n-3 Polyunsaturated fatty acids throughout the cancer trajectory: influence on disease incidence, progression, response to therapy and cancer-associated cachexia

Vickie E. Baracos1*, Vera C. Mazurak1 and David W. L. Ma2

1Department of Oncology, Division of Palliative Medicine, University of Alberta, Edmonton, Alberta, Canada T6G 2P5
2Faculty of Nutrition, Texas A&M University, College Station, Texas, USA

Evidence from epidemiological studies suggests that diets rich in n-3 PUFA may be associated with reduced cancer risk. These observations have formed the rationale for exploring the mechanisms by which n-3 PUFA may be chemoprotective and have resulted in significant advances in our mechanistic understanding of n-3 PUFA action on tumour growth. Various interrelated and integrated mechanisms may be at work by which n-3 PUFA influence cancer at all stages of initiation, promotion, progression, and neoplastic transformation. More recently, experimental studies have reported enhanced tumour cell death with chemotherapy when fish oil is provided while toxic side effects to the host are reduced. Furthermore, cancer-associated wasting has been shown to be attenuated by fish oil supplementation. Clinical evidence suggests that the n-3 PUFA status of newly diagnosed cancer patients and individuals undergoing chemotherapy is low. Therefore, both the disease itself and therapeutic treatments may be contributing factors in the decline of n-3 PUFA status. Dietary supplementation to maintain and replenish n-3 PUFA status at key points in the cancer disease trajectory may provide additional health benefits and an enhanced quality of life. The present review will focus on and critically examine current research efforts related to the putative anti-cancer effects of n-3 PUFA and their suggested ability to palliate cancer-associated and treatment-associated symptoms.

Cancer: Polyunsaturated fatty acids: Fish oil: Chemotherapy: Cachexia: Drug resistance

Introduction

Adults in Western countries consume on average 75 to 150 g and 30 to 45 % of their total energy from fat, a smaller proportion of which consists of long-chain PUFA. The essential fatty acids, linoleic (18 : 2 \( n \)-6) and \( \alpha \)-linolenic (18 : 3 \( n \)-3) acids, cannot be synthesised by mammalian cells, and so must be obtained from the diet. Linoleic acid can be elongated and desaturated to arachidonic acid whereas enzyme conversion from linolenic acid forms eicosapentaenoic acid (EPA; 20 : 5 \( n \)-3) which can be further metabolised to docosahexaenoic acid (DHA; 22 : 6 \( n \)-3). In Western diets, n-6 fatty acids are the predominant PUFA as they are widely distributed in most plant oils (for example, maize, safflower-seed, sunflower-seed), margarines, and animal fat, whereas dietary sources of \( \alpha \)-linolenic acid are comparatively limited. \( \alpha \)-Linolenic acid is found in flaxseed, soyabean and rapeseed oils. Long-chain n-3 PUFA, EPA and DHA, can be synthesised from \( \alpha \)-linolenic acid in man, and also consumed in the diet from marine fish oils (for example, herring, mackerel, tuna, sardine) and in the oils extracted from the livers of fish which live in warmer waters (for example, cod). The n-6 and n-3 fatty acids compete in biochemical pathways and have metabolically distinct and opposing physiological functions.

Epidemiological and experimental evidence link n-3 PUFA to reduced cancer risk. This work has centred on the anti-cancer effects of n-3 PUFA derived from fish oils containing EPA and DHA. Several recent reviews have addressed in detail the array of mechanisms attributable to n-3 PUFA to inhibit cancer growth during the initiation and promotion stages of cancer development (Jiang et al. 1998; Bartsch et al. 1999; Bougnoux, 1999; Rose & Connolly, 1999, 2000; Simopoulos, 1999, 2002; Woutersen et al. 1999; Stoll, 2001; Hardman, 2002; McEntee & Whelan, 2004).

Abbreviations: COX, cyclo-oxygenase; DHA, docosahexaenoic acid; DOX, doxorubicin; EPA, eicosapentaenoic acid; MDR, multiple drug resistance; PgP, phosphoglycoprotein; PIF, proteolysis-inducing factor; PKC, protein kinase C.

* Corresponding author: Dr Vickie E. Baracos, fax +1 780 432 8425, email Vickie.baracos@ualberta.ca
2002; Chajes & Bougnoux, 2003). An exciting new implication of n-3 PUFA use in cancer treatment has emerged in recent years from in vitro and animal studies that suggest a potential interaction between n-3 PUFA and cancer chemotherapy. n-3 PUFA have been reported to enhance chemotherapy-induced tumour cell death and also reduce the toxic side effects of chemotherapy on host tissues. Finally, cachexia is a common paraneoplastic syndrome and n-3 PUFA may interfere with the catabolic signal-transduction pathways implicated in cancer cachexia and attenuate the loss of weight and lean body mass in patients with advanced cancer (Barber & Fearon, 2001; Ross & Fearon, 2002). It is also important to note that neurotransmitters and hormones that may be involved in various stages of cancer progression, including disease-induced anorexia, have also been reported to be affected by n-3 fatty acids. However, this discussion is beyond the scope of the present paper and has been reviewed elsewhere (Meguid et al. 2000; Wang et al. 2002; Das et al. 2003; Laviano et al. 2003). In the light of suggestions that n-3 PUFA status in cancer patients may be poor, these beneficial effects of n-3 PUFA may underpin an argument for dietary supplementation to maintain and replenish n-3 PUFA status at key points in the cancer disease trajectory. The present review will focus on and critically examine current research efforts related to the putative anti-cancer effects of n-3 PUFA and their suggested ability to palliate cancer- and treatment-associated symptoms.

**Dietary polyunsaturated fatty acids: prevention and risk reduction of cancer**

There are several prevailing streams of thought regarding the contribution of dietary fat to cancer. Animal studies in particular have allowed the delineation of these points through the use of diets with defined fat components. The quantity and energy contribution of fat to energy intake and the composition of that fat independently influence cancer risk and the growth of established tumours. Within fat composition, the importance of the PUFA:saturated fatty acids ratio in the diet as well as the levels of different specific fatty acids on tumour incidence, growth and anti-tumour immune responses have been recognised (Sasaki et al. 1998; Robinson et al. 2001). The balance between n-3 and n-6 PUFA is important, but this may also extend to other biologically active fatty acids such as conjugated linoleic acid (Wahle & Heys, 2002), oleic acid (Bartsch et al. 1999), and individual fatty acids within the n-3 and n-6 PUFA family.

**Epidemiological and experimental evidence**

The results from epidemiological studies examining the effect of fish consumption on the development of human cancer has been reviewed extensively (de Deckere, 1999; Cohen, 2000; Weisburger, 2000; Zock, 2001; Temple, 2002; Dennis et al. 2003; Terry et al. 2003). Although studies suggest a beneficial and protective effect of fish oil consumption, overall, epidemiological evidence cannot be used to definitively conclude that n-3 PUFA consumption is chemopreventive. There are two significant shortcomings inherent in most epidemiological studies. Reviewed collectively, studies fail to demonstrate that cancer risk or tumour burden is lowered by n-3 PUFA consumption. Second, such approaches do not rigorously address mechanisms of action. The inconsistent findings reflect the difficulty in evaluating the relative contribution of minor fatty acids in the diet which are not easily dissected from a large matrix of confounders. The list of confounders appears endless and highlights the many challenges in interpreting these types of studies.

Given the recognised limitations of various epidemiological study formats (i.e. case–control, retrospective and prospective studies; Temple, 2002; Dennis et al. 2003), the fact remains that there is a trend that suggests a role for dietary n-3 PUFA in cancer prevention. Whereas the epidemiological data have primarily provided correlative evidence for cancer prevention and risk reduction, experimental data from animal models and cell lines demonstrate that dietary n-3 PUFA can inhibit tumour growth in a variety of cancer models such as breast, colon and prostate (Clarke et al. 1999; Connolly et al. 1999; Chen & Istfan, 2000; Chung et al. 2001). A large body of in vitro and animal model data links n-3 PUFA to physiological and morphological changes that reduce tumour weight, size and multiplicity.

Various mechanisms may be at work by which n-3 PUFA can prevent cancer at all stages of initiation, promotion, progression, and then neoplastic transformation of benign tumours to malignant tumours. A survey of the literature reveals a multiplicity of mechanisms currently being investigated, broadly related to apoptosis, cell proliferation, cell signalling, gene regulation, angiogenesis and metastasis; these are summarised in Fig. 1. Well-studied mechanisms have been discussed in detail in several recent reviews (Jiang et al. 1998; Bartsch et al. 1999; Bougnoux, 1999; Rose & Connolly, 1999b, 2000; Simopoulos, 1999, 2002; Woutersen et al. 1999; Stoll, 2001; Hardman, 2002; McEntee & Whelan, 2002; Chajes & Bougnoux, 2003); therefore, we will highlight, or summarise in brief, areas of emerging interest.

**Apoptosis.** There is keen interest to understand how n-3 PUFA may alter the balance between cell growth and cell death. Experimental evidence suggests that the absolute or relative levels of n-6 and n-3 PUFA can either promote tumour cell growth, or promote apoptosis, respectively. A variety of mechanisms may be involved in orchestrating apoptosis by n-3 PUFA (Fig. 1). Mitochondrial-mediated apoptosis is an important facet of programmed cell death (Ravagnan et al. 2002). An emerging area of importance is the involvement of n-3 PUFA in modulating mitochondrial-mediated apoptosis regulated by the Bcl-2 family of proteins (Ravagnan et al. 2002). This family of proteins promotes and inhibits apoptosis by regulating the release of downstream effectors from the mitochondria. In rats fed fish or maize oil in combination with fibre, the combination of fish oil and fibre was shown to increase colonocyte mitochondrial membrane potential, cytochrome C release and caspase-3 activity, hallmarks of mitochondrial-mediated apoptosis (Hong et al. 2002). In a rat model of colon cancer, apoptosis was enhanced in fish-oil-fed animals which expressed lower levels of the anti-apoptotic protein, Bcl-2.
(Hong et al. 2003). Yamamoto et al. (1999) reported that EPA enhances the expression of pro-apoptotic (Bax and Bcl-xs) and reduces the expression of anti-apoptotic (Bcl-2 and Bcl-xL) proteins in mammary cancer cell lines. Studies in Jurkat leukaemic (Siddiqui et al. 2001) and HT-29 colonic (Clarke et al. 1999) tumour cell lines have shown that exposure to DHA and EPA enhanced caspase-3 activation, an activator of apoptosis. In colonic HT-29 cancer cells, Bcl-2 expression is strongly down regulated by DHA, which is in part mediated by increased lipid peroxidation (Chen & Istfan, 2000). These studies provide new mechanistic insight into the action of n-3 PUFA, demonstrating the ability to shift the balance between pro- and anti-apoptotic Bcl-2 proteins in favour of cell death in cancer cells.

Cell signalling via lipid rafts and caveolae. A large number of research reports suggest that n-3 PUFA can modulate cell physiology by affecting signalling-transduction pathways and the expression of genes. A variety of signalling molecules such as protein kinase C (PKC; Pandian et al. 2001), Ras (Davidson et al. 1999; Collett et al. 2001), cell-cycle proteins (Chen & Istfan, 2001), eicosanoids (Hughes-Fulford et al. 2001), transcription factors (Thoennes et al. 2000; Chung et al. 2001; Liu et al. 2001) and translation factors (Palakurthi et al. 2000) have been identified as cellular targets of n-3 PUFA (Fig. 1).

The integration and regulation of many cell-signalling events at the level of the plasma membrane is now known to involve structures known as lipid rafts and caveolae. Lipid rafts and caveolae are characterised as highly ordered microdomains of the plasma membrane enriched in cholesterol and sphingolipids (Liu et al. 2002; Pike et al. 2002; Pike, 2003). Many signal-transduction proteins are now known to preferentially localise to these microdomains. Altering the lipid composition of rafts has been reported to affect cell-signalling pathways (Swinnen et al. 2003) as well as the localisation, and hence functionality, of resident proteins. Incorporation of n-3 PUFA into lipid rafts of immune cells has been shown to profoundly disrupt T-cell receptor protein assemblage and functionality both in vitro (Stulnig et al. 1998, 2001) and in vivo (Fan et al. 2003). This suggests a mechanism by which n-3 PUFA can attenuate inflammatory processes. Therefore, modification of the lipid composition in these microdomains by n-3 PUFA may also have a significant effect on signalling pathways in cancer cells.

Angiogenesis and metastasis. There is some evidence suggesting that n-3 PUFA may be beneficial in inhibiting angiogenesis and metastasis; however, the exact mechanisms have yet to be clearly defined. At more progressive stages of tumour development n-3 PUFA have been reported to have anti-angiogenic effects in breast cancer (Rose & Connolly, 1999a, 2000) and anti-metastatic effects in colon and breast cancer models (Iwamoto et al. 1998; Senzaki et al. 1998), preventing the formation of new blood vessels to support tumour growth and expansion to secondary sites. The mechanisms by which n-3 PUFA inhibit these processes are not known, but may involve NO synthesis (Narayanan et al. 2003), the synthesis of bioactive metabolites from n-3 PUFA (Tsujii et al. 2003), or lipoxygenase-derived metabolites (Pasqualini et al. 2003).

Overall, recent investigations have focused on the role of...
cyclo-oxygenase (COX) expression and the use of selective COX inhibitors as anti-angiogenic and anti-metastatic agents (Amano et al. 2003; Shappell et al. 2003; Woods et al. 2003; Yamauchi et al. 2003). The use of highly novel models, such as the prostaglandin receptor knockout mouse model, has identified a role for a prostaglandin receptor in angiogenesis (Amano et al. 2003). These studies demonstrate a role for COX and eicosanoids in tumour development. Moreover, these observations suggest an influential role for n-3 PUFA in modulating COX function and eicosanoid synthesis as a mechanism for inhibiting angiogenesis and metastasis; however, a clear beneficial effect has yet to be determined in man. In fact, there is evidence suggesting that n-3 PUFA have no effect on metastasis (Griffini et al. 1998). It is possible that there are limitations to the degree in which n-3 PUFA can be efficacious in affecting the outcome of very-late-stage tumour development. Further studies in this area are needed to address this potentially beneficial role of n-3 PUFA.

**Gene regulation.** We have briefly highlighted several possible mechanisms of action by which n-3 PUFA affect tumour growth, and one may question how it is possible for these elements to simultaneously result in diverse changes in cell metabolism. This is certainly a complex issue, as one realises that n-3 PUFA do not necessarily have to act at a single critical juncture in cell physiology to explain all the phenomenology. The accumulated experimental evidence so far has linked n-3 PUFA to very compartmentalised segments of inter-related and integrated biochemical processes. Therefore, understanding how integrated pathways and processes behave when n-3 PUFA are supplemented will be critical in substantiating the health benefits of n-3 PUFA in cancer. The advent of genomics technologies has created the potential for greater understanding of the mechanism of action. Microarray technologies now provide the capability to assess the expression of thousands of genes simultaneously, yielding a cancer expression profile (Birkenkamp-Demtroer et al. 2002; Mariadason et al. 2002; Rhodes et al. 2002). Only two microarray studies have been published on the chemoprotective effects of n-3 PUFA. Using Caco-2 colon cancer cells, microarray analyses revealed that apoptosis-, cell cycle-, NO- and eicosanoid-related genes were affected by n-3 PUFA treatment, consistent with a chemoprotective role of n-3 PUFA against colon cancer (Narayanan et al. 2001, 2003). These exciting studies are at the forefront of, and most probably foreshadow, more studies that will increase our understanding of n-3 PUFA effects at the molecular level. This provides the potential to identify relational interactions between genes that are either up regulated or down regulated in response to n-3 PUFA. Furthermore, genes previously not considered for their relevance or yet to be identified gene products, known affectionately as EST, can be identified for further study.

**n-3 Polyunsaturated fatty acids enhance anti-tumour effects of some chemotherapeutic agents**

A clearer understanding and subsequent exploitation of cell properties and proteins involved in apoptotic and proliferative pathways has led to the development of potent cytotoxic agents effective at reducing tumour growth, but not without toxic side effects. The toxicity inherent in these drugs requires that dosages be limited and delivered in conjunction with adjuvant therapies or strategies to enhance the toxicity of drugs to tumour cells at low doses and/or offer protection to non-target tissues. n-3 PUFA are reported to exhibit both of these properties and therefore may be highly useful during cancer chemotherapy.

**Efficacy of chemotherapy and n-3 polyunsaturated fatty acids**

Certain PUFA enhance the cytotoxicity of several widely used anti-neoplastic agents including anthracyclines, cisplatinum, alkylating agents, irinotecan and bleomycin (for a review, see Conklin, 2002). As is the case for cancer prevention and inhibition of cancer progression, there are potentially several inter-related and integrated mechanisms that impart benefits associated with anti-neoplastic therapies. To date, most of these studies have been performed *in vitro* and in animals (summarised in Table 1); however, a rationale may be forthcoming for movement of these observations to the clinic. This section will review the most recent literature that has investigated the interaction of n-3 PUFA with cytotoxic drugs for cancer treatments.

**Membranes and proteins.** Much of the recent work citing improved sensitivity to cytotoxic drugs has arisen from the investigations into how n-3 PUFA aid in overcoming multiple drug resistance (MDR). Inherent, or, more commonly, acquired MDR has been defined as cross resistance to a broad spectrum of structurally and functionally unrelated cytotoxic drugs (Davies et al. 1999) which render chemotherapy treatments ineffective. The ability of tumour cells to survive and adapt to unfavourable conditions is an inherent feature of malignancy and understanding the mechanisms of how this occurs may be useful in developing new targets of intervention. MDR is associated with several alterations in cellular properties including changes in drug transport (Cowan et al. 1986), overexpression of drug efflux pumps (Arancia et al. 2001) and elevated levels of antioxidant enzymes (Batist et al. 1986; Gottesman & Pastan, 1993; Kumar & Das, 1995; Abulrob et al. 2000). Importantly, compared with cell lines that are sensitive to chemotherapy drugs, drug-resistant cell lines differ in their ability to take up supplemental fatty acids (Davies et al. 1999) and exhibit lower α-linolenic, γ-linolenic and DHA content in their membrane phospholipids (Das et al. 1998). Cells expressing MDR exhibit elevated levels of cholesterol, glycosphingomyelin and sphingomyelin in their cell membranes, lipids that are characteristically associated with membrane microdomains, caveolae and rafts (for reviews, see Lavie et al. 1999; Lavie & Liscovitch, 2000; Senchenkov et al. 2001).

Experimental studies have shown that n-3 PUFA increase the cytotoxicity of several anti-neoplastic agents that work through diverse and varied mechanisms (Conklin, 2002), suggesting that there may be several means by which n-3 PUFA are able to modulate this response. The addition of fatty acids to media or to the diet results in their subsequent enrichment in membranes and tissues, including...
Yamn (2001) (MDA-MB-231, SK-Br3, (navelbine) and paclitaxel alone or with fatty following order: 18 : 3

The studies that have emerged exemplify the importance of determining the types of tumour-drug combinations that may be most responsive to interventions with n-3 PUFA (Table 1).

<table>
<thead>
<tr>
<th>Author</th>
<th>Model</th>
<th>Drug</th>
<th>Design</th>
<th>Main findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Das et al. (1998)</td>
<td>Vincristine-resistant and sensitive HeLa variants (KB-Ch®-3-1) human cervical carcinoma cells</td>
<td>Vincristine, cisplatinum, and doxorubicin</td>
<td>Cells cultured with or without fatty acids (DHA, 20 : 3n-6, AA, EPA, 18 : 3n-6, 18 : 2n-6 and 18 : 3n-3) Cells were supplemented with 10 ng [3H]-vincristine/ml.</td>
<td>Increased cytotoxicity by 18 : 3n-6 and EPA; increased uptake and retention of vincristine by 18 : 3n-3; 18 : 3n-6 EPA- and DHA-treated cells</td>
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<tr>
<td>Germain et al. (1998)</td>
<td>MDA-MB-231, breast cancer cells</td>
<td>DOX and Mitoxantrone</td>
<td>Cells supplemented with EPA, DHA, 20 : 4n-6, 18 : 3n-6, 18 : 3n-3, 18 : 2n-6 or 18 : 1n-9 with and without oxidants</td>
<td>Increased DOX cytotoxicity by DHA and further increase by addition of oxidants</td>
</tr>
<tr>
<td>Davies et al. (1999)</td>
<td>Anthracine-sensitive and -resistant breast (MCF-7) and urothelial (MGH-U1) cell lines</td>
<td>Anthracines and related drugs: DOX; epipodophyllotoxins; doxorubicin, methotrexate</td>
<td>Incubated with 18 : 3n-6 for 24 h before drug treatment. Flow cytometry and confocal microscopy assessed drug uptake and localisation</td>
<td>Flow cytometry showed no increase in drug uptake with 18 : 3n-6 treatment. Increase in methotrexate uptake in resistant cell lines treated with 18 : 3n-6 using confocal microscopy</td>
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<tr>
<td>Hardman et al. (2000); Liu &amp; Tan (2000)</td>
<td>Nude mice injected with A549 lung tumour cells P388 and P388/DOX-resistant mouse leukaemia cell line</td>
<td>DOX + Fe</td>
<td>Maize oil (20 %) or fish oil (19 % +1% maize oil) diets. Cells treated with combinations of 18 : 3n-6, DHA, DOX and antioxidants</td>
<td>Increased tumour regression by fish oil Following pretreatment of cells with 18 : 3n-6 or DHA; sensitivity to DOX in resistant cells; increase in some antioxidant enzyme activities in cells Addition of antioxidants to media reduced level of antioxidant enzymes to normal</td>
</tr>
<tr>
<td>Bradley et al. (2001)</td>
<td>Madison 109 (M109) small-cell tumour lines cultured in CD2F1 mice</td>
<td>DHA-paclitaxel conjugate or paclitaxel</td>
<td>Drugs were administered (intravenously) once daily for 5 d</td>
<td>No regression with paclitaxel alone; increased regression with conjugate; increased retention of paclitaxel in cells by conjugate</td>
</tr>
<tr>
<td>Menendez et al. (2001)</td>
<td>Breast cancer cells (MDA-MB-231, SK-BR3, 4T/7D and MCF-7)</td>
<td>Paclitaxel, vinorelbine (navelbine) and gemcitabine</td>
<td>Cells supplemented with paclitaxel alone or with fatty acids (18 : 1n-9, 18 : 2n-6, 18 : 3n-3, 18 : 3n-6, EPA or DHA) before and after paclitaxel treatment</td>
<td>Increased drug cytotoxicity in the following order: 18 : 3n-6 &gt; 18 : 3n-3 &gt; 20 : 5n-3 &gt; 22 : 6n-3 &gt; LA = paclitaxel alone. Increased cytotoxicity of navelbine but not gemcitabine by 18 : 3n-6</td>
</tr>
<tr>
<td>Yam et al. (2001)</td>
<td>C57 BL/6J mice inoculated with Lewis lung carcinoma</td>
<td>Cisplatin</td>
<td>Dietary treatments comprised chow, fish oil (4 %), with or without antioxidants. Diets were fed before tumour inoculation and fish oil diet switched to an antioxidant-supplemented diet after cisplatin treatment</td>
<td>Decreased metastasis with fish oil diet; decreased metastasis following cisplatin treatment was achieved with antioxidant- and fish-oil-supplemented diets</td>
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DHA, docosaexaenoic acid; AA, arachidonic acid; EPA, eicosapentaenoic acid; DOX, doxorubicin; LA, linoleic acid.

Phosphoglycoprotein (Pgp) is a transporter whose activity is highly influenced by the lipid microenvironment (Lavie & Liscovitch, 2000). Pgp facilitates the movement of cytotoxic drugs out of the cell (Abulrob et al. 2000) and is overexpressed in MDR (Goldstein et al. 1992; Gottesman & Pastan, 1993). While this protein has been identified as a target for drug development, at present, agents known to modify the action of Pgp, such as doxorubicin (DOX), are not potent enough at the doses required to avoid toxic effects (Sikic, 1997). n-3 PUFA have been reported to increase the accumulation and retention of DOX in both drug-resistant and drug-sensitive cell lines via a Pgp-dependent mechanism (Rudra & Krokan, 2001). PKC is the intracellular pathway that phosphorylates and acti-
vates PgP. Tumours exhibiting MDR have been reported to exhibit higher PKC activity (Das et al. 1998; Cha et al. 2001); this offers an additional target for novel cytotoxic drugs. For example, some genotoxic drugs work by reducing PKC activity (Cha et al. 2002). EPA and DHA are known modulators of the PKC activation pathway (Nakamura & Nishizuka, 1994; Verstovsek et al. 1998; Mirnikjoo et al. 2001; Nair et al. 2001) and subsequently may alter the function of other proteins and enzymes that rely on this intracellular pathway. Whether or not n-3 PUFA are effective in overcoming MDR via a PKC-related mechanism is beginning to be explored.

Other cell types exhibiting MDR show reduced levels of p53, a cell-cycle inhibitor (Das et al. 1998; Davies et al. 1999; Menendez et al. 2001) that is an essential component of the apoptotic process induced by anti-cancer drugs. Tumours that do not express p53 are resistant to therapies and continue to grow. Drugs have been developed to up regulate p53 expression in tumours to make them more sensitive to treatment (Davies et al. 1999). The interaction of these drugs with n-3 PUFA has not yet been explored and may hold promise in future experimental studies.

**Formation of peroxides**

Several lines of evidence have evolved to support a role for PUFA in promoting an oxidative environment in the presence of cytotoxic drugs to create an environment toxic to tumour cells (for a review, see Conklin, 2002). Recent literature has confirmed previous reports that the supplementation of n-3 fatty acids reduces the expression of endogenous antioxidant enzymes in tumours both *in vitro* (Davies et al. 1999) and in an animal model (Hardman et al. 2001), thereby creating the potential for the development of a more oxidative environment. Suppressing enzymes such as glutathione peroxidase in the tumour has been reported to be a more important factor in reducing tumour growth than the formation of peroxides induced by the drug (Hardman et al. 2002).

Despite the evidence for a role of peroxidation in promoting tumour death (Germain et al. 1998; Cognault et al. 2000; Menendez et al. 2001; Yam et al. 2001), not all studies support these findings (Rudra & Krokan, 2001). Cytotoxic effects of acrolein, a metabolite of the cytostatic drug cyclophosphamide, were not related to lipid peroxidation in a glioblastoma cell line even though oxidative damage occurred in a lung tumour cell line in the same study (Rudra & Krokan, 2001). Another study reported that the addition of 18 : 3 n-6 and DHA to P388 cells increased the expression of tumour antioxidant enzymes, an effect that was abrogated by adding antioxidants to the media (Liu & Tan, 2000). Differences in the timing of administration of fish oil and/or antioxidants, the type of drug used and the characteristics of the cell lines employed may explain the inconsistent results. Drug-sensitive and -resistant cell lines have been reported to respond differently to an oxidative environment and also differ in their ability to take up supplemental fatty acids into their membranes as well as their ability to up regulate antioxidant enzymes to protect against peroxidation (Kumar & Das, 1995). Further work is needed to define the optimal oxidative environment for promoting tumour cell death in the presence of fish oil. In a highly oxidative environment, such as that induced by some cytotoxic agents, supplemental fatty acids may be oxidised before they are incorporated into tumours (Yam et al. 2001), thereby reducing their mechanism of action. On the other hand, supplementing antioxidants to the host before treatment may reduce peroxidation intended by the drug. Studies employing diets that contain low amounts of PUFA have reported no effect on tumour growth and number when oxidative agents are utilised (Cognault et al. 2000). Therefore, lipid peroxidation as a mechanism of enhanced tumour death may only be relevant in the presence of high amounts of dietary PUFA. Feeding a fish oil-enriched diet fed before and supplementing with vitamins E and C after cyclophosphamide treatment was reported to have the most effective anti-metastatic effect in an animal model (Yam et al. 2001). Hrelia et al. (2002) reported that extracts derived from green tea, which act as antioxidants, counteract the membrane modification of cardiomyocytes induced by DOX and argue a point for the concurrent supplementation of n-3 PUFA and antioxidants. Taken together, these studies emphasise the possible importance of the oxidative status of the host before, during and after cytotoxic treatments as additional potential factors involved in tumour cytotoxicity. It is of note that many toxic side effects, such as cardotoxicity, mutagenesis, pulmonary fibrosis, neutropenia, reduced natural killer cell activity, anorexia and cachexia (Clemens et al. 1997; Sidorenko et al. 1998; Theile & Kemper, 1998), are related to the exhaustion of built-in host antioxidant mechanisms following chemotherapy treatment. It would seem important to further clarify the optimal conditions for enhanced cytotoxic effects to the tumour while preventing harmful side effects by adjusting the redox balance of the host. Results from the diverse models and agents that are being actively investigated (Table 1) will provide insight into how fish oils alter tumour biology and interact with diverse anti-neoplastic agents.

**n-3 Fatty acids and palliation of cancer: associated symptoms**

**n-3 Polyunsaturated fatty acids limit the toxic side effects of chemotherapy**

Gastrointestinal, haematological and cardiac toxicities limit the dose at which anti-cancer drugs can be administered. Chemotherapy regimens are toxic, and significant numbers of patients experience sufficient toxicity to have their doses reduced and/or delayed or require hospitalisation for toxic side effects. Several recent studies report an association between dietary fish oil supplementation and the attenuation of side effects associated with anti-neoplastic therapies (summarised in Table 2).

In a series of cell culture and animal studies, Hardman et al. (1999, 2000, 2001, 2002) have accumulated evidence to support a protective effect of fish oil on host tissues during chemotherapy. CPT-11 (irinotecan) has a dose-limiting toxic effect of severe diarrhoea associated with pathological changes in the large and small intestines as well as haematological toxicities. In an earlier report, Hardman et al.
Table 2. Experimental studies of the protective effects of n-3 fatty acids to the host during chemotherapy

<table>
<thead>
<tr>
<th>Author et al. (Year)</th>
<th>Model</th>
<th>Drug</th>
<th>Design</th>
<th>Main findings</th>
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<tbody>
<tr>
<td>Hardman et al. (1999)</td>
<td>MCF-7 transplanted into female Harlan Sprague–Dawley nude mice (nu/nu)</td>
<td>Irinotecan (CPT-11)</td>
<td>Tumour bearing mice were fed 7 % maize oil (n-6), 4 % maize oil + 3 % fish oil or 1 % maize oil + 6 % fish oil</td>
<td>Increased CPT cytotoxicity by fish oil; decreased intestinal pathology with fish oil</td>
</tr>
<tr>
<td>Ogilvie et al. (2000)</td>
<td>Dogs with lymphoma</td>
<td>DOX</td>
<td>Dogs with lymphoma were fed menhaden fish oil + arginine or soyabean oil (control)</td>
<td>Increased number of disease-free days and survival time for stage III dogs but not stage IV; increased survival time in dogs with highest serum 22 : 6n-3</td>
</tr>
<tr>
<td>Bradley et al. (2001)</td>
<td>Madison 109 (M109) small-cell lung tumours in CD2F1 mice</td>
<td>DHA–paclitaxel conjugated with EPA or paclitaxel</td>
<td>Drugs were administered (intravenously) once daily for 5 d</td>
<td>Decreased hind limb paralysis with conjugate</td>
</tr>
<tr>
<td>Cha et al. (2002)</td>
<td>BDF1 mice injected with L1210 leukaemia cells</td>
<td>Ara-C</td>
<td>Mice were fed diets for 14 d before tumour inoculation. Animals were fed chow with combinations of high and low safflower-seed oil and high and low DHA diets</td>
<td>Beneficial effect on survival, haematology and tumour burden observed at low levels of DHA; however, at plasma concentrations of 100 µg-DHA these beneficial effects were reversed and increased host toxicity and decreased tumour cytotoxicity were observed</td>
</tr>
<tr>
<td>Hardman et al. (2001)</td>
<td>MDA-MB-231 transplanted into Harlan Sprague–Dawley nude mice (nu/nu)</td>
<td>DOX</td>
<td>Tumour-bearing animals fed 5 % maize oil or 2 % maize oil + 3 % fish oil concentrate (34 % EPA, 24 % DHA, and 10 % other n-3)</td>
<td>Increased lipid peroxidation by DOX + fish oil; decreased tumour growth by DOX + fish oil; decreased weight loss with DOX + fish oil</td>
</tr>
<tr>
<td>Hardman et al. (2002)</td>
<td>Mice were provided diets for 2 weeks before delivering chemotherapy</td>
<td>Irinotecan (CPT-11)</td>
<td>Mice were fed diets modified to contain: 10 % (w/w) maize oil + 0 % AAFA™, 9 % maize oil + 1 % AAFA™, 8 % maize oil + 2 % AAFA™, or 7 % maize oil + 3 % AAFA™</td>
<td>2 % AAFA™ in the diet of the CPT-11-treated mice resulted in reduced gastrointestinal toxicities, prevented liver hypertrophy, increased erythrocyte leucocyte counts, reduced haematological toxicities and resulted in maintenance of normal grooming behaviour</td>
</tr>
</tbody>
</table>

DOX, doxorubicin; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.

*AAFA™ is an n-3 fatty acid product that contains a total of 55 % EPA plus DHA.

(1999) reported enhanced tumour regression by the co-administration of CPT-11 with fish oil and have subsequently reported fewer toxic effects to the host in the same model. Low-fat diets (7 %, w/w) comprised of maize oil only, 3 % or 6 % fish oil were fed to tumour-bearing mice. Following irinotecan treatment, the intestinal physiology of the mice fed fish oil appeared similar to the mice who received no drug treatment whereas the mice fed maize-oil diets displayed considerable intestinal pathology (Hardman et al. 2000). A follow-up study employing 2 % fish oil confirmed the protective effects of fish oil on gastrointestinal tissues (Hardman et al. 2002). The same investigators have reported similar findings using DOX as the cytotoxic agent (Hardman et al. 2001). Higher numbers of lymphocytes and erythrocytes and less weight loss were observed in tumour-bearing mice fed a fish-oil concentrate at 3 % compared with those fed a 5 % maize-oil diet. Therefore, the gastrointestinal and haematological toxicities that limit the dose of these drugs appear to be attenuated when dietary fish oil accompanies the treatment.

One study has investigated the role of fish oil in relation to cardiac toxicity. Tumour-bearing rats fed 15 % sardine oil were given epirubicin treatment with or without lipid peroxide inhibitors or inducers. The group receiving fish oil had significantly reduced growth and number of tumours, and no additional cardiac toxicity was observed (Germain et al. 1999).

While alopecia is not a dose-limiting toxic effect per se, it is a common side effect that is distressing to the patient and has few acceptable treatment options. Cytotoxic drugs designed to induce apoptosis in tumour cells also induce death in hair follicle cells resulting in hair loss. Alopecia induced by Ara-C and etoposide was prevented by the addition of DHA to the diet in an animal model. All animals not provided dietary DHA developed alopecia (Takahata et al. 1999).

While evidence from these few animal studies are generally positive, difficulty arises in the interpretation of studies where basal or control diets are either not representative of human diets or that, based on their fatty acid composition, would be expected to promote tumour progression. Maize oil has been commonly used as the source of fat in control diets in animal studies. While a maize-oil-based diet is not likely to induce an essential fatty acid deficiency per se, the
skewed n-6:n-3 PUFA ratio in maize oil may be supportive of tumour growth or altered immune functions (Black et al. 1992; Rose & Connolly, 1993; Simonsen et al. 1998; Klein et al. 2000; Furukawa et al. 2002; Calder, 2003). The balance of n-6 and n-3 PUFA rather than the total amount of fat consumed may be more important in determining cancer risk. A study employing 5 or 10 % (w/w) safflower-seed diets containing 1-5 or 3 % (w/w) DHA, respectively, reported reduced erythrocyte concentrations and lower survival when plasma levels of DHA reached 100 µM (Cha et al. 2002). Conversely, benefits were seen at lower levels, suggesting that there may be an upper threshold limit for the beneficial effects of fish oil. In the one trial that fed dietary levels of fish oils that could be attained in man (Hardman et al. 2002), gastrointestinal toxicity was reduced; however, the control diet in that study was not relevant to man and, in fact, could be expected to promote inflammatory processes. Furthermore, the mechanisms of the drug in question and its interactions with specific nutrients may impact on the efficacy of fish oils. In the animal studies discussed, tumours were established before dietary intervention took place and drugs were administered some time afterwards. This design is of relevance to address supplementation as an adjuvant to therapy and may help address the question of whether long-chain PUFA may become conditionally essential during chemotherapy treatments. With several inter-related mechanisms proposed for the enhanced cytotoxic effects and protective host effects that potentially impact on many physiological systems, it would seem important to further clarify optimal conditions for growth inhibition and tissue protection that are relevant to the human situation.

**n-3 Polynsaturated fatty acids alter catabolic signal transduction and attenuate wasting**

A majority of patients with advanced cancer experience a progressive syndrome of wasting known as cachexia. The end results of the wasting syndrome, emaciation and functional loss, remain hallmarks of advanced neoplastic disease. The wasting syndrome is typified by hyper-catabolism and accelerated degradation of skeletal muscle protein and adipose tissue which is often accompanied by anorexia, early satiety, weakness, anaemia, suppression of immune functions and oedema. Together with pain, cachexia is one of the most frequent and devastating symptoms that affect cancer patients. There have been a few reports of the inhibition of cancer-associated wasting by n-3 PUFA in animal models and clinical investigations. The idea of employing n-3 PUFA to attenuate protein catabolism in cancer-associated wasting syndromes is based on the understanding that systemic inflammation, cytokines and eicosanoids are central drivers of protein breakdown.

**Research in animal models.** Wasting has been attenuated in several animal models of cachexia by dietary fish oil supplementation (Table 3). Tisdale and co-workers have reported the suppression of wasting, muscle protein catabolism and muscle protease induction by feeding fish oil to mice bearing the MAC16 adenocarcinoma (Whitehouse et al. 2001; Tisdale, 2001). Similar effects have been shown for methylcholanthrene-induced sarcoma (Jho et al. 2002) and ApcMin/+ mice (Whelan et al. 2002). Tumour growth is inhibited by n-3 fatty acid supplementation in some models, and this would be expected to attenuate wasting indirectly (Whitehouse & Tisdale, 2001; Jho et al. 2002). Although in some cases this may provide a partial explanation of reduced weight loss and tissue wasting, it seems clear that the anti-cachectic effect is larger than would be expected based on the magnitude of the reduction of tumour growth.

**Mechanisms for attenuation of wasting by n-3 polyunsaturated fatty acids.** The proposed mechanisms of attenuation of wasting by n-3 PUFA centre largely around catabolic signal transduction by cytokines, eicosanoids and tumour-derived proteolysis-inducing factor (PIF). The response of the body to cancer has many parallels with systemic inflammation (Argiles et al. 2000; Barber et al. 2000; Kotler, 2000; Wigmore et al. 2001). This has been very clearly resolved in animal models to significantly involve both cytokines and eicosanoids in cancer-associated wasting. The participation of pro-inflammatory cytokines including IL-1-β, IL-6, TNF-α and interferon-γ has been positively established by changing cytokine production or activity using experimental approaches such as passive immunisation with antibodies to cytokines, cytokine receptor knockout mice, or animals over-expressing soluble receptor isoforms (Argiles & Lopez-Soriano, 1999; Kotler, 2000; MacDonald et al. 2003).

**Eicosanoids.** Eicosanoids are key inflammatory mediators that have also been implicated in cancer cachexia (Ross & Fearon, 2002). Eicosanoids appear to lie in the catabolic signal-transduction pathways of both cytokines and a tumour-derived proteolysis-inducing glycoprotein (Ross & Fearon, 2002). Eicosanoids derived from n-3 PUFA have up to 100-fold less biological potency in the induction of cellular responses than those derived from arachidonic acid and are usually associated with decreased inflammatory responses (Calder, 2003). Eicosanoids are produced by some tumours (Zweifel et al. 2002; Badawi & Badr, 2003) as well as by the host during immune responses. Some of the evidence for the involvement of eicosanoids in the inflammatory process, which underlies cancer-associated wasting, has come from studies examining the action of non-steroidal anti-inflammatory drugs, including traditional non-steroidal anti-inflammatory drugs as well as agents developed to selectively block the synthesis of the inducible pro-inflammatory prostaglandins (Cahn et al. 2000). Thus, one clear mechanism whereby n-3 PUFA might influence catabolism would be through modification of the eicosanoid axis resulting in a reduction in the inflammatory response. Alternatively, they may alter the production of eicosanoids involved in the signalling pathways of various catabolic mediators, such as the PIF (Lorite et al. 2001; Tisdale, 2003).

**Proteolysis-inducing factor.** Tumour-derived PIF is a novel molecule that mediates catabolism in mice bearing the MAC16 adenocarcinoma (Cariuk et al. 1997). This proteolysis-inducing glycoprotein of tumour origin consists of
Table 3. n-3 Fatty acid effects on anorexia cachexia syndrome: animal and human studies

<table>
<thead>
<tr>
<th>Author</th>
<th>Cancer type</th>
<th>Study design</th>
<th>Main finding</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Animal studies</strong></td>
<td></td>
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<tr>
<td>Smith <em>et al.</em> (1999)</td>
<td>Murine C_{2}C_{12} myoblasts</td>
<td>Effect of PIF in vitro and modulation by EPA</td>
<td>Decreased protein synthesis and degradation by PIF. Pre-incubation with EPA inhibited protein degradation. Increased arachidonate release, PGE_2, PGF_2_a, 5-, 12- and 15-HETE synthesis by PIF, 15-HETE identified as the specific eicosanoid mediator of PIF</td>
</tr>
<tr>
<td>Sauer <em>et al.</em> (2000)</td>
<td>Hepatoma 7288CTC cell line implanted into Buffalo rats</td>
<td>Assessed for fatty acid transport and 13-HODE formation</td>
<td>Decreased fatty acid uptake and release of 13-HODE by EPA. Decreased [\textsuperscript{3}H]-thymidine incorporation by EPA. Addition of 13-HODE, forskolin, pertussis toxin and 8-Br-cAMP reversed the inhibition by EPA. Receptor for n-3 fatty acids is a G-protein involved in signalling via cAMP pathway</td>
</tr>
<tr>
<td>Islam-Ali <em>et al.</em> (2001)</td>
<td>MAC16 adenocarcinoma implanted into NMRI mice</td>
<td>Mice were or were not fed EPA</td>
<td>Increased weight-lossing mice had: lipid-mobilising factor; increased adenylyt cyclase formation; expression G proteins. Treatment with EPA reduced G protein ratio to normal</td>
</tr>
<tr>
<td><strong>Human studies</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Barber <em>et al.</em> (1999b)</td>
<td>Weight-losing patients with advanced pancreatic cancer</td>
<td>Patients received two cans/d of a nutritional supplement enriched with 1:1 g EPA and 0:46 g DHA v. no supplement v. healthy controls</td>
<td>Increased weight in supplemented group (median 1 kg); decreased weight in unsupplemented group (median 2:8 kg); increased production of negative acute-phase proteins (albumin, pre-albumin and transferrin) in supplemented group suggesting an improvement in nutritional status and/or attenuation of acute-phase protein response</td>
</tr>
<tr>
<td>Barber <em>et al.</em> (1999a)</td>
<td>Weight-losing patients with advanced pancreatic cancer</td>
<td>Patients received two cans/d of a nutritional supplement enriched with 1:1 g EPA and 0:46 g DHA v. no supplement v. healthy controls over 7 weeks</td>
<td>Patients consuming supplement exhibited increased weight gain over 7 weeks (median 2:5 kg); decreased resting energy expenditure. No change in serum C-reactive protein concentrations. Increased Karnofsky performance and appetite scores</td>
</tr>
<tr>
<td>Barber <em>et al.</em> (2001)</td>
<td>Weight-losing patients with advanced pancreatic cancer</td>
<td>Patients consumed a nutritional supplement providing 2510 kJ (600 kcal) and 2 g of EPA per d for 3 weeks</td>
<td>Decreased IL-6; increased serum insulin concentration; decreased cortisol:insulin ratio; decreased proportion of patients excreting proteolysis-inducing factor</td>
</tr>
<tr>
<td>Bruera <em>et al.</em> (2003)</td>
<td>Weight-losing patients of mixed tumour types</td>
<td>Patients consumed 1:8 g EPA/d and 1:2 g DHA/d as capsules for 14 d</td>
<td>No differences in weight loss, appetite, nutritional status or symptoms compared with placebo</td>
</tr>
<tr>
<td>Burns <em>et al.</em> (1999)</td>
<td>Weight-losing advanced cancer of mixed types</td>
<td>Maximum tolerable dosage of fish oil capsules (378 mg EPA and 249 mg DHA) was determined</td>
<td>Maximum tolerable dose as high as 0.3 g/kg per day with minimal side effects (i.e. 21 g/d for 70 kg individual)</td>
</tr>
<tr>
<td>Wigmore <em>et al.</em> (2000)</td>
<td>Weight-losing patients with advanced pancreatic cancer</td>
<td>Patients consumed 95 % EPA in capsules; 1 g/d for 1 week, 2 g/d in second week, and 4 g/d in third week, and 6 g/d thereafter</td>
<td>Patients became weight stable with EPA supplementation, but no significant difference in energy intake was observed. 6 g treatment did not appear to have a greater effect relative to a previous trial using 2 g/d</td>
</tr>
<tr>
<td>Fearn <em>et al.</em> (2003)</td>
<td>Weight-losing patients with advanced pancreatic cancer</td>
<td>Patients instructed to consume two cans of a supplement providing 1300 kJ (310 kcal), 16 g protein, 6 g fat with or without 1:1 g EPA + antioxidants</td>
<td>Weight loss was abrogated in both treatment groups. Providing n-3 fatty acids resulted in weight gain, gain of lean tissue and improved quality of life</td>
</tr>
</tbody>
</table>

PIF, proteolysis inducing factor; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.
a short polypeptide chain which is highly glycosylated. When muscle cells or animals are treated with the purified factor, intense protein catabolism is elicited (Cariuk et al. 1997; Lorite et al. 1998; Todorov et al. 1999). The mechanism of action of this factor is suggested to involve an eicosanoid, 15-HETE. The protein catabolism induced by PIF is markedly attenuated by the administration of EPA (Smith & Tisdale, 2003; Tisdale, 2003). An identical factor found in man (Cabral-Manzano et al. 2001; Tisdale, 2001) appears to be present in a broad spectrum of cancer patients (i.e. carcinomas of the breast, lung, ovary, melanoma, gastrointestinal system) with active weight loss, but is absent in cancer patients who are not losing weight or in weight-losing patients with benign disease (Wigmore et al. 2000; Cabral-Manzano et al. 2001; Tisdale, 2001). These results possibly represent the discovery of a critical factor responsible for cancer cachexia in man. While several anti-inflammatory therapies exist that may be appropriate for cancer-associated cachexia (MacDonald et al. 2003), n-3 PUFA appear to intersect a number of pathways that would impart benefits to ameliorate the wasting syndrome.

Safety and efficacy of n-3 polyunsaturated fatty acids in clinical trials

n-3 PUFA are safe compounds. Safety was evaluated in a phase I trial, where participants with terminal cancer took up to 0-3 g EPA/kg body weight, well beyond the 2-4 g/d doses used in clinical trials (Burns et al. 1999). Even at high doses, side effects were relatively mild and included bloating, ‘a fishy taste’ and loose bowel movements. Similarly, early phase II trials conducted by Fearon and co-workers involving patients with pancreatic tumours not otherwise on chemotherapy receiving fish oil capsules were promising (Barber et al. 1999b; Wigmore et al. 2000). Significant improvements in weight, function and, to a lesser extent, appetite were reported. Of particular interest, in contrast to megestrol acetate, lean body mass increased. A randomised investigation of fish oil v. a maltodextrine placebo (Gogos et al. 1998) reported an improvement in survival and in immune function with an increase of the CD4:CD8 ratio (Gogos et al. 1998) in a mixed group of malnourished patients with generalised malignancy.

Following their series of phase II trials, Fearon and co-workers carried out a phase III double-blind randomised placebo-controlled trial, again enlisting weight-losing pancreatic cancer patients who had not undergone chemotherapy treatment. The source of n-3 in this study was a liquid enteral formula to be taken at a dose that would provide 2-2 g EPA and 0.9 g DHA. The control treatment was an isoenergetic, isonitrogenous supplement without EPA and DHA. An improvement in survival was not demonstrated in the n-3-treated group but, once again, these authors reported an improvement of lean body mass in those patients who were taking in excess of 1-5 g EPA daily (Barber & Fearon, 2001). The most recent study from this group involved 200 patients with advanced pancreatic cancer (Fearon et al. 2003) randomised to the fish-oil-containing enteral supplement or an identical placebo containing n-6 fatty acids. Again, n-3 PUFA supplementation was associated with an increased lean body mass in a dose-dependent fashion. A recently completed randomised, double-blind study was conducted cooperatively by the Northwest Clinical trials group NCI and NCI-Canada and this has been published as a preliminary report (Jatoi et al. 2003). These investigators compared the n-3 nutrient supplement used by Fearon’s group (and the same placebo) with megestrol, and the two agents in combination, in an advanced cancer patient population. The EPA supplement scored as well as megestrol acetate on overall weight gain, survival and global quality of life; however the proportion of patients gaining 10 % or more of body weight was higher in the patients receiving megestrol acetate alone. Changes in lean body mass were not investigated (Jatoi et al. 2003).

Polyunsaturated fatty acid and n-3 status of the cancer patient

The fatty acid status of cancer patients at diagnosis and throughout the disease trajectory has rarely been determined. Although a few studies have assessed nutritional status at select time-points during the disease (Table 3), no studies have systematically assessed changes in fatty acid status throughout the disease and/or treatment progression.

At diagnosis

Population studies illustrate the large variation of n-3 levels in blood and cell lipids in individuals from different countries and regions (de Deckere, 1999; Amiano et al. 2002; Augustsson et al. 2003; Terry et al. 2003). Thus it seems clear that any reports of PUFA status in cancer patients at any stage must be interpreted in the light of national or regional context. In a comprehensive review of intakes of fish and marine fatty acids in relation to hormone-related cancers, Terry et al. (2003) point out that the difference between the highest and lowest n-3 intakes internationally are as much as 15-fold (i.e. 1500, 400 and 100 mg/d in Japan, Scandinavian countries and North America, respectively). The dietary n-6:n-3 fatty acids ratio shows a similar degree of variation.

The fatty acid status of patients at the time of a cancer diagnosis can be assumed to be a function of previous dietary intakes and the effect, if any, specific to disease and disease stage. Only one study to date has reported plasma fatty acid levels in patients with a new diagnosis of pancreatic, non-small-cell lung and stomach or oesophageal cancer (Zuidgeest-van Leeuwen et al. 2002). The disease stage was not reported; however, up to 23 % of patients had metastatic cancer at diagnosis. These were compared with normal healthy controls from a local population (The Netherlands). This report suggests impairments in essential fatty acid metabolism as the levels of fatty acids of the n-6 and n-3 series typically were lower than those of healthy subjects; however, the degree of difference and the specific fatty acids that are affected appear to vary between cancer types.

During therapy

Fatty acid metabolism and fatty acid status during treatment for cancer are not well characterised. In a pilot study by our group, we reported a selective depletion of long-
chain PUFA in plasma and membrane phospholipid by a high-dose combination of cyclophosphamide, taxotere and 5-fluorouracil (MacDonald et al. 2003). Although preliminary, this study reported very low levels of long-chain PUFA (both n-6 and n-3 fatty acids) and markedly skewed n-6:n-3 ratios in plasma and cell phospholipid after cytotoxic treatment. Cytotoxic agents have been reported to interfere with the metabolism of PUFA (Marra et al. 1986) and may limit the endogenous production of EPA and DHA from 18 : 3n-3, and of 20 : 4n-6 from 18 : 2n-6. It would seem important to examine the elongation and desaturation activity of 18-carbon fatty acids in cancer patients, as impairment of this function may result in the conditional essentiality of long-chain PUFA (Bordoni et al. 1999). The effects of various chemotherapies on fatty acid status need to be more fully documented and this may support an argument to attempt to increase PUFA levels before chemotherapy or to include supplementation with post-chemotherapy re-alimentation. It seems possible that the depletion of PUFA is a side effect of chemotherapy that reduces n-3 status and thereby potentiates the side effects of subsequent cycles of chemotherapy, as well as inflammation and catabolic processes.

At advanced disease stages

Very little data have been collected on the fatty acid status of patients with advanced cancer. In a recent study (Pratt et al. 2002) we compared the fatty acid status of patients with advanced cancer with healthy subjects. Patients with locally recurrent or metastatic cancer of mixed primary sites were recruited from palliative care services. The median survival for the overall group was 14-2 (s.e. 1-9) weeks, reflecting a patient population with very advanced disease. Most strikingly, plasma phospholipids and most individual fatty acids in the phospholipids of advanced cancer patients were about 30 % of the levels observed in healthy subjects with the essential fatty acids, 18 : 2n-6 and 18 : 3n-3, present in plasma phospholipid at 29 and 27 % respectively. The mead acid (20 : 3n-9):20 : 4n-6 ratio, often used as an indicator of essential fatty acid deficiencies, was elevated in the cancer patients (0.3 (s.e. 0.04) μg/ml) compared with the healthy subjects (zero). In neutrophils, elevated arachidonic acid in all phospholipid classes was observed in patients with advanced cancer and contributed to a higher n-6:n-3 fatty acid ratio in these cells. These results suggest a potentially important depletion of n-3 and n-6 PUFA in advanced cancer and, within that, a tendency towards elevated n-6:n-3 ratios, at least in neutrophils (Pratt et al. 2002).

While very limited in overall scope, these data suggest that the fatty acid status of patients newly diagnosed with cancer may be poor and that disease progression, poor dietary intakes and cytotoxic therapy all contribute to the progressive decline of fatty acid status during disease progression. In some cases, this may amount to a frank deficiency of essential fatty acids. In countries with low levels of marine fish in the diet and high n-6 fatty acid intakes, the fatty acid status may be enormously skewed towards n-6 fatty acids. A more comprehensive follow-up of fatty acid status of cancer patients throughout all stages of the disease trajectory is clearly warranted.

Cautions, limitations and suggestions for further research

What remains ambiguous in epidemiological studies has been clarified through experimental studies to define a role for n-3 fatty acids in reducing tumour growth. The mechanisms of action are now being described in precise biochemical and molecular terms. The advent of techniques that can dissect the response of genes to specific nutrients has created the potential for a clearer understanding of mechanisms and new targets of interventions in the coming future.

The role of n-3 fatty acids in mediating enhanced cytotoxicity and host protection remains an emerging field with several unanswered questions. While evidence from the few animal studies are generally positive, difficulty arises in drawing conclusions from studies where diets are not representative of human diets or that lack relevance to the human situation. Further studies must employ physiologically relevant diets and model clinical situations to clarify the role of n-3 PUFA in improving response to treatment. Furthermore, the mechanisms of the drug in question and its interactions with specific nutrients may impact on the efficacy of fish oils to increase toxic effects to the tumour while protecting the host tissues.

The optimal conditions for enhanced tumour death with host protection has not been well characterised due, in part, to the variety of experimental models that have been utilised. It appears, however, that the redox status of the host may be a factor in determining the efficacy of anti-neoplastic therapies. In light of this, further studies may emphasise a need for clearer nutritional recommendations to emerge for patients undergoing chemotherapy as many patients are self-prescribing antioxidant therapies before, during and after treatments. The impact this may have on the efficacy of the treatment in question is not known at this time. Clarification of these issues may be sought by adjusting the balance of pro-oxidants, such as fish oil, and antioxidants at different treatment stages in animal models that are appropriate human comparisons. With several inter-related mechanisms proposed for the enhanced cytotoxic effects and protective host effects that potentially impact on many physiological systems, it would seem important to further clarify the optimal conditions for these effects.

At present, little is known about the nutritional and fatty acid status of cancer patients at different points in the disease trajectory and this area is only beginning to be explored. It seems possible that the depletion of PUFA is a side effect of chemotherapy that reduces n-3 PUFA status and thereby potentiates the side effects of subsequent cycles of chemotherapy, as well as inflammation and catabolic processes. This makes one ponder if nutritional status and essential fatty acid status were ‘optimal’ at diagnosis, and could be maintained throughout serial therapies, improving tumour killing while experiencing fewer side effects. Assessments of nutritional status continuously from the time of presentation and diagnosis would greatly enhance studies investigating the role of nutritional status in relation to cancer and therapies. Furthermore, it is necessary to determine whether the long-chain n-3 PUFA
become essential in the diet during cancer treatment, due to reduced de novo synthesis or increased utilisation.

A conclusion arising from experimental studies is that muscle wasting falls into distinct sub-types when considered at the level of humoral mediators (Baracos, 2000, 2001) which may very well be analogous to what occurs in cancer patients; however, the full profile of putative catabolic mediators in cancer cachexia has never been detailed in any cancer patient population. This may be important to discriminate between patients exhibiting different catabolic mechanisms during clinical assessment in order to apply appropriate interventions, and it would appear that n-3 PUFA status would be a key variable to include in clinical assessments.

Further exploration of the mechanisms of MDR, as well as cell growth and death regulators, will lead to the discovery of key subcellular targets for drug development and clarify the role of n-3 PUFA. Taking into account the many anti-tumour properties of n-3 PUFA, enhanced cytotoxicity of chemotherapeutic drugs to tumours and some evidence of protection to host tissues, n-3 fatty acid supplementation may be established as an effective adjuvant for current chemotherapy protocols.

The information on fatty acid status and fatty acid supplementation in patients with advanced cancer, taken together, provides a rationale for developing dietary recommendations based on formal assessments of fatty acid status and fatty acid requirements. This is particularly pertinent in countries where n-3 fatty acid intakes are generally low and n-6/n-3 ratios are high. Current intakes of total n-3 fatty acids are about 1·6 g/d (0·7 % of energy intake) in the USA (Kris-Etherton et al. 2002). Of this, α-linolenic acid accounts for about 1·4 g/d, and only 0·1 to 0·2 g/d comes from EPA and DHA. Formal population-based dietary recommendations for n-3 fatty acids have been made by the WHO and the North Atlantic Treaty Organization and also exist in several countries including Canada, Sweden, the UK, Australia and Japan. Typical recommendations for EPA+DHA are 0·3 to 0·5 g/d, and for α-linolenic acid are 0·8 to 1·1 g/d. Dietary reference intakes for energy and macronutrients were recently released by the Food and Nutrition Board, Institute of Medicine, and The National Academies (USA), in collaboration with Health Canada. These institutions placed the acceptable macronutrient distribution range for α-linolenic acid at 0·6 to 1·2 % of energy, or 1·3 to 2·7 g/d on the basis of a 8370 kJ (2000 kcal) diet (Kris-Etherton et al. 2002). This is nearly ten times the current intake of EPA+DHA of typical North Americans. In view of suggestions that the n-3 PUFA status in cancer patients may be even poorer than in the general population, it would appear obvious that dietary supplementation to maintain and replenish n-3 PUFA status at key points in the cancer disease trajectory is required. Dietary recommendations for such supplementation have not been adopted by cancer centres or agencies, and the number and type of supplements and n-3-containing products suited to the patient populations are very limited. For those wishing to advise upon or implement n-3 supplementation, the American Heart Association position paper on fish oil and n-3 fatty acid consumption (Kris-Etherton et al. 2002) is a useful reference.

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Current perspectives on n-3 PUFA and cancer


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