaling attenuated age- and oxidative stress-dependent genomic instability (Figures S1I, S1J, 3O, and S1K). Together, these results indicate a mechanism where amino acids are able to affect mutation frequency and thus genomic instability, at least in part, by activation of the Tor-Sch9 and Ras/PKA pathways and decreased stress resistance (Figure 3P).

Low Protein Intake and Weight Maintenance in Old Mice

Based on the observed opposite effects of a low protein diet in subjects 50–65 years old versus those 66 and older and on the major drop in BMI and IGF-1 levels after age 65, we

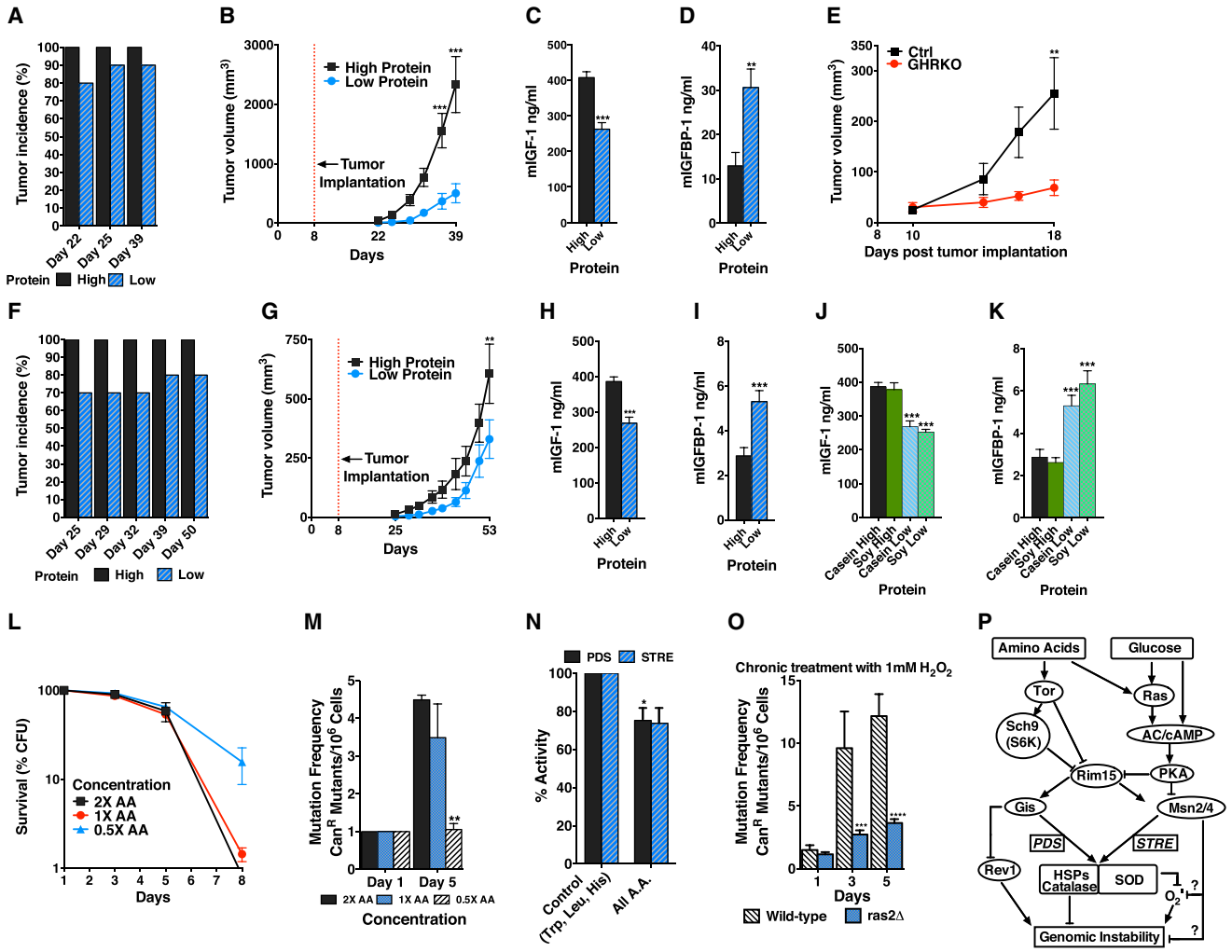

Figure 3. Effects of Proteins and Amino Acids on Tumor Progression or DNA Damage in Mouse and *S. cerevisiae* Models

(A) Tumor incidence in 18-week-old male C57BL/6 mice implanted with 20,000 melanoma (B16) cells and fed either a high protein (18%; n = 10) or low protein (4%; n = 10) diet.

(B) B16 tumor volume progression in 18-week-old male C57BL/6 mice fed either a high protein (n = 10) or low protein (n = 10) diet.

(C) IGF-1 at day 16 in 18-week-old male C57BL/6 mice fed either a high protein (n = 5) or low protein (n = 5) diet.

(D) IGFBP-1 at day 16 in 18-week-old male C57BL/6 mice fed either a high protein (n = 10) or low protein (n = 10) diet.

(E) B16 melanoma tumor progression in 10-month-old female GHRKO mice (n = 5) versus age-matched littermate controls (Ctrl; n = 7).

(F) Tumor incidence in 12-week-old female BALB/c mice implanted with 20,000 breast cancer (4T1) cells and fed either a high protein (18%; n = 10) or low protein (7%; n = 10) diet.

(G) 4T1 breast cancer progression in 12-week-old female BALB/c mice fed either a high protein (n = 10) or low protein (n = 10) diet.

(H) IGF-1 at day 16 in 12-week-old female BALB/c mice fed either a high animal protein (n = 5) or low animal protein (n = 5) diet.

(I) IGFBP-1 at day 16 in 12-week-old female BALB/c mice fed either a high animal protein (n = 10) or low animal protein (n = 10) diet.

(J) IGF-1 at day 16 in 12-week-old female BALB/c mice fed either a high soy protein (n = 5) or low soy protein (n = 5) diet.

(K) IGFBP-1 at day 16 in 12-week-old female BALB/c mice fed either high soy protein (n = 10) or low soy protein (n = 10) diet.

(L and M) Survival (L) and DNA mutation frequency (M) of yeast exposed to a 0.5×, 1×, or 2× concentration of a standard amino acid mix.

(N) PDS and STRE activity in yeast grown in media containing only Trp, Leu, and His compared to those grown in the presence of all amino acids.

(O) *RAS2* deletion protects against oxidative stress-induced genomic instability measured as DNA mutation frequency (Can^r) in wild-type (DBY746) and *ras2Δ* mutants chronically exposed to 1 mM H_2O_2.

(P) A model for the effect of amino acids on aging and genomic instability in *S. cerevisiae*. Amino acids activate the Tor-Sch9 and Ras-cAMP-PKA pathway also activated by glucose and promote age- and oxidative stress-dependent genomic instability in part via reduced activity of Gis1 and Msn2/4. In all graphs, data points represent the mean of the biological replicates ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001.

hypothesized that older subjects on a low protein diet may become malnourished and unable to absorb or process a sufficient level of amino acids. To test this possibility in mice, we fed young mice (18 weeks old) and old mice (24 months old) with isocaloric diets containing either 18% or 4% animal protein. A very low protein diet was purposely selected to reveal any sensitivity to protein restriction in an old organism. Whereas old mice maintained on a high protein diet for 30 days gained

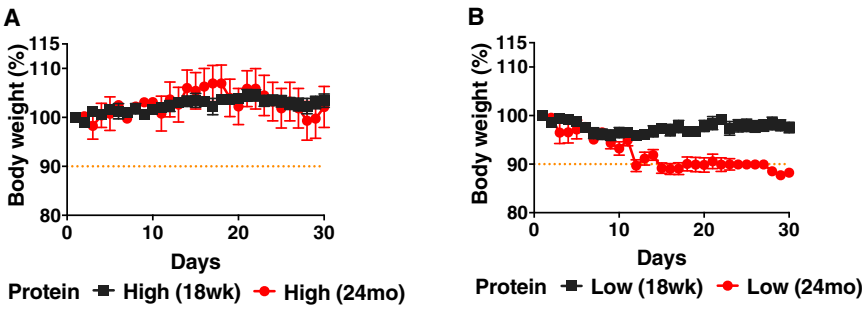

Figure 4. Effect of Protein Intake on Body Weight in Young and Old Mice

(A) Young (18-week-old; n = 10) and old (24-month-old; n = 6) C57BL/6 mice fed a high (18%) protein diet.

(B) Young (18-week-old; n = 10) and old (24-month-old; n = 6) C57BL/6 mice fed a low (4%) protein diet. Data points represent mean ± SEM.

weight, old but not young mice on a low protein diet lost 10% of their weight by day 15 (Figures 4A and 4B), in agreement with the effect of aging on turning the beneficial effects of protein restriction on mortality into negative effects.

DISCUSSION

Here, using a major nationally representative study of nutrition in the United States population, our results show that among those ages 50 and above, the level of protein intake is associated with increased risk of diabetes mortality, but not associated with differences in all-cause, cancer, or CVD mortality. Nevertheless, we found an age interaction for the association between protein consumption and mortality, both overall and from cancer, with subjects ages 50–65 years potentially experiencing benefits from low protein intake, and subjects ages 66+ experiencing detriments. This may explain why previously the strong association between protein intake, IGF-1, disease, and mortality has been poorly understood and controversial (Saydah et al., 2007). Furthermore, among 2,253 subjects, the risks of all-cause and cancer mortality for those with high protein intake compared to the low protein intake group were increased even further for those who also had high levels of IGF-1. This is in agreement with previous studies associating IGF-1 levels to various types of cancer (Giovannucci et al., 2003; Guevara-Aguirre et al., 2011; Pollak et al., 2004).

Notably, our results showed that the amount of proteins derived from animal sources accounted for a significant proportion of the association between overall protein intake and all-cause and cancer mortality. These results are in agreement with recent findings on the association between red meat consumption and death from all-cause and cancer (Fung et al., 2010; Pan et al., 2012). Previous studies in the U.S. have found that a low carbohydrate diet is associated with an increase in overall mortality and showed that when such a diet is from animal-based products, the risk of overall as well as cancer mortality is increased even further (Fung et al., 2010; Lagiou et al., 2007). Our study indicates that high levels of animal proteins, promoting increases in IGF-1 and possibly insulin, is one of the major promoters of mortality for people age 50–65 in the 18 years following the survey assessing protein intake.

Our results from yeast and mice may explain at least part of the fundamental connection between protein intake, cancer, and overall mortality by providing a link between amino acids, stress resistance, DNA damage, and cancer incidence/progression. In mice, the changes caused by reduced protein levels had an effect potent enough to prevent the establishment of 10%–30% of tumors, even when 20,000 tumor cells were already present at a subcutaneous site. Furthermore, the progression of both melanoma and breast cancer was strongly attenuated by the low protein diet, indicating that low protein diets may have applications in both cancer prevention and treatment, in agreement with previous studies (Fontana et al., 2006, 2008; McCarty, 2011; Youngman, 1993).

Although protein intake is associated with increased mortality for adults who were middle-aged at baseline, there was also evidence that a low protein diet may be hazardous for older adults. Both high and moderate protein intake in the elderly were associated with reduced mortality compared to that in the low protein group, suggesting that protein intake representing at least 10% of the calories consumed may be necessary after age 65 to reduce age-dependent weight loss and prevent an excessive loss of IGF-1 and of other important factors. In fact, previous studies have noted that an increased protein intake and the resulting increase in IGF-1 may prove beneficial in older adults (Heaney et al., 1999), and the switch from the protective to the detrimental effect of the low protein diet coincides with a time at which weight begins to decline. Based on previous longitudinal studies, weight tends to increase up until age 50–60, at which point it becomes stable before beginning to decline steadily by an average of 0.5% per year for those over age 65 (Villareal et al., 2005; Wallace et al., 1995). We speculate that frail subjects who have lost a significant percentage of their body weight and have a low BMI may be more susceptible to protein malnourishment. It is also possible that other factors such as inflammation or genetic factors may contribute to the sensitivity to protein restriction in elderly subjects, in agreement with our mouse studies.

Although other studies have noted age-associated declines of nutrient absorption in rodents related to changes in the pH microclimate, impaired adaptive response in the aged gut, and changes in the morphology of the intestine, there is still no clear association between food absorption and mortality (Chen et al., 1990; Woudstra and Thomson, 2002). In humans, some studies have shown that dietary protein digestion and absorption kinetics are not impaired in vivo in healthy, elderly men. However, these studies have also reported increased splanchnic extraction of amino acids, which might result in decreased availability to peripheral tissues, and speculated that in the case of low protein intake or increased protein requirement, the limited systemic availability of dietary amino acids may contribute to decreased muscle protein synthesis (Boirie et al., 1997; Koopman et al., 2009). Furthermore, in humans factors like poor dentition, medication, and psychosocial

issues also play a significant role in rates of malnourishment (Woudstra and Thomson, 2002).

IGF-1 has been previously shown to decrease at older ages (Iranmanesh et al., 1991), possibly increasing the risk of frailty (Lamberts et al., 1997) and mortality (Cappola et al., 2003). Thus our findings may explain the controversy related to IGF-1 and mortality indicating that a minimum level of proteins and possibly IGF-1 is important in the elderly, or that low circulating IGF-1 reflects a state of malnourishment, frailty, and/or morbidity (Maggio et al., 2007). In fact, inflammation and other disorders are known to decrease IGF-1 levels, raising the possibility that the low protein and low IGF-1 group may contain a significant number of both malnourished and frail individuals having or in the process of developing major diseases (Fontana et al., 2012).

There are some limitations to our study, which should be acknowledged. First, the use of a single 24 hr dietary recall followed by up to 18 years of mortality assessment has the potential of misclassifying dietary practice if the 24 hr period was not representative of a participant's normal day. However, 93% of our sample reported that the 24 hr period represented a normal day. We also include this variable as a control in our analysis. Furthermore, the 24 hr dietary recall has been shown to be a valid approach to identify the "usual diet" of subjects (Blanton et al., 2006; Conway et al., 2004; Coulston and Boushey, 2008; Prentice et al., 2011). While we must admit that the lack of longitudinal data on dietary consumption is a potential limitation of our study, study of dietary consistency over six years among older people revealed little change over time in dietary habits (Garry et al., 1989). Another study looking at dietary habits over 20 years showed that while energy intake decreased for protein, fat, and carbohydrates as people aged, the decreases were equal across the three types (Flynn et al., 1992).

Another limitation of our study is classification of respondents into protein intake groups and small sample sizes, especially for analyses involving diabetes mortality among persons without diabetes at baseline, or participants in the IGF-1 subsample. As a result, our hazard ratios and 95% confidence intervals may be much larger than what would have been seen with a larger sample size. Nevertheless, one would expect a small sample size to decrease statistical power and make it harder to detect associations. Therefore, our ability to detect significance indicates that the associations between protein and mortality are robust. Furthermore, the lower limits of the 95% confidence intervals from our mortality analyses were well above 1.0, signifying that the increased risk is probably large. Finally, given these limitations, our study was strengthened by its use of reliable cause-specific mortality data, as well as its inclusion of a large nationally representative sample, a feature often missing from the previous literature.

Overall, our human and animal studies indicate that a low protein diet during middle age is likely to be beneficial for the prevention of cancer, overall mortality, and possibly diabetes through a process that may involve, at least in part, regulation of circulating IGF-1 and possibly insulin levels. In agreement with other epidemiological and animal studies (Estruch et al., 2013; Linos and Willett, 2007; Michaud et al., 2001; Willett, 2006), our findings suggest that a diet in which plant-based nutrients represent the majority of the food intake is likely to maximize health benefits in all age groups. However, we propose that up to age 65 and possibly 70, depending on health status, the 0.7 to 0.8 g of proteins/kg of body weight/day reported by the Food and Nutrition Board of the Institute of Medicine, currently viewed as a minimum requirement, should be recommended instead of the 1.0–1.3 g grams of proteins/kg of body weight/day consumed by adults ages 19–70 (Fulgoni, 2008). We also propose that at older ages, it may be important to avoid low protein intake and gradually adopt a moderate to high protein, preferably mostly plant-based consumption to allow the maintenance of a healthy weight and protection from frailty (Bartali et al., 2006; Ferrucci et al., 2003; Kobayashi et al., 2013).

EXPERIMENTAL PROCEDURES

Nutrient Intake for Human Data

Nutrient intake data are based on reports of food and beverage intake during a 24 hr period. Data were collected via an automated, microcomputer-based coding system, with information on over 80 nutrients. There are several advantages to using this method for collecting dietary data. Given that the time elapsing between consumption and recall is short, participants are typically able to recall more information. Also, unlike many other reporting methods, 24 hr dietary recall relies on data collection after consumption, reducing the potential for assessment to alter dietary behaviors (Coulston and Boushey, 2008). Furthermore, 24 hr recalls have been shown to be a stronger estimate of total energy and protein consumption compared to the commonly used food frequency questionnaires (Prentice et al., 2011), and have also been shown to be a more valid measure of total energy and nutrient intake than both the Block food-frequency questionnaire and the National Cancer Institute's Diet History Questionnaire (Blanton et al., 2006). Finally, this approach has been found to accurately assess energy, protein, fat, and carbohydrate intake, regardless of body mass index (Conway et al., 2004).

Epidemiological Mortality Follow-Up

Mortality data were available from the National Death Index. Information for 113 potential underlying causes of death (UCOD-113) was used to determine all-cause mortality, cardiovascular mortality, cancer mortality, and diabetes mortality.

Statistical Analysis for Human Data

Cox proportional hazard models were used to estimate the association between intake of calories from protein on subsequent all-cause, CVD, cancer, and diabetes mortality, with the latter three run using competing-risks structures. Next we tested the interaction between age and protein consumption on the association with mortality. Based on these results, we categorized subjects into two age groups (50–65 years and 66+ years), which were used in the remainder of the analyses. age-stratified proportional hazard models were used to estimate the association of percent calories from protein with mortality within the two age groups and examine whether the relationship was influenced by percent of calories from fat, percent of calories from carbohydrates, or animal protein. Hazard models were re-estimated for the IGF-1 subsample to determine whether including IGF-1 changed the association between protein intake and mortality. Finally, proportional hazard models were used to examine the interaction between protein and IGF-1 and to calculate predicted hazard ratios for each protein group at various IGF-1 levels, to determine whether protein intake is differentially associated with mortality depending on levels of IGF-1. All analyses were run using sample weights, accounting for sampling design, and controlling for age, race/ethnicity, education, sex, disease status, smoking, dietary changes, attempted weight loss, and total calorie consumption.

Cancer Models in Mice

All animal experiments were performed according to procedures approved by USC's Institutional Animal Care and Use Committee. To establish a subcutaneous cancer mouse model, we injected 18-week-old male C57BL/6 mice as well as 10-month-old GHRKO mice, age-matched littermate control mice with B16 melanoma cells, and 12-week-old female BALB/c with 4T1 breast

cancer cells. Before injection, cells in log phase of growth were harvested and suspended in serum-free, high-glucose Dulbecco's modified Eagle's medium (DMEM) at 2×10^5 or 2×10^6 cells/ml, and 100 μl (2×10^4 cells per C57BL/6 or BALB/c mouse; 2×10^5 cells per GHRKO mouse) was subsequently injected subcutaneously in the lower back. All mice were shaved before subcutaneous tumor injection. Tumor incidence was determined by palpation of the injected area, and tumor size was measured using a digital Vernier caliper starting 10–15 days postimplantation. The experiments for C57BL/6 and BALB/c mice ended at different time points based on USC IACUC-approved humane endpoint criteria for tumor size and ulceration.

SUPPLEMENTAL INFORMATION

Supplemental Information includes two figures, seven tables, Supplemental Results, and Supplemental Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.cmet.2014.02.006.

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Obesity, Visceral Adiposity, and Prostate Cancer: What Is the Role of Lifestyle Interventions?

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Excess body fat has long been recognized as a serious health risk, with the World Health Organization identifying obesity as a global health epidemic more than 20 years ago.[1] Since that time, research has continued to demonstrate the negative impact of excess body fat by showing associations between obesity and metabolic syndrome, type 2 diabetes, cardiovascular disease, sleep-related breathing abnormalities, infertility, osteoarthritis, and liver and gallbladder disease.[2] Obesity has also previously been associated with certain cancers, particularly cancers targeting the colon, breast (among postmenopausal women), endometrium, esophagus, and kidneys, as well as advanced prostate cancer. Although targeting obesity has remained critical, research is also focusing on the importance of the location of fat tissue, with particular concerns centering on visceral fat.[3,4] High levels of visceral fat have been associated with a large number of medical conditions, including insulin resistance, type 2 diabetes, hypertension, and cardiovascular disease, as well as all-cause mortality.[3-6]

In novel research building upon this evidence for the importance of the distribution of body fat to the risk of disease,[3-6] Dickerman et al[7] conducted a prospective examination of whether fat distribution was associated with prostate cancer outcomes. Working with cohort data from the Age, Gene/Environment Susceptibility–Reykjavik (AGES-Reykjavik) study, the investigators used computed tomography imaging of fat in the abdomen and thigh as well as prostate cancer outcomes to identify associations between visceral fat and the risk of advanced disease and between thigh subcutaneous fat and the risk of fatal prostate cancer. The association between visceral fat and advanced and fatal disease held, and indeed was stronger, among men with a lower body mass index (BMI). Although the study presents an advancement in considerations of fat distribution and prostate cancer, it is important to consider 2 points. First, the BMI cutoff for lean men was based on participant median values. As such, men with BMIs under 27 kg/m^2 were considered lean when, traditionally, this classification would include both normal-weight men and overweight men, who would likely have higher visceral fat. This BMI categorization may influence the generalizability of findings. Second, fat in multiple locations (ie, visceral fat and thigh subcutaneous fat) was associated with clinically relevant prostate cancer. This supports existing evidence for the link between body fatness, regardless of location, and advanced prostate cancer indicated previously.

The findings from Dickerman et al's research,[7] in addition to existing evidence on obesity and prostate cancer, highlight the need to examine lifestyle interventions that target fat loss in promoting optimal prostate cancer outcomes. Two lifestyle behaviors frequently examined within weight-loss research are diet and exercise. These behaviors are targeted because of their influence on energy balance.[8-11] When each lifestyle behavior is considered separately, diet is a commonly used and effective method for targeting weight loss because exercise has limited impact on total weight loss.[12,13] Although many types of diets have been studied (eg, low-carbohydrate, low-fat, high-protein, and Mediterranean diets), a recent meta-analysis has found that a low-carbohydrate/high-protein dietary intervention results in the greatest weight loss.[12]

However, in light of the strong emphasis in the article on the findings associating visceral fat and advanced prostate cancer among men with lower BMIs and in light of existing knowledge on the detrimental impact of visceral fat, there is also a need to determine what behavior may be most effective in targeting this regional fat distribution. In these cases, exercise may be particularly beneficial.[10,14,15] For example, in research comparing overweight men with high and low levels of fitness, those with higher levels of fitness had significantly lower visceral fat, and this suggests

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that physical activity targeting fitness may be critical in reducing visceral fat.[16] Indeed, regardless of total body weight or weight loss, increases in physical activity, more specifically exercise, have been related to significant decreases in visceral fat.[10,14]

Because of the importance of both behaviors in targeting fat loss identified as necessary by the current analysis from the AGES-Reykjavik study, it may be most beneficial to frame lifestyle interventions for prostate cancer with an approach that combines both behaviors—in this case, diet plus exercise. Existing research and meta-analyses support the beneficial impact of combining both behaviors for achieving greater weight loss.[17,18] The goal of intervening with these combined behaviors would be to reduce body fat, with a particular emphasis on visceral fat, without a decline in muscle mass. As a result, although many interventions use aerobic-based approaches, resistance training may be important for achieving optimal changes in body composition.[17]

In light of the findings by Dickerman et al[7] and the existing body of knowledge on diet and exercise, new opportunities arise for knowledge development and practice for researchers and clinicians. First, researchers could benefit from including measures that assess visceral fat as outcomes in interventions, whereas clinicians would benefit from collecting knowledge of fat location in addition to the total weight. This information, alongside existing outcome measures such as inflammatory markers, may be beneficial in indicating whether prostate cancer risks are being targeted during treatment.

Second, the research provides support for the importance of the timing of lifestyle interventions. According to the information provided by Dickerman et al[7] on the cohort in the AGES-Reykjavik study, optimal benefits may be experienced prior to diagnosis through early preventative interventions. Within the AGES-Reykjavik study, the men who demonstrated higher visceral fat also had lower self-reported physical activity during youth and midlife. Although a direct association was not made, through knowledge of the importance of diet and physical activity in targeting fat, interventions should potentially prioritize targeting these lifestyle factors during youth or emerging adulthood in order to decrease the risk of prostate cancer. Indeed, research on physical activity and prostate cancer suggests that adolescence and young adulthood may be optimal years for preventive behaviors such as physical activity.[19] In sum, the evidence linking fat and particularly distribution to prostate cancer outcomes opens a number of directions for enhancing the direction and quality of lifestyle interventions.

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IN PERSPECTIVE

Obesity and cancer: A mechanistic overview of metabolic changes in obesity that impact genetic instability

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Abstract

Obesity, defined as a state of positive energy balance with a body mass index exceeding 30 kg/m^2 in adults and 95th percentile in children, is an increasing global concern. Approximately one-third of the world's population is overweight or obese, and in the United States alone, obesity affects one in six children. Meta-analysis studies suggest that obesity increases the likelihood of developing several types of cancer, and with poorer outcomes, especially in children. The contribution of obesity to cancer risk requires a better understanding of the association between obesity-induced metabolic changes and its impact on genomic instability, which is a major driving force of tumorigenesis. In this review, we discuss how molecular changes during adipose tissue dysregulation can result in oxidative stress and subsequent DNA damage. This represents one of the many critical steps connecting obesity and cancer since oxidative DNA lesions can result in cancer-associated genetic instability. In addition, the by-products of the oxidative degradation of lipids (e.g., malondialdehyde, 4-hydroxynonenal, and acrolein), and gut microbiota-mediated secondary bile acid metabolites (e.g., deoxycholic acid and lithocholic acid), can function as genotoxic agents and tumor promoters. We also discuss how obesity can impact DNA repair efficiency, potentially contributing to cancer initiation and progression. Finally, we outline obesity-related epigenetic changes and identify the gaps in knowledge to be addressed for the development of better therapeutic strategies for the prevention and treatment of obesity-related cancers.

KEYWORDS
DNA damage, DNA repair, genetic instability, obesity, oxidative stress

Abbreviations: $^{\bullet}$OH, hydroxyl radical; 4-HNE, 4-hydroxynonenal; 5-mC, 5′-methyl-cytosine; 8-OH-dG, 8-hydroxy-2′-deoxyguanosine; 8-oxo-dG, 8-oxo-7,8-dihydro-2′-deoxyguanosine; Acr, acrolein; AP, apurinic/apyrimidinic; APE1, apurinic/apyrimidinic endonuclease 1; APNG, alkyl-*N*-purine glycosylase; ATM, adipose tissue macrophage; BA, bile acids; BER, base excision repair; BMI, body mass index; BPDE, Benzo(a)pyrene diol epoxide; C/EBPβ, CCAAT/enhancer-binding protein-β; CA, cholic acid; CDCA, chenodeoxycholic acid; CHO, Chinese hamster ovary; CRC, colorectal cancers; CS, cockayne syndrome; CSA, cockayne syndrome group A; CSA, cockayne syndrome group B; CXCL-9, C-X-C motif chemokine ligand 9; DCA, deoxycholic acid; DPCs, DNA-protein crosslinks; DSBs, DNA double-strand breaks; *E. Coli*, Escherichia coli; EAC, esophageal adenocarcinoma; FEN1, flap endonuclease 1; FLT3-ITD, FMS-like tyrosine kinase receptor; FXR, farnesoid X receptor; Gro-α, growth-regulated oncogene-alpha; H_2O_2, hydrogen peroxide; HCC, hepatocellular carcinoma; HDACs, histone deacetylases; HFD, high-fat diet; HNE-dG, 6-(1-hydroxyhexanyl)−8-hydroxy-1,N(2)-propano-2′-deoxyguanosine; HR, homologous recombination repair; HSCs, hepatic stellate cells; ICLs, inter-strand crosslinks; IKKβ, Inhibitor of kappa B kinase β; IL, interleukin; JNK, c-Jun N-terminal kinase; LCA, lithocholic acid; M_1dG, 3-(2-deoxy-β-D-erythro-pentofuranosyl)pyrimido[1,2-α]purin-10(3H)-one deoxygunanosine; MDA, malondialdehyde; MLH1, MutL homolog 1 colon cancer, nonpolyposis type 2; mM, millimolar; MMR, mismatch repair; MMS, methyl methanesulfonate; MNNG, methyl-*N*′-nitro-*N*-nitrosoguanidine; MutS, mutator S; MUTYH, mutY glycosylase homologue; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NEIL, nei endonuclease VIII-like; NER, nucleotide excision repair; NFκB, nuclear factor κ-light-chain-enhancer of activated B cells; NHBE, normal human bronchial epithelia; NHLF, normal human lung fibroblasts; Nox 4, NADPH oxidase 4; $O_2^{\bullet}$, superoxide radical; OGG1, 8-oxoguanine DNA glycosylase; PARP, poly-ADP ribose polymerase; PdG, propanodeoxyguanosine; Pol α, DNA polymerase α; Pol β, DNA polymerase β; PPAR-γ, peroxisome proliferator-activated receptor-gamma; PUFA, polyunsaturated fatty acids; PXR, pregnane X receptor; ROS, reactive oxygen species; SASP, senescence-associated secretory phenotype; SSBs, single-strand breaks; T2DM, type II diabetes; TC-NER, transcription-coupled nucleotide excision repair; TNF-α, tumor necrosis factor-α; topo II, topoisomerase II; XPA, xeroderma pigmentosum complement group A; α-OH-Acr-dG, α-hydroxy-1,N2-propano-2′-deoxyguanosine; γ-OH-Acr-dG, γ-hydroxy-1,N2-propano-2′-deoxyguanosine; μM, micromolar.

1 | INTRODUCTION

The present global burden of issues affecting human health is not limited to infectious disease, as noncommunicable disease, such as obesity is the fifth leading global risk factor for mortality.[1] The global prevalence of children and adults being overweight or obese has increased by ~47% and ~28%, respectively, between 1980 and 2013.[2] Overweight and obesity are defined as the accumulation of excess body fat, which can have a negative effect on health. The body-mass index (BMI), measured as a ratio of weight in kilograms to the square of the height in meters, is often used as a tool for assessing overall adiposity. Adults with a BMI of 25.0 to 29.9 kg/m^2 are considered overweight, whereas a BMI of greater than 30 kg/m^2 is considered as obese. BMI-for-age percentiles define whether a child or young adult is overweight (85th to 95th percentile) or obese (>95th percentile).[3] With one-third of the world's population being overweight or obese, both developed and developing countries are coping with the challenges posed by this epidemic.[4]

Apart from the psychological challenges such as depression, anxiety, eating disorders, and low self-esteem,[5] obesity is also associated with a number of psychiatric disorders.[6] High adiposity also elevates the risk for developing other serious diseases and health conditions such as metabolic syndrome, diabetes, hypertension, dyslipidemia, hyperglycemia, sleep disorders, cardiovascular disease, and osteoarthritis.[7] Evidence from several epidemiological studies have suggested that a significant increase in the amount of body fat is associated with an increased risk of various cancers, including endometrial, breast, colon, kidney, gallbladder, liver, and melanoma to name a few.[8-11] In fact, according to a 2012 population-based study, ~4% of all cancer cases in adults worldwide may be attributed to high BMI levels.[12]

Meta-analysis data from epidemiological studies have enabled us to identify the existence of a link between obesity and several diseases. But owing to the differences in metabolic profiles, genetics, and the environment to which an individual is exposed, mechanistic studies are required to determine the physiological and pathological alterations instigated at the molecular level. For example, there are several metabolic and cellular alterations that are thought to link obesity and cancer, including (1) chronic low-level systemic inflammation due to elevated levels of cytokines[13]; (2) insulin resistance due to high levels of insulin and insulin-like growth factor-1, resulting in altered insulin signaling[14]; (3) dysregulation of adipokines, for example, leptin and adiponectin[15]; (4) elevated levels of estrogen in adipose tissue[16]; (5) increased lipids and alterations in lipid signaling[17]; and (6) oxidative stress leading to cellular and molecular alterations, including DNA damage.[18]

The molecular mechanisms that underlie the effects of obesity on tumor formation are complex and certainly involve multiple pathways. An important factor associated with obesity-induced metabolic dysregulation is alterations in the adipose tissue.[19] Adipose tissue is a dynamic endocrine organ, which apart from serving as an energy reservoir, has biochemical and metabolic functions that are implicated in cancer incidence and progression.[20,21] Many groups have found that p53 levels are elevated in adipose tissue under conditions of chronic nutrient abundance. Contrary to its role as a tumor suppressor, the sustained activation of *p53* signaling has been linked to the development of insulin resistance and adiposity. Activation of *p53* also contributes to the induction of inflammatory cytokines that can promote the initiation and progression of cancer.[22-24]

Another pathway that seems to associate obesity with cancer development is genetic instability.[25] Many studies have indicated that obesity-related modulations in DNA damage and/or repair pathways may be involved in obesity-induced genetic instability.[26] However, little is known about the impact of obesity on DNA damage and DNA repair mechanisms that play critical roles in tumor initiation and progression. A better understanding of the relationship between obesity and cancer at the molecular level is important as it may aid in the development of effective therapies and noninvasive interventions that can help attenuate the obesity-associated cancer risk.

Studies have shown that oxidative stress and lipid dysregulation in obese individuals can result in elevated levels of reactive oxygen species (ROS), which in turn can cause oxidative DNA damage directly or via the formation of reactive lipid peroxidation by-products (e.g., malondialdehyde [MDA], 4-hydroxynonenal [4-HNE], and acrolein [Acr]), and secondary bile acid (BA) metabolites (e.g., deoxycholic acid [DCA] and lithocholic acid [LCA]).[18,27-29] Another aspect central to obesity-induced carcinogenesis is the inefficient repair and/or failure of DNA repair mechanisms to process ROS-induced and other obesity-associated metabolite-mediated DNA lesions, which can result in genetic instability.[26] Recent studies have also suggested that obesity-induced epigenomic reprogramming can serve as signatures for the prediction of the development of obesity-related metabolic disorders and cancers.[30-32] This review specifically focuses on obesity-associated alterations in DNA damage (including oxidative DNA damage, and DNA damage induced by lipid peroxidation by-products and secondary BA metabolites), and DNA repair mechanisms that may result in genetic instability and increased cancer risk (Figure 1).

2 | DNA DAMAGE

2.1 | Obesity-induced oxidative stress promotes DNA damage

Formation of free radicals is a consequence of many endogenous biochemical processes, as well as the result of exposure to external factors. The presence of unpaired electrons in free radicals such as the hydroxyl radical ($^{\bullet}OH$) and the superoxide radical ($O_2^{\bullet-}$) makes them unstable and highly reactive.[33] Biological macromolecules such as nucleic acids, proteins, carbohydrates, and lipids are potential targets of free radicals or ROS, which can result in perturbations to cell and tissue homeostasis.[34] This process of oxidative stress can increase the potential for tissue damage; thus, cells have evolved a

variety of antioxidative defense mechanisms.[35] However, the onset of several pathological conditions such as obesity, diabetes, and cardiovascular disease can disturb this balance leading to additional systemic oxidative stress.[36,37] Several studies in mice and humans have confirmed a positive correlation between systemic oxidative stress and fat dysregulation in adipose tissue, resulting in obesity-related metabolic disorders.[27,38,39]

Adipose tissue is the preferred site of excess fat storage during nutrient abundance. However, the expansion capacity of adipose tissue is finite and when a critical limit is reached, lipid spillage from adipocytes and accumulation in other metabolically harmful ectopic tissues can occur, resulting in lipid toxicity.[40] Environmental and genetic factors can modulate the capacity of adipose tissue to increase in mass.[41] Moreover, the regulation of the fat-storing capacity of adipose tissue, while maintaining homeostasis during obesity, is dependent on the ability of existing adipocytes to expand (hypertrophy) and/or preadipocytes to differentiate into adipocytes (adipogenesis).[42] For example, several studies in mouse models and humans have shown that hypertrophic expansion of adipocytes resulted in adipose tissue dysregulation associated with impaired insulin sensitivity, disturbed adipokine secretion, and induction of inflammation due to the activation and infiltration of immune/inflammatory cells.[43]

Using genetically engineered and/or diet-induced mouse models of obesity and insulin resistance, Prieur et al[44] showed that lipid-induced toxicity might serve as a link between obesity and inflammation due to adipose tissue macrophage (ATM) polarization. The infiltration of macrophages and the induction of anti-inflammatory M2 markers, in association with the remodeling of adipose tissue, may protect against progressive early-stage positive energy balance. However, later stages of obesity characterized by hypertrophic expansion of adipocytes can lead to ATM class switching from anti-inflammatory M2 to proinflammatory M1 cytokines, such as tumor necrosis factor-α (TNF-α), interleukin IL-1β, IL-6, leptin, visfatin, resistin, angiotensin II, and monocyte chemoattractant protein-1.[45-49] In adipose tissue, these proinflammatory cytokines can promote insulin resistance either directly by inhibiting insulin signal transduction via serine phosphorylation of the insulin receptor,[50,51] or indirectly by disrupting the insulin signaling pathways via the c-Jun N-terminal kinase (JNK) and the I-kappa B kinase β (IKKβ)/nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) pathways.[52] In addition, proinflammatory cytokines such as IL-1, TNF-α, and interferon gamma can stimulate the production of ROS stimulating oxidative stress.[53,54]

Though the inherent mechanism linking adipose tissue dysfunction and oxidative stress is unclear, various studies have suggested that the production of ROS and free radical generation is increased in adipose tissue in the obese state.[27,55] The role of oxidative stress in adipogenesis is not yet clearly understood. For example, while elevated oxidative stress in aging can inhibit preadipocyte differentiation,[56] in obesity, ROS, and free radicals can promote preadipocyte differentiation.[57] To support a role of oxidative stress in the promotion of adipogenesis in obesity, Lee et al[58] demonstrated that redox-induced DNA binding of CCAAT/enhancer-binding protein-β (C/EBPβ) is crucial for the clonal expansion and differentiation of adipocytes. Further, a study in nondiabetic obese Zucker fatty rats also showed that the association of obesity with a reduced redox state could promote differentiation of preadipocytes.[59]

The parallel increase of ROS levels with the expansion of adipocytes has also been found to be associated with the upregulation of NADPH oxidase 4 (Nox4).[27,60,61] Although some studies have provided evidence for the protective effect of Nox4,[62,63] others have reported Nox4-mediated ROS as a molecular switch promoting the differentiation of preadipocytes, the onset of insulin resistance, and adipose tissue inflammation.[60,64-66] Additionally, increased expression of *Nox4* in white adipose tissue can result in the production of H_2O_2, which can be converted to •OH radicals via the Fenton and Haber-Weiss reactions in the presence of Fe^{2+} and Cu^{2+} ions, eventually leading to oxidative DNA damage.[27] Weyemi et al[67] have reported that the *H-Ras* oncogene positively regulated Nox4, resulting in the production of ROS, the induction of DNA double-strand breaks (DSBs), and subsequent cellular senescence. In an obese mouse model, gain-of-function mutations in the *H-Ras* oncogene were found to be associated with nonalcoholic fatty liver

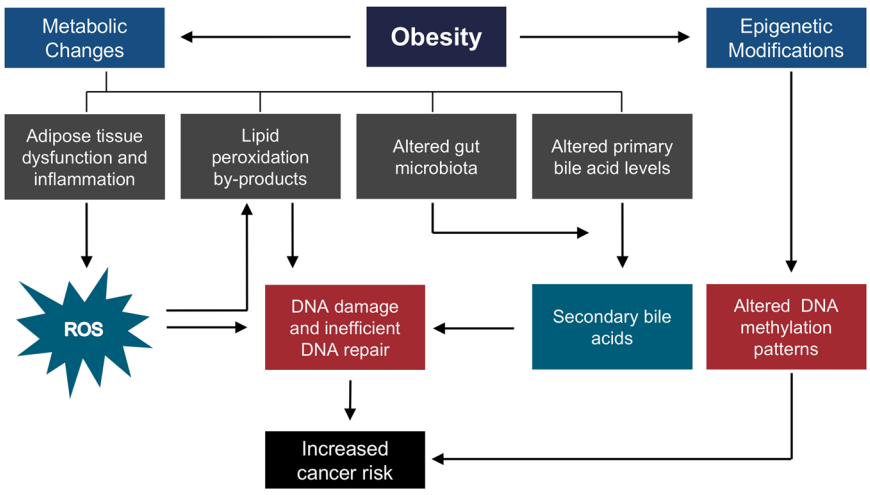

FIGURE 1 Schematic overview of the impact of obesity on genetic instability. ROS, reactive oxygen species [Color figure can be viewed at wileyonlinelibrary.com]

disease (NAFLD) and hepatocellular carcinoma (HCC).[68] Obesity has also been reported to be a risk factor for an aggressive acute myeloid leukemia phenotype containing an internal tandem duplication of the FMS-like tyrosine kinase receptor (FLT3-ITD). It has been shown that FLT3-ITD-expressing cell lines stimulated Nox-mediated ROS generation that resulted in error-prone DNA repair or DNA repair that may contribute to drug resistance.[69-72]

Thus, there are several obesity-related mechanisms of increased ROS production which can result in the formation of mutagenic lesions in the DNA such as 8-hydroxy-2′-deoxyguanosine (8-OH-dG) or 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxo-dG)[18,73] (Figure 2A).

Oxidized guanine bases (e.g., 8-oxo-dG) are sensitive biomarkers for the elevated levels of oxidative stress during obesity and carcinogenesis.[74-78] The induction and distribution of oxidative DNA damage depends on a number of factors, including but not limited to, chromatin structure, the accessibility of DNA repair proteins,[79] the DNA sequence[80] and context (e.g., guanines are more susceptible to oxidation due to their low redox potential compared with other bases),[81] alternative DNA structures, which are known sources of genetic instability[82,83] (e.g., hairpin-forming triplet-repeat sequences are hypersensitive to oxidative damage),[84,85] and metal ions.[86]

During DNA replication, DNA polymerases can misincorporate an A opposite 8-oxo-dG with a frequency of 10% to 75%, resulting in GC to TA mutations.[87] Other mutations that can result from guanine oxidation products include GC to AT or GC to CG mutations.[75,88,89] Such base substitution events have been frequently observed in the *RAS* oncogene and the *p53* tumor suppressor gene in skin, lung, and breast cancers.[90-92] Formation of 8-oxo-dG lesions adjacent to 5′-methyl-cytosine (5-mC) may inhibit the binding of methyl-binding proteins, thereby interfering with various steps of chromatin condensation, resulting in epigenetic alterations.[93] Oxidative DNA damage may also result in other cellular effects such as microsatellite instability, frameshift mutations, and acceleration of telomere shortening, leading to cellular senescence.[94,95]

The abundance and stability of 8-oxo-dG lesions and their mutagenic potential during DNA replication may represent one of the many critical steps connecting metabolic disorders and cancer.[96] However, the correlation between BMI and 8-oxo-dG levels is not clear. For example, one study found a negative correlation between BMI and 8-oxo-dG levels and concluded that occupational stress combined with social and lifestyle factors increased cancer risk.[97] Whereas another study showed no correlation existed between BMI and 8-oxo-dG levels in urine samples and suggested that urban living might contribute to elevated urinary 8-oxo-dG levels.[98] However, a 1-year follow-up study in obese patients showed significantly reduced antioxidant enzymes (e.g., superoxide dismutase, catalase, and glutathione peroxidase) and increased oxidized/reduced glutathione ratios in peripheral blood mononuclear cells in addition to elevated serum and urinary 8-oxo-dG levels.[99]

DNA replication of guanine oxidation products may result in somatic mutations that can cause proliferation of smooth muscle cells contributing to the etiology of atherosclerosis plaque formation, a risk factor of obesity.[100,101] Obesity is also associated with NAFLD, and ROS-induced oxidative DNA damage that may contribute to chronic conditions, such as liver fibrosis and HCC in NAFLD patients.[102-104] The molecular mechanisms connecting obesity, ROS, and cancer are unclear; however, obesity-stimulated oxidative stress, contributing to DNA damage and consequently to genetic instability, appears to play an important role.

2.2 | Obesity-induced lipid peroxidation by-products promote DNA damage

The microenvironment created by the dysregulation of adipose tissue allows for a continuous cycle where increased oxidative stress and inflammation together stimulate the induction of signaling molecules involved in transduction pathways maintaining adipogenesis and metabolic homeostasis. ROS-mediated oxidative degradation of polyunsaturated fatty acids (PUFA), that is, lipid peroxidation, results in unsaturated reactive aldehyde by-products such as MDA (Figure 2B), 4-HNE (Figure 2C) and Acr (Figure 2D).[28] Both MDA and 4-HNE levels have been found to be increased in obese patients.[27,74] In fact, it has been reported that MDA levels in newborns positively correlated with maternal BMI and were also found to be elevated in obese children.[105,106] These electrophilic molecules can form adducts with nucleophilic centers of phospholipids, proteins, and nucleic acids, potentially altering their biological functions.[107] Of these, MDA is thought to be the most mutagenic, and 4-HNE the most genotoxic of all lipid peroxidation by-products.[108]

2.2.1 | Malondialdehyde

MDA reacts with DNA to form 3-(2-deoxy-β-D-erythro-pentofuranosyl)pyrimido[1,2-α]purin-10(3H)-one-deoxygunanosine (M_1dG) adducts[109,110] (Figure 2B). M_1dG has been detected in DNA from human leukocytes, liver, pancreas, and breast tissues typically at levels from 2 to 150 per 10^8 bases.[111-113] The cytotoxic and mutagenic effects of MDA have been demonstrated in *Escherichia coli* (*E. coli*) and mammalian cells.[114-116] For example, transfection of MDA-adduct-containing plasmid DNA into mammalian cells stimulated a ~15-fold increase in mutation frequency over background levels.[115]

The high reactivity of MDA toward CG-rich sequences has been evidenced by the prevalence of MDA-induced base-pair substitutions or frameshift mutations at repetitive CG base-pairs in mammalian cells.[117] MDA adducts can also form DNA interstrand crosslinks (ICLs) which can block DNA replication and transcription. In addition to point mutations, these lesions can also result in large insertions and deletions during their processing and repair.[115] Further, unrepaired MDA-induced DNA lesions have been shown to result in frameshift mutations, point mutations, strand breaks, cell cycle arrest, and cellular apoptosis.[115-119]

MDA levels have been used as a biomarker for measuring seminal oxidative stress and were found to be positively correlated with oxidative guanine adduct levels.[120,121] Hosen et al[121] measured

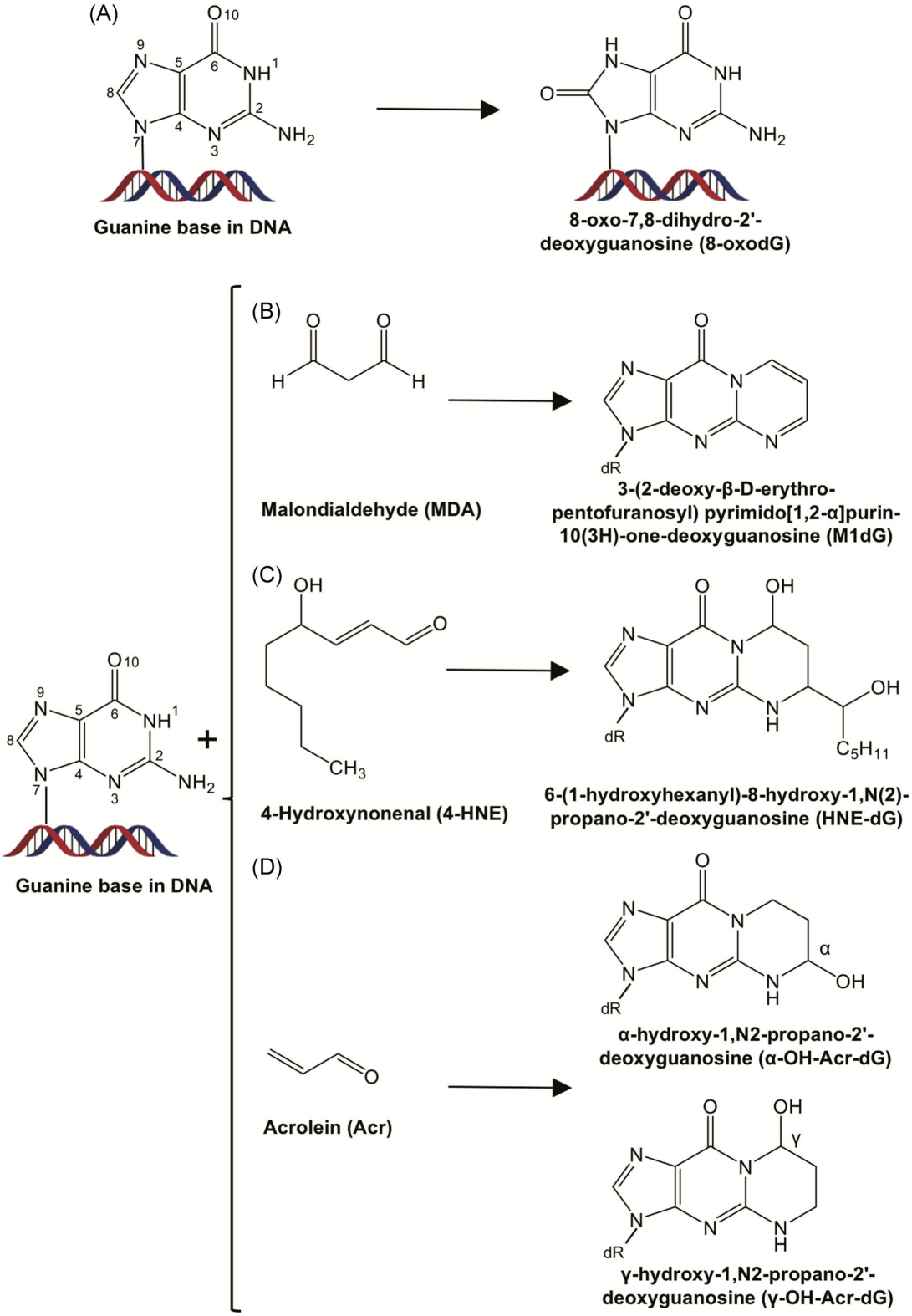

FIGURE 2 Chemical structures of dG adducts in DNA generated endogenously via oxidation (A), and reactions of lipid peroxidation by-products with DNA (B-D). A, 8-oxo-dG. B, M_1dG. C, HNE-dG. D, α-OH-Acr-dG and γ-OH-Acr-dG. 8-oxo-dG, 8-oxo-7,8-dihydro-2′-deoxyguanosine; α-OH-Acr-dG, α-hydroxy-1,N2-propano-2′-deoxyguanosine; γ-OH-Acr-dG, γ-hydroxy-1,N2-propano-2′-deoxyguanosine; dG, deoxyguanosine; HNE-dG, 6-(1-hydroxyhexanyl)−8-hydroxy-1,N(2)-propano-2′-deoxyguanosine; M_1dG, 3-(2-deoxy-β-D-erythro-pentofuranosyl)pyrimido[1,2-α]purin-10(3H)-one-deoxyguanosine [Color figure can be viewed at wileyonlinelibrary.com]

seminal MDA levels in 41 infertile male subjects, and found that oxidative stress-induced sperm DNA damage was associated with the pathogenesis of male infertility. Further, several epidemiological studies indicated an increased infertility risk that correlated with increased levels of MDA in obese males compared with their nonobese counterparts.[122,123]

2.2.2 | 4-Hydroxynonenal

Another by-product of lipid peroxidation, 4-HNE, is a reactive aldehyde that acts as a second messenger of free radicals.[124] At low intracellular concentrations (<2 μM), 4-HNE has been reported to promote cell survival and proliferation, but at higher concentrations (10-60 μM) it can result in mutagenesis (e.g., GC to AT mutations) and can function as a genotoxic agent (e.g., facilitating the formation of sister chromatid exchange, micronuclei formation, and DNA fragmentation) in mammalian cells.[125,126] 4-HNE can also cause inhibition of DNA synthesis leading to cytotoxicity.[127] The electrophilic nature of 4-HNE results in interactions with DNA bases with different specificities (G>C>A>T), leading to the formation of adducts that can interfere with DNA replication and transcription.[128] 4-HNE can be further metabolized by liver enzymes to an epoxide that can interact with DNA to form exocyclic etheno-guanine-cytosine and -adenine adducts,[129] where the major bulky exocyclic adduct in various human and rat tissues is 6-(1-hydroxyhexanyl)−8-hydroxy-1,N(2)-propano-2′-deoxyguanosine (HNE-dG; Figure 2C).[130-132] Some stereoisomers of 4-HNE can form ICLs, which covalently link the two complementary strands of DNA, impeding DNA replication and transcription.[133]

A role for HNE-dG in the etiology of HCC has been reported,[134] and the tumor suppressor gene *p53* is frequently mutated at codon 249 in HCC.[135-138] Using a wild-type p53 human lymphoblastoid cell line, Hussain et al[139] found that 4-HNE can bind to the third guanine of codon 249 (–AGG–) of *p53*, resulting in G to T transversions. The *p53* gene was mapped for 4-HNE-DNA adducts using the UvrABC nuclease, an *E. coli* nucleotide excision repair (NER) enzyme, and the results revealed the preferential formation of 4-HNE adducts at the second guanine in codons 174 and 249, which contain the same sequence –G AGG* C–. Perhaps due to the chromatin structure *in vivo*, 4-HNE more frequently binds to the mutational hotspot in codon 249 than 174, providing a growth advantage to liver cells, contributing to HCC.[136,140]

2.2.3 | Acrolein

Acr, a carcinogenic by-product of lipid peroxidation, can form two exocyclic DNA adducts, α- and γ-hydroxy-1,N2-propano-2′-deoxyguanosine (α-OH-Acr-dG and γ-OH-Acr-dG; Figure 2D), in human cells under conditions of oxidative stress.[141-144] Both adduct types, α-OH-Acr-dG and γ-OH-Acr-dG can induce base substitution mutations and can inhibit DNA synthesis.[145,146] Acr-DNA adducts are thought to form predominantly at guanine residues, specifically in G-rich sequences, though Acr adducts with dC, dA, and dT have also been reported.[147-150] A preference for Acr-DNA adduct formation has been shown at CpG sites containing methylated cytosines (5-mC), which are cytotoxic and mutagenic in Chinese hamster ovary (CHO) cells and can exert dose-dependent effects in NER-deficient human xeroderma pigmentosum complement group A (XPA) cells when compared with NER-proficient human fibroblast cells.[150]

The interactions of Acr with newly synthesized histones such as H4K12ac and H3K9 and K14ac has been reported. For example, Acr can interact with lysine residues 5 and 12 of histone H4 to form adducts that subsequently reduce histone acetylation. This can prevent certain protein-protein interactions ultimately affecting chromatin assembly.[151,152] Acr can also form ICLs or DNA-protein crosslinks (DPCs),[153] which have been implicated in lung, liver and bladder cancers.[154-156]

2.3 | Obesity-induced secondary BA metabolites promote DNA damage

Several studies have reported an association between obesity and alterations in the gut microbiome in mouse models and in humans.[157,158] However, a meta-analysis report suggested that this association was more robust in animal models than in the human cohorts studied. The variations in metabolism and the immune system from person to person may result in the inability of these studies to tease out the microbial differences (and causes and effects of obesity and microbiome alterations) in such cohort studies.[159] Nevertheless, the gut microbiota found in the intestinal epithelium of the host, through symbiotic interactions can assist in digestion and nutrient metabolism,[160] preservation of the structural integrity of the gut mucosal barrier to promote caloric extraction from the diet,[161,162] xenobiotic and drug detoxification, antimicrobial activities, and other homeostatic functions such as modulation of the immune system and modulation of apoptosis.[163,164]

Gut microbiota play an important role in digestion, and due to this function have been implicated in obesity and metabolic syndrome.[165,166] Studies in animal models and in humans have shown that obesity is associated with phylum-level changes in the microbiota. For example, metagenomic analyses of gut bacteria in mice and humans have shown increases in the Firmicute to Bacteroidete ratio in obese subjects compared with their lean counterparts.[167-169] However, Duncan et al[170] were unable to confirm this observation in humans, indicating variation in study outcomes related to the complexity of lifestyle and other factors.

Gut microbiota also participate in the production of bioactive metabolites such as BAs that signal through their cognate receptors to regulate the host metabolism.[171] The oxidation of cholesterol to a primary BA in the liver is mediated by cytochrome P-450 enzymes.[172] In the liver, the primary BA, cholic acid (CA), and chenodeoxycholic acid (CDCA) produced by humans, and CA and muricholic acid produced by mice, conjugate with glycine and/or taurine to form bile salts before being secreted in the bile.[171] In the intestine, small amounts of the bile salts can undergo dehydroxylation by gut microbial populations with active BA-inducible genes to

produce secondary bile metabolites such as DCA (Figure 3A) and LCA (Figure 3B).[173-175] Bacteria that can convert primary BA into secondary metabolites have been identified in *Clostridium* (clusters XIVa and XI) and in *Eubacterium*, both genera belonging to the Firmicutes phylum.[173,176-178] In addition to their important roles in cholesterol homeostasis,[172] BA can directly bind and activate the farnesoid X receptor (FXR), the pregnane X receptor, and the vitamin D receptor to regulate lipid, glucose, and drug metabolism in liver and/or the intestine, which is frequently exposed to BA at relatively high concentrations.[179-183]

Differences in BA production and physiology in obese mice and humans have been reported. In obese (ob/ob) mice, hyperglycemia can stimulate the overexpression of *CYP7A1* in the liver leading to increased synthesis and altered BA composition.[184] Conversely, studies in Fxr-deficient mice ($Fxr^{-/-}$) bred on a genetically obese background ($ob^{-/-}Fxr^{-/-}$) or $Fxr^{-/-}$ mice fed on a high fat diet (HFD) showed resistance to weight gain compared with their respective ob/ob and wild-type control mice.[185,186] A recent study examining 32 obese, nondiabetic human subjects for the effects of obesity and insulin resistance on BA synthesis and transport, reported a substantial increase in BA synthesis markers and variations in BA serum levels along with defective hepatic BA transport.[187] Obesity is also associated with an altered representation of bacterial genes and metabolic pathways. For example, by modulation of the hepatic FXR signaling, a primary sensor for endogenous BA, gut bacteria not only regulate secondary bile-salt metabolism, but also hepatic BA synthesis.[185,188] Thus, the microbiota-BA interactions play important roles in obesity and other metabolic disorders.[171]

One of the earliest studies suggesting the carcinogenic potential of BA, particularly secondary BA in mice was published in 1940.[189] Since that time, much research has been done to elucidate the roles of secondary bile metabolites in obesity-related gastrointestinal and pancreatic cancers.[29,190] Epidemiological studies have shown that consumption of a diet containing high fat and a high intake of beef products was associated with high fecal levels of the secondary BAs DCA and LCA, similar to patients with carcinoma of the colon.[191,192] The inability to accurately measure the different forms of secondary BAs may limit direct interpretation of such results; however, several mechanistic studies described below also provide support for the carcinogenic effects of DCA and LCA in rodents and humans.

Several bacterial studies have revealed the mutagenic effects of BA and their metabolites. Through single-gel electrophoresis assays, Ames tests, fluctuation assays, and reversion assays in various *Salmonella* strains, research groups have identified the formation of DSBs, single-strand DNA breaks, abasic sites, frameshift mutations, base-pair substitutions, and point mutations in response to bile-salt exposure.[193-198] Results have shown that frameshift mutations (−1) were a result of the adaptive SOS response to the stressor BA, and point mutations were largely comprised of nucleotide substitutions (GC to AT transitions), suggesting that exposure to BA salts can result in oxidative DNA damage.[196]

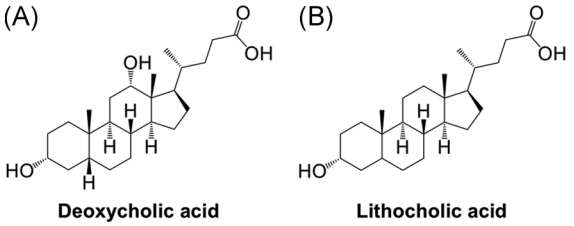

FIGURE 3 Chemical structures of secondary bile acids. A, Deoxycholic acid. B, Lithocholic acid

2.3.1 | Deoxycholic acid

DCA, a hydrophobic secondary metabolite of CA, is known to be a stress inducer and at higher than normal levels, DCA can adversely affect colon epithelial cells.[199] A diet high in fat has been associated with high levels of DCA, which can cause oxidative DNA damage, including modified bases, DSBs, aneuploidy, chromosomal alterations, micronuclei formation, and mitotic aberrations.[200-203] For example, DCA-induced mutations in the *K-Ras* gene in colon epithelial cells can provide a proliferative advantage to those cells, contributing to carcinogenesis.[204]

In obese mice, the levels of DNA-damaging DCA were found to be elevated in the enterohepatic circulation, and this was thought to be due to their altered gut bacterial metabolism.[191,205] DCA has been shown to induce a senescence-associated secretory phenotype (SASP) in hepatic stellate cells (HSCs) via upregulation of the *53BP1* and *p21* genes. HSCs can also secrete inflammatory and tumor-promoting factors (e.g., IL-6, growth-regulated oncogene-*alpha* [Gro-α] and C-X-C motif chemokine ligand 9 [CXCL-9]) in the liver, thereby facilitating the development of HCC in mice after carcinogen exposure.[206,207] Reducing the production of DCA, by either reducing primary to secondary BA converting *Clostridium cluster* XI or XIVa bacterial strains by antibiotic treatment, or by decreasing 7α-dehydroxylation activity with difructose anhydride III, substantially reduced the development of HCC in obese mice.[207] In nonalcoholic steatohepatitis (NASH) patients, the induction of SASP has been observed, indicating that the DCA-SASP axis may play a role in the development of obesity-associated HCC in humans.[208] In another experiment involving the incorporation of radiolabeled thymidine into hepatocyte DNA, DCA was found to inhibit DNA synthesis, suggesting that this secondary BA metabolite may affect DNA repair and cellular proliferation.[209]

Shi et al[210] demonstrated the effects of DCA on the gastric epithelial cell line (GES-1) *in vitro*. Among other factors, DCA downregulated the core histone H2A.1. This histone is involved in chromatin structure, which is important in DNA repair, DNA replication and transcription, and chromosomal stability. Since obesity has been associated as a risk factor for gastritis[211-213] and DCA is upregulated in the obese state, it is plausible that DCA might be a contributing factor to obesity-related gastric cancers.

DCA has also been implicated in the development of Barrett adenocarcinoma. DCA treatment of Barrett's epithelial cells increased the formation of DSBs as evidenced by γ-H2AX staining, and

stimulated the phosphorylation of proteins in the NF-κB signaling pathway.[214,215] Burnat et al[216] found that DCA upregulated NFκB-mediated induction of cyclooxygenase-2 and downregulated the DNA repair glycosylases, 8-oxoguanine DNA glycosylase (OGG1) and MutY glycosylase homologue (MUTYH), in Barrett's epithelial cancer cells. These findings suggest that DCA exerts its carcinogenic potential by increasing DNA damage levels.

2.3.2 | Lithocholic acid

LCA, the other major secondary metabolite of BA, is known to function as an oncometabolite. Generally, postsynthesis in the liver, most of the primary BA and its secondary metabolites enter the enterohepatic circulation before passing through the small intestine for reabsorption by the intestinal lumen. The unabsorbed metabolites are brought back to the liver for secretion into the bile.[217] LCA on the contrary, is partly absorbed in the intestine and transits to the colon where it is concentrated, and has been found to be excreted in feces at levels of up to 200 μM.[218] This may promote tumor initiation in colon epithelial cells by forming adducts with DNA.[219] Kulkarni et al[220] showed that within one hour of treating mouse lymphoblastoma L1210 cells with LCA (at 250 μM), single-strand breaks (SSBs) appeared in DNA, which were subsequently repaired when the cells were incubated in LCA-free media. Similar results of DNA damage and repair were observed in an LCA-induced insult study in colon epithelium crypt cells involved in colon carcinogenesis.[221] Treatment of colon cells with LCA over a range of physiologically relevant concentrations (25-300 μM) also led to DNA strand breaks as assessed by comet assays.[222,223]

LCA can promote methyl-*N*'-nitro-N-nitrosoguanidine (MNNG)-induced carcinogenesis in rat colon epithelial cells, presumably by inhibiting the base excision repair (BER) enzyme, DNA polymerase β (pol β), which plays an essential role in repairing DNA damage induced by MNNG.[224,225] The demonstration that BER was inhibited by LCA has provided a possible mechanism for its role as an oncometabolite.[225,226] LCA is also an inhibitor of topoisomerase II (topo II), which simultaneously breaks and rejoins DNA strands to manage the supercoiling during DNA metabolic processes such as DNA repair. Three-dimensional computational analysis revealed interaction sites for LCA with pol β at amino acids Lys60, Leu77, and Thr79, and interaction sites for LCA with topo II were identified at Lys720, Leu760, and Thr791.[227] The identification of these binding sites should prove helpful for the development of selective DNA repair inhibitors in cancer therapy.[228]

3 | DNA REPAIR

The DNA repair machinery is crucial to maintain genome stability, and thus eukaryotic cells have evolved several highly conserved mechanisms to remove DNA damage.[229] Depending on the complexity of the damage, the repair proteins/pathways work in a concerted fashion targeting different types of DNA lesions to maintain genome stability. While some lesions involving alkylation of the bases can be reversed by direct methyltransferase repair,[230] more complex lesions require repair mechanisms that involve a number of proteins that can excise damaged bases and resynthesize DNA. For example, BER removes many types of DNA lesions that result in modification of single bases using specialized DNA glycosylases and apurinic/apyrimidinic (AP) endonucleases.[231] Proteins in the NER pathway process helix-distorting bulky adducts, for example, those created by exposure to UV radiation.[232] DSBs can be processed by a variety of homologous or nonhomologous pathways; for example, breaks with nonhomologous ends can be processed via nonhomologous end-joining repair mechanisms,[233] whereas homologous recombination repair (HR) is guided by sequence homology[234] often provided by a sister chromatid during S-phase repair. Mismatch repair (MMR) processes base-base mismatches, and small loops or insertion/deletion mispairs generated during replication and recombination.[235]

Although the molecular mechanisms underlying the obesity-cancer link are poorly understood, there is some mechanistic evidence to suggest that inefficient repair of DNA adducts formed as a result of obesity-induced metabolic changes may contribute to carcinogenesis.

3.1 | Repair of oxidative stress-induced DNA lesions

Obesity results in altered lipid homeostasis, cellular oxidative stress, and inflammation that can result in DNA damage and overwhelm or reduce the repair capacity and/or accuracy of various DNA repair mechanisms. Over time, the accumulation of unrepaired DNA lesions and/or the error-generating repair of DNA lesions can result in mutations and genetic alterations involved in the etiology of many diseases, including cancer.[236,237]

One of the critical markers of oxidative stress in accumulated fat is elevated levels of Nox4 and ATM class switching from anti-inflammatory to proinflammatory cytokines which can stimulate ROS,[27,53] resulting in the formation of oxidative DNA damage lesions such as 8-oxo-dG.[18,73] To limit mutagenesis and cytotoxicity, 8-oxo-dG lesions are primarily repaired by OGG1 via the BER mechanism.[238] OGG1 cleaves the N-glycosidic bond between the sugar and the base, followed by endonucleolytic cleavage and subsequent gap-filling with limited specificity toward the 8-oxo-dG lesions on the daughter strand.[231] Mutations and gene polymorphisms in the human OGG1 gene have been found to be associated with an increased risk of developing various cancers.[239] Moreover, mice deficient in OGG1 ($OGG1^{-/-}$) have been found to accumulate 8-oxo-dG lesions in an age-related and tissue-specific manner.[240] Rozalski et al[241] have shown a ~26% reduction in urinary levels of 8-oxo-dG in $OGG1^{-/-}$ mice compared with wild-type mice suggesting the important role of OGG1 glycosylase.

However, several groups have reported the existence of back-up BER glycosylases.[242,243] As an example, DNA constructs containing a single 8-oxo-dG lesion incubated in mammalian cell extracts were primarily repaired by BER via pol β-dependent removal and

gap-filling of a single nucleotide. However, 8-oxo-dG lesions were also repaired in pol β-deficient cells, suggesting the existence of an alternative pathway(s) for their removal.[244] Other glycosylases that may be involved in processing 8-oxo-dG include the Nei endonuclease VIII-like or NEIL family of glycosylases (NEIL1, NEIL2, and NEIL3),[243,245] and evidence has suggested potential roles for the NER and MMR mechanisms to compensate for deficient OGG1 activity in processing these lesions.[246,247]

The role of BER glycosylases in modulating cellular and whole-body energy homeostasis has been reported. For example, Sampath et al[248,249] showed that mice deficient in the BER enzymes, DNA glycosylase NEIL1 or OGG1, had increased susceptibility to the development of metabolic syndrome. A recent report by the same group indicated protection from diet-induced obesity in mice over-expressing mitochondrial OGG1, further reinforcing the idea that BER plays an important role in body weight regulation.[250]

GC to TA transversion mutations can result during DNA replication past 8-oxo-dG lesions. Using immunofluorescence experiments in mammalian cells, van Loon and Hübscher demonstrated the involvement of MUTYH in recognizing these lesions. MUTYH generates an AP site, which is subsequently processed by AP endonuclease 1 (APE1). This is followed by the incorporation of deoxycytidine triphosphate in the single nucleotide gap by DNA pol λ, replication protein A, proliferating cell nuclear antigen, and finally ligation of the DNA strand by DNA ligase I and flap endonuclease 1 (FEN1).[251]

Cockayne syndrome (CS) group A (CSA) and B (CSB) proteins involved in the transcription-coupled repair subpathway of NER (TC-NER) have also been implicated in processing 8-oxo-dG lesions by several groups.[252-256] Compared to control human cells, primary fibroblasts from CS patients were unable to repair ionizing radiation-induced 8-oxo-dG lesions, suggesting that unrepaired oxidative DNA lesions might contribute to neurodegeneration in CS patients.[255]

MMR has been reported as an alternative pathway for processing 8-oxo-dG lesions. For example, MMR-deficient mouse cells treated with oxidizing agents or low-dose ionizing radiation showed elevated levels of 8-oxo-dG compared to MMR-proficient wild-type cells.[257,258] In addition, oxidized purines in MMR-deficient cells have been implicated in frameshift mutations resulting in microsatellite instability.[259] Though the precise mechanism is unclear, human MUTSα (a dimer of MSH2 and MSH6) preferentially recognized 8-oxoG-containing duplexes with an unpaired C or T, suggesting a role for MMR in repairing 8-oxo-dG lesions.[260,261] In response to oxidative stress-induced DNA damage, loss of key MMR genes has been reported. MSH2-deficient mouse embryonic fibroblasts contained higher levels of both basal and ROS-induced 8-oxo-dG lesions when compared with wild-type cells.[257]

Unsurprisingly, 8-oxo-dG is also an important biomarker for the prognosis and progression of colorectal cancers (CRC), a risk factor of obesity,[262] and MMR status is used to classify CRC into different subtypes.[263] Epigenetic inactivation of the *MutL homolog 1, colon cancer, nonpolyposis type 2* (*MLH1*) gene promoter and germline mutations in the MMR genes resulted in microsatellite instability, which occurs in 10% to 20% of CRC cases.[264-268] However, obesity alone presented a poor prognosis for overall survival in patients with CRC, as obese patients were found to be ~40% less likely to have a deficient MMR status compared to normal-weight CRC patients.[269,270] In fact, obese CRC patients exhibited a prognostically unfavorable proficient MMR molecular subtype characterized with chromosomal instability.[266,271]

Several groups have reported the mutagenic effects of oxidative stress-induced 8-oxo-dG lesions in DNA regions with repetitive sequences capable of adopting alternative DNA structures. For example, we have observed stimulation of oxidative stress and induction of mutations in and around mirror-repeat sequences capable of forming alternative DNA structures in a diet-induced obese mouse model. Other groups have found that when the repair of 8-oxo-dG lesions occurred at or near triplet-repeat sequences such as CAG or CTG or CGG, GCC or GAA, slipped DNA or hairpin structures can form transiently within the sequence, which was improperly processed by the BER machinery,[272-275] impacting deletion or expansion of the repeats. This error generating repair of 8-oxo-dG within triplet-repeat sequences may be involved in disease etiology, as triplet-repeat expansions have been implicated in many neurological disorders.[274]

3.2 | Repair of lipid peroxidation by-product-induced DNA lesions

The by-products of lipid peroxidation generally have long half-lives and can diffuse from their sites of formation across membranes, thus acting as second messengers of oxidative stress (e.g., 4-HNE).[124,276] Their electrophilic nature enables them to react with nucleophilic functional groups in DNA, and sulfhydryl, guanidine, and amino groups in proteins.[277] Aldehydes such as (MDA and 4-HNE) often form covalent mutagenic exocyclic adducts with the free $-NH_2-$ in the DNA bases leading to genetic instability, and subsequently contributing to the development of various cancers.[276]

3.2.1 | Malondialdehyde

Chemically stable propanodeoxyguanosine (PdG)-DNA adducts have been used as an *in vitro* model for unstable M_1dG adducts as they are structurally analogous. In both *E. coli* and mammalian cell systems, PdG-DNA adducts have been shown to be repaired by the NER mechanism,[278] and that formamidopyrimidine glycosylase or 3-methyladenine glycosylase do not play roles in the removal of M_1dG adducts.[116,278] Transformation of M_1dG adduct-containing plasmids in NER-deficient *E. coli* (LM103) cells resulted in an ~threefold increase in mutation frequency compared with the NER-proficient wild-type (LM102) cells. Further, without a functional NER system, the half-life of the M_1dG adducts was longer, likely contributing to the increased mutagenicity.[116] Interestingly, when an M_1dG adduct-containing plasmid was replicated in human fibroblasts deficient in NER (XP12BE and XP12RO), no increase in mutation frequency

was observed relative to NER-proficient wild-type cells (GM00637F).[115] A possible explanation for this result could be the presence of another repair mechanism in human cells that is capable of removing the M_1dG adducts in the absence of NER.

TC-NER has been implicated in the processing of endogenous lipid peroxidation by-products such as 4-HNE.[279] Thus, a potential role for TC-NER in the processing and repair of M_1dG adducts has also been examined. When present in the transcribed strand of a DNA template, M_1dG was able to arrest T7 RNA polymerase and mammalian RNA polymerase II. Also, an M_1dG adduct opposite to a C posed a stronger block to transcription than when present opposite to a T. These results suggest that M_1dG-induced DNA adducts might have negative implications for patients with CS.[280]

The non-bulky nature of M_1dG adducts might prevent them from being efficiently recognized by NER, making them substrates for MMR. Johnson et al[281] transfected an M_1dG-containing M13 phage vector into MMR-deficient *E. coli* cells and found them to be recognized by mutator S (MutS), which appeared to compete with NER for processing these lesions. In the absence of MutS, M_1dG-DNA adducts were repaired by NER, thereby reducing their mutagenic potential. The researchers used surface plasmon resonance assays and gel mobility shift assays to show that MutS interacted with M_1dG when located opposite to T with a similar affinity to a GT mismatch, suggesting that M_1dG adducts can also be recognized and repaired by the MMR system.

Singh et al[282] tested the role of the *E. coli* dioxygenase enzyme, AlkB, in repairing Acr and MDA adducts in single-stranded and double-stranded DNA. AlkB is an alpha-ketoglutarate-dependent hydroxylase, which is involved in the direct reversal of alkylation damage.[283] Using high-resolution quadrupole time-of-flight mass spectrometry, they found that AlkB repaired MDA-induced single-stranded DNA lesions more efficiently than double-stranded DNA lesions and the repair efficiency depended on the base opposite the lesion.[282]

3.2.2 | 4-Hydroxynonenal

Hydroxynonenal can form bulky exocyclic HNE-dG-DNA adducts that are primarily repaired by the NER mechanism,[284] as evidenced by significantly higher levels of 4-HNE-dG-induced mutations in NER-deficient human and *E. coli* cells compared to NER-proficient cells.[285] Choudhury et al[286] demonstrated the efficient repair of HNE-dG adducts in human cell nuclear extracts in a dose- and time-dependent manner. The NER proteins recognized and repaired HNE-dG adducts more efficiently (~2.4-fold) than UV adducts (the canonical NER substrate), which might explain the relatively low levels of HNE-dG-induced DNA lesions in mammalian cells.

CSB, a protein required for TC-NER, has been reported to be involved in the processing of HNE-dG lesions. Compared to CSB-proficient wild-type human fibroblast cells (CS1AN/pc3.1-CSBwt), treatment of CSB-deficient human fibroblast cells (CS1AN/pc3.1) with 4-HNE at physiological concentrations (1-10 μM), resulted in hypersensitivity and enhanced sister chromatid exchanges. Endogenous HNE-induced DNA adducts can also elicit DNA replication and transcription arrest. At concentrations of 1-20 μM *in vitro*, 4-HNE exposure was found to dephosphorylate CSB in a dose-dependent manner, resulting in increased CSB ATPase activity and activation of TC-NER. However, at higher concentrations (100 and 200 μM), 4-HNE significantly inhibited CSB ATPase activity, which led to cell death.[279] This affect was thought to be due to the formation of direct adducts with the CSB protein. Through Michael adduction, 4-HNE can react with the amino group of lysine, the imidazole group of histidine, and the sulfhydryl group of cysteine.[107,287,288] If the adducted proteins are part of the DNA repair machinery, then the removal of HNE-dG adducts could be inhibited or inefficient, contributing to cytotoxicity and carcinogenicity. For example, NER was inhibited in *in vitro* host-cell reactivation assays using a human colon and lung epithelial cells and cell extracts treated with 4-HNE, BPDE-, or UV-damaged DNA, all of which are substrates for NER.[289]

Winczura et al[290] examined the modulation of BER enzymes by 4-HNE. Pretreatment of the human fibroblast cell line, K21, with physiological concentrations of 4-HNE hypersensitized the cells to hydrogen peroxide (H_2O_2), and the alkylating agent, methyl methanesulfonate (MMS). Direct exposure of purified BER enzymes to high concentrations (1-2 mM) of 4-HNE showed inhibitory effects on alkyl-N-purine glycosylase (APNG) and thymine DNA glycosylase; whereas, OGG1 glycosylase and APE1 were unaffected. Exposure of 4-HNE pretreated K21 cells to H_2O_2 and MMS resulted in the accumulation of SSBs, suggesting that 4-HNE also inhibited DNA ligation.

3.2.3 | Acrolein

Acr-dG adducts have been shown to be repaired by the NER pathway in both *E. coli* and mammalian cells.[145,291,292] However, the repair kinetics of Acr-dG was shown to be much slower than HNE-dG in human colon cancer cells. This was suggested to be due to the poor excision efficiency of Acr-dG adducts, such that their repair was inhibited in the presence of HNE-dG.[293] These results support the observation that the *in vivo* levels of Acr-dG adducts were two orders of magnitude higher than HNE-dG adducts.[132,144] α-OH-Acr-dG adducts have been found to be better substrates for excision repair than γ-OH-Acr-dG adducts, and thus are repaired more efficiently.[150] Interestingly, Acr-adducts are repaired by NER proteins in normal human bronchial epithelia (NHBE) cells and normal human lung fibroblasts (NHLF), but the treatment of NHBE and NHLF cell lysates with Acr decreased the capacity of NER to remove Acr-adducts within the *p53* gene and the overall genome of these cells.[294] Following treatment with Acr, the gene expression levels of *XPA*, *XPC*, *hOGG1*, *PMS2*, and *MLH1* were unaffected, but protein degradation was increased, perhaps via Michael addition with the amino acids lysine, cysteine, and histidine.[142] These findings suggested that Acr treatment could inhibit NER, BER, and MMR by the degradation of repair proteins in these pathways, contributing to its mutagenic and carcinogenic potential.[291]

In *E. coli*, Pol I catalyzes the error-free translesion synthesis past Acr-dG adducts, whereas in eukaryotic cells, various translesion synthesis polymerases participate in catalyzing bypass of the Acr-dG adduct, often resulting in mutations.[292,295] Lee et al[156] showed that endogenous (e.g., oxidative stress-induced lipid peroxidation) and exogenous (e.g., tobacco smoke) sources of Acr can cause bladder tumors in a rat model. Treatment of urothelial cells with Acr revealed that it induced degradation of repair proteins, thereby inhibiting the NER and BER pathways, implicating it in tobacco-smoke induced bladder cancers. Interestingly, a meta-analysis study showed that obesity is associated with an ~10% increase in the risk of bladder cancer.[296] Though smoking did not seem to play a role, studies on the contribution of obesity-induced increases in oxidative stress-induced Acr levels are required to understand the molecular mechanisms involved in obesity-associated bladder cancers.

Like MDA, Acr-dG adducts can also be repaired by AlkB. Interestingly, the γ-OH-Acr-dG adducts were oxidatively dealkylated more efficiently by AlkB than α-OH-Acr-dG adducts in both open- and closed-ring forms.[282] Acr was also shown to inhibit DNA polymerase α (Pol α), and influence DNA methylation by either inhibiting the repair enzyme O6-methylguanine-DNA-methyltransferase or DNA methylase by covalently binding to guanine residues and modifying DNA such that it is no longer a substrate for these enzymes.[297,298]

3.3 | Repair of secondary BA metabolite-induced DNA lesions

The DNA damaging effects of BA and their metabolites leading to carcinogenesis have been reported,[29,190-192,299] but their effects on DNA repair mechanisms are not well understood. In *Salmonella enterica*, bile salts have been reported to induce oxidative SOS responses when the lesions impaired DNA replication, and when single-stranded DNA was generated by BER or dam-directed MMR during the processing of the oxidative DNA lesions. Their repair can generate SSBs which can be converted to DSBs during DNA replication. However, the replication block can be overcome by the dinB polymerase via translesion synthesis, followed by the action of recA, recBCD, and PolIV enzymes that repair the DSBs via homologous recombination.[195,196,300] Bernstein et al[301] have observed similar SOS-induced DNA repair responses to bile salts in *E. coli*. Importantly, bile-salt-induced mutagenesis in bacterial species colonizing the intestine may lead to chronic infection or adaptation giving rise to antibiotic-resistant strains.[301,302]

Of note, the molecular mechanisms that underlie the cellular responses to BA salts in mouse models do not always mirror the mechanisms found in human carcinogenesis. Factors limiting the applicability of rodent data to humans may be due to: (1) differences in rodent and human gut physiology; (2) the shorter life span of rodents, such that the experimental exposure time is insufficient to recapitulate the long-term effects of BA metabolites in humans; (3) the carcinogenic effects of BA metabolites often manifest at particular sites and under specific physiological conditions; and (4) BA metabolites are carcinogenic in humans, while they show tumorigenic effects in animal studies, suggesting that they may be tumor promoters only and not carcinogens in rodents.[29,303]

3.3.1 | Deoxycholic acid

The bile-salt, deoxycholate, has been found to be associated with colon cancer in individuals on a high-fat diet.[304] To better understand this finding, Romagnolo et al[305] treated colon epithelial cells *in vitro* with sodium deoxycholate at a low noncytotoxic concentration (10 μM), and found that the expression of *BRCA1*, which is an important protein for DNA repair and apoptosis, was induced; whereas, at higher cytotoxic concentrations (≥100 μM) of sodium deoxycholate, *BRCA1* expression was inhibited. These observations correlated with *BRCA1* gene expression levels in colon cancer patients, suggesting that clonal selection of colon epithelial cells resistant to apoptosis (in part due to BA-induced loss of BRCA1) contributed to colon carcinogenesis.

Kandell et al[299] demonstrated the induction of unscheduled DNA repair synthesis when human fore-skin fibroblasts were exposed to CDCA and DCA. In addition, UV4 CHO cells deficient in excision repair and DNA crosslink removal were more sensitive to sodium chenodeoxycholate than wild-type cells, and EM9 CHO cells deficient in single-strand DNA repair were more sensitive to sodium deoxycholate than wild-type cells. In response to sodium-deoxycholate-induced DNA damage, human Jurkat cells and rat epithelial cells were found to overexpress the stress response proteins NFκB and poly-ADP ribose polymerase (PARP), thereby facilitating DNA repair and protecting the cells from bile-salt-induced apoptosis.[306] Conversely, Glinghammar et al[199] showed that the treatment of human HCT 116 and HT-29 colon cancer cells with DCA led to the activation of caspase-3 and cleavage of PARP, subsequently resulting in apoptosis.

The exposure of Barrett's epithelial cells to DCA was shown to increase the production of ROS.[215] Among other cellular changes, this led to the accumulation of oxidative DNA damage-induced mutations, perhaps contributing to the etiology of esophageal adenocarcinoma (EAC). Recently, Hong et al[307] investigated the role of the BER protein, APE1, on the survival of mutant p53 EAC cells in response to BA. They found that overexpression of APE1 in EAC cells suppressed BA-induced DSBs, perhaps by enhancing BER and regulating stress responses, which reduced JNK- and p38-associated apotposis.

3.3.2 | Lithocholic acid

LCA is a potent inhibitor of Pol β, a key enzyme required for the BER mechanism,[226,228] and other DNA polymerase enzymes (Pol α, Pol δ, and Pol ε) involved in DNA replication and repair.[226] Inefficient BER can result in intermediates that require BRCA2-dependent HR for their repair. Stachelek et al[308] found that LCA reduced the efficiency of BER by inhibiting Pol β in EUFA423 BRCA2-deficient human fibroblasts. LCA was found to inhibit the formation of a stable

pol β-DNA complex, thereby suppressing the DNA polymerase and 5′-deoxyribose phosphate lyase activities of pol β. These effects were synergistic with the cytotoxic effects of the alkylating agent temozolomide, which can methylate guanine residues in DNA. This synergism depended on the conversion of BER-induced SSBs into DSBs during replication, as evidenced by γ-H2AX immunofluorescence. Cotreatment with LCA and temozolomide eventually led to cell death likely due to the accumulation of unrepaired DSBs in BRCA2-deficient cells, suggesting that BRCA2-deficient cancers may be an attractive target for synergistic drug therapy.

4 | OBESITY-INDUCED EPIGENETIC CHANGES

Several studies have been published on the role of epigenetics in the etiology of obesity. It is only recently that we have begun to understand that the obesogenic state, apart from eliciting a DNA damage and repair response, can also result in epigenetic alterations. Studies have shown that our environment influences changes in gene expression, sometimes leading to the onset of disease, but due to genome-wide epigenetic reprogramming, the trans-generational inheritance of these changes is not well defined.[309]

Epigenetic marks are tissue-specific and include DNA methylation and histone modifications. Since all the tissues in the body depend on the uptake of glucose and lipids, their cellular states are affected directly or indirectly by increases in the nutrient levels during obesity. As mentioned previously, in the adipose tissue, the increase in lipids is accommodated via hyperplasia under the regulation of the adipogenic transcription factor C/EBPβ. During adipocyte differentiation, C/EBPβ can be hypermethylated whereas, the promoter region of the fatty acid storage regulator, peroxisome proliferator-activated receptor-γ (PPAR-γ), can be hypomethylated.[310] There is evidence that histone deacetylases (HDACs) are also involved in adipocyte differentiation. Specifically, HDAC9 was found to negatively regulate adipogenesis in a deacetylase-independent manner.[311] Rodents fed on a HFD have been shown to undergo chromatin modifications in the liver and at regulatory regions near genes associated with metabolism and insulin signaling.[312,313]

Mendelson et al[314] performed a microarray-based analysis of DNA from whole blood samples from 3743 adult participants to study the association between BMI and differential methylation over 400, 000 CpG sites. They identified BMI-related differential methylation at 83 of these CpG sites (replicating across cohorts), which were associated with concurrent expression of genes involved in lipid metabolism. Though the cross-sectional nature of the data makes it difficult to draw causal relationships between BMI and DNA methylation, the results provide evidence to warrant further investigation to establish the mechanistic relationship between the two. Other research groups have performed similar epigenome-wide association studies in human blood samples from a variety of study cohorts with similar results.[30,315] Interestingly, Wahl et al[30] using methylation array analyses of blood and tissue samples from 5387 individuals, identified 187 CpG sites with altered methylation patterns that were associated with BMI. These methylation changes led to alterations in the expression of several genes involved in lipid metabolism, substrate transport, and inflammation. They also identified 62 markers that were associated with Type II Diabetes (T2DM), with one marker in the ATP Binding Cassette Subfamily G Member 1 locus involved in insulin secretion and β-cell function showing the strongest association. This association was observed to be independent of the canonical T2DM risk factors, supporting the authors' view that changes in obesity-related DNA methylation might represent a predictive measure for obesity-related disorders, such as T2DM.

Obesity-associated reprogramming of the epigenome via DNA methylation may alter the expression of genes that inhibit tumor progression thereby increasing cancer risk. As an example, the epigenetic silencing of tumor suppressor genes by estrogen in mammary epithelial cells in obese women may contribute to the development of or drive the progression of breast cancer.[31] Similarly, obesity-induced dysregulation of DNA methylation in endometrial epithelial cells may result in endometrial cancer development in obese women.[32]

5 | CONCLUSIONS

The dearth of studies correlating DNA damage, DNA repair, genetic instability and cancer in overweight or obese humans (or in nonhuman animal models) makes it challenging to draw direct causal links. However, the available mechanistic studies have proved useful in postulating hitherto unexplained obesity-cancer links via genetic instability.

Many gaps in knowledge still exist as to which stage(s) of increases in body weight initiate cellular responses leading to increases in oxidative stress, and subsequent DNA lesion formation and DNA damage responses. Information is limited regarding the consequences of obesity-associated DNA damage on oncogenes and malignant transformation of cells. Studies utilizing genetic and/or diet-induced obesity animal models deficient in, or overexpressing a specific DNA repair protein, will assist in a better understanding of the molecular mechanisms underlying the obesity-cancer link. Further research focus is also needed to investigate the association between oxidative DNA damage and modification of DNA methylation during obesity. Information provided by such studies will assist in the design of personalized therapies targeted to specific pathways involved in DNA damage, DNA repair, epigenetic modifications, and genetic instability associated with obesity. As we and many other research groups continue to investigate obesity-induced genetic instability and its potential contribution to cancer development, the findings will assist in the development of improved therapeutic strategies for the prevention and treatment of obesity-related cancers.

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CONFLICT OF INTERESTS

The authors declare that there are is conflict of interests.

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