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Plenary Session 3: Fatty acids and health and disease

Dietary PUFA and cancer

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The aim of the present paper is to give a brief overview on the role of dietary fat in carcinogenesis and as possible anticancer agents. Dietary fat is an essential nutrient and important source for the essential fatty acids (FA), linoleic and α-linolenic acids, which contribute to proper growth and development. However, dietary fat has been associated with the development of colorectal, breast, prostate, endometrial and ovarian cancers, with the type and quality of fat playing an underlying role. Tumour growth is the disruption of the homoeostatic balance regulating cell differentiation, proliferation and apoptosis and is associated with altered lipid metabolism. Animal cancer models and human cancer biopsy tissue demonstrate that a characteristic lipid profile is associated with the growth and development of neoplastic lesions. This entails alterations in membrane cholesterol, phospholipid and PUFA metabolism. Particularly, alterations in cell membrane FA metabolism involving the *n*-6 and *n*-3 PUFA, are associated with changes in membrane structure, function, cellular oxidative status, activity of enzymes and signalling pathways. These events are a driving force in sustaining the altered growth of cancerous lesions and provide unique targets for intervention/cancer modulation. Challenges in utilising FA in cancer modulation exist regarding intake and effect on cell structure and biochemical interactions within the cell in the prevention of cancer development. Therefore, utilising dietary PUFA in a specific *n*-6:*n*-3 ratio may be an important chemopreventive tool in altering the growth characteristics of cancer cells.

PUFA: Cancer: Cell survival: Liver: Oxidative stress: Lipid peroxidation

Cancer development (carcinogenesis) is a multistage process, whereby a normal cell is transformed or altered into cells expressing the malignant phenotype. This process is the breakdown or dysfunction of finely controlled processes such as cell differentiation, proliferation and programmed cell death (apoptosis) and the regulatory feed-back mechanisms involved[1,2] leading to the characteristic rapid growth and spread of cancer cells[3]. In the struggle against cancer, an ideal strategy would be to regain control of these deregulated processes, such as apoptosis and cell proliferation. In this regard, experimental and epidemiological evidence have indicated the potential of bioactive food components which can be effective in the prevention of cancer.

Many recognised health problems are associated with excessive intake of dietary fat, such as obesity, insulin resistance, CHD and certain types of cancer[4–6]. With regard to dietary fat, the Western diet is characterised by an increased energy intake and decreased energy expenditure, increased SFA, *n*-6 fatty acid (FA) and *trans* FA with decreased intake of *n*-3 FA, vegetables and fruit[5,6]. Dietary components such as vegetables,

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fruit, grains, fibre, certain micronutrients and certain FA have been associated with a protective effect against the development of certain diseases such as cancer[7]. Amongst these, interest in PUFA of the *n*-3 family has gained prominence regarding their effects on cell structure and survival.

Importance of the cell membrane

The study of biological membranes regarding function and structure is important as they are the very boundaries within which life exists, separating the vast array of biochemical reactions that define a living cell from the extracellular world. Within the cell, membranes also organise and separate these biochemical reactions from each other, generating compositionally and morphologically distinct compartments. The membrane of a cell is important in maintaining a homoeostatic environment in the cell, serving as a gateway for nutrients and creating a signalling pathway for cellular communication. The properties and functionality of the cell membrane is dependent on particular constituent lipid components such as the phospholipids, cholesterol, proteins and FA. Phospholipids are not symmetrically distributed between the outer and inner membrane leaflets and this feature appears to be integral for correct functioning. Studies involving the erythrocyte membrane indicate that most of the phosphatidylethanolamine (PE) and phosphatidylserine are situated within the inner or cytoplasmic membrane leaflet, while most of the phosphatidylcholine (PC) and sphingomyelin are found in the outer leaflet[8,9].

This characteristic asymmetric lipid distribution of cell membranes is important in the physiological functioning of the membrane. Events such as translocation of phosphatidylserine from the inner to the outer membrane leaflet of erythrocytes or vascular endothelium promotes blood coagulation and translocation of phosphatidylserine and PE to the outer membrane leaflet is a signal for apoptosis and removal of an injured or damaged cell[10,11]. Therefore, disruption of the membrane phospholipid distribution or the PC:PE ratio as well as FA content can change the responsiveness of the cell to normal physiological control mechanisms.

As components of cellular phospholipids, FA are the main structural building blocks of all cell membranes[12,13]. Amongst the FA, the SFA, monounsaturated FA and PUFA as well as interaction between these classes play important roles in maintaining the integrity and functioning of the cell membrane. Changes in the FA content, especially with regard to PUFA and SFA, can affect the fluidity of cell membranes, ultimately affecting membrane function[14]. Certain FA are also substrates for the synthesis of eicosanoids which are involved in the regulation of cell growth, cell activity and cell function[15]. Functionally, FA also affect gene expression by binding to and regulating the activity of nuclear receptors controlling the major regulatory networks that impact cell metabolism and signalling mechanisms[16].

As cell membranes are susceptible to dietary modulation, particularly of the *n*-3 family, the chemopreventive mechanisms of these FA are multiple. Mechanisms include the suppression of $C_{20}:4n$-6-derived eicosanoid production, enhancement of apoptosis, inhibition of tumour cell proliferation and an increase in anti-angiogenic activity[17]. The incorporation and association of *n*-6 and *n*-3 FA with membrane dynamics involving cholesterol, phospholipids and membrane proteins, also significantly impact on signal transduction pathways and cell-to-cell communication in the regulation of cell growth. Therefore, changes in the cellular lipid composition can affect the structural and functional properties of membranes, thereby altering the growth characteristics of neoplastic cells. Membrane components such as a cholesterol and FA are also of particular importance to certain membrane substructures such as lipid 'rafts' or 'domains' which play important roles in cell signalling[18].

Dietary fatty acids and cancer in human subjects

In 1956, Hugh Sinclair[19] claimed that most of the 'diseases of civilisation' such as CHD, thrombosis, cancer, diabetes, inflammation and skin diseases are caused by a disturbance in fat metabolism. The main reason for the occurrence of these diseases was associated with the increased intake of processed foods rich in SFA and *trans* FA and a subsequent alteration in essential FA intake, such as a shift towards a higher *n*-6 FA intake. However, the clarity surrounding the association of saturated fats and CVD is being questioned[20]. During the 1970s the beneficial health effects of *n*-3 FA gained recognition and since then a growing body of evidence from *in vitro* studies in cell cultures, animal cancer models, epidemiological and clinical studies in human subjects has provided evidence to support their use in the prevention of cancers such as in the colon, breast and prostate[17]. In contrast, a high dietary intake of *n*-6 FA is associated with an increased risk for the development of cancer[6]. Research into the beneficial health effects of the *n*-3 PUFA were fuelled by the 'classical' studies undertaken by Dyerberg, Bang and Hjorne which indicated that high-fish-consuming populations such as the Inuits and Danes have low cancer incidence rates[21]. Further epidemiological studies have shown a protective association regarding *n*-3 PUFA and cancer risk. In a Japanese population-based study, the *n*-3 PUFA (EPA, $C_{20}:5n$-3; docosapentaenoic acid, $C_{22}:5n$-3; DHA, $C_{22}:6n$-3) were associated with protection against developing hepatocellular carcinoma[22], whereas another population-based prospective study undertaken by the Japanese Public Health Center demonstrated an inverse relationship between the intake of marine *n*-3 PUFA and the risk of colorectal cancer, even though the association was only significant in the proximal site of the colon[23]. Large studies, such as the European Prospective Investigation into Cancer and the United States' Physicians Health study, involving thousands of participants have confirmed the protection against

cancer afforded by *n*-3 PUFA, particularly signifying a decrease in the risk of colorectal cancers[24,25]. In a case–control study, erythrocyte levels of EPA and DHA were inversely associated with breast cancer risk[26]. Further detailed reviews concerning *n*-3 PUFA and cancer are given by Gerber[27] and Stephenson *et al.*[28].

Fatty acid and cancer: mechanisms of control

FA may influence carcinogenesis through various mechanisms and it is therefore important to understand the control of FA over these processes, thereby determining possible intervention points.

Diets containing high levels of *n*-6 FA, such as in corn oil, have been shown to enhance the development of tumours[6,29,30]. Most of these effects have been attributed to the metabolism and cell signalling properties of arachidonic acid ($C_{20}:4n$-6). The deregulation or disturbance in $C_{20}:4n$-6 metabolism has been connected to a large number of pathological disorders, indicating the need for tight control over the metabolism of this FA[31]. Therefore control over $C_{20}:4n$-6 metabolism may be an important factor in controlling cellular proliferation and apoptosis and therefore cancer cell survival. In this regard, the effect of *n*-3 PUFA on chemoprevention has been shown to be effective *in vivo* and *in vitro*. Mechanisms involved have been demonstrated to involve the competitive incorporation of *n*-3 FA in cell membranes, to be involved in signal transduction by acting as ligands of certain nuclear receptors, competing in the synthesis of eicosanoids via the cyclooxygenase-2 pathway, enhancing apoptosis and cell differentiation, and altering the cellular oxidative status by enhancing lipid peroxidation[32].

Altered lipid profile in liver cancerous tissue

Understanding the alterations in the lipid profile of cancerous tissue will enable possible modulation strategies utilising PUFA in chemoprevention. Studies from animal cancer models and human cancer biopsy tissue demonstrate that a characteristic lipid profile is associated with the growth and development of neoplastic lesions[33,34]. This profile entails alterations in membrane cholesterol, phospholipid and PUFA metabolism resulting in a shift in $C_{20}:4n$-6 phospholipid distribution and a decreased cellular oxidative status. Cancerous tissue from a rat liver cancer model and biopsy tissue from human patients with liver cancer showed similarities in the altered lipid profile associated with liver cancer development[34,35].

Alterations in the PUFA content of liver cancerous tissue, encompass a decrease in the *n*-3 PUFA resulting in an increased *n*-6:*n*-3 PUFA ratio, increased $C_{18}:1n$-9 (oleic acid), increase in $C_{20}:4n$-6, increased $C_{20}:4n$-6/ $C_{20}:5n$-3 and decreased $C_{20}:4n$-6 PC:PE ratios. A shift in the $C_{20}:4n$-6 PC:PE ratio could indicate a higher availability of $C_{20}:4n$-6 for eicosanoid synthesis and cell signalling or a compensatory shift in phospholipid distribution to maintain membrane stability and functionality. Cholesterol was increased, whereas the phospholipid PC:PE ratio decreased. Alterations in the membrane phospholipid and cholesterol levels, affecting the membrane cholesterol:phospholipid and PC:PE ratios, will influence membrane structure, fluidity and functionality. Disruption of the typical phospholipid and cholesterol asymmetrical arrangement may affect cellular signalling influencing events such as apoptosis[11,12]. Changes in these parameters are also known to regulate the function of Δ6-desaturase enzyme, a key rate-limiting enzyme in FA metabolism[36]. Various studies have demonstrated an impairment of the Δ6-desaturase in cancer tissue affecting PUFA synthesis[37,38]. Lipid peroxidation was decreased in conjunction with altered levels of the antioxidant glutathione (reduced form) as well as altered activity of the antioxidant enzymes, i.e. glutathione reductase and peroxidase, superoxide dismutase and catalase. These changes result in the maintenance of a low cellular oxidative status thereby creating a protective environment for cancer cell survival. The level of lipid peroxidation has also been found to influence tumour growth[39] and, together with changes in the membrane lipid status, is likely to play an important role in the abnormal cellular growth which prevails in cancerous tissue. These events are highlighted as a driving force sustaining the altered growth characteristics of cancerous lesions together with a diet high in *n*-6 PUFA playing an underlying stimulating role. The integrity of the cellular membrane is therefore important in the normal functioning of the cell and its responses to external growth stimulatory and/or inhibitory factors.

In the rat liver cancer model, the altered lipid parameters associated with hepatocyte nodule development mimicked normal cellular proliferation in regenerating liver[34]. However, a major difference between the two proliferating tissue compartments was the persistence of the lipid changes in the hepatocyte nodules suggesting that nodule lipid metabolism escapes the regulatory mechanisms required for normal cellular homoeostasis during proliferation. This indicates that the impaired regulation of lipid metabolism may be responsible for the enhanced proliferation and altered growth pattern in hepatocyte nodules.

Modulation of lipid profile by dietary PUFA and effect on cancer cell development

It can be questioned whether the characteristic lipid profile in cancerous tissue can be modulated by dietary PUFA thereby affecting cancer cell development. Utilising the rat liver cancer model it was demonstrated, dependent on the dietary PUFA composition and stage of intervention, that the development of early preneoplastic cancer lesions (nodules) can be modulated.

Modulation of rat hepatocyte nodules

To determine the effect of dietary PUFA on hepatocyte development in rat liver, the fat component of the rodent AIN 93 diet[40] was modified reflecting varying low *n*-6:*n*-3 FA dietary ratios and fed to rats with carcinogen-induced hepatocyte nodules[41]. Different fat sources consisting of sunflower oil (S; 250:1 *n*-6:*n*-3 FA ratio) or S in combination with γ-linolenic acid-80 (GLA-80) and/or EPA-55 fish oil to obtain the sunflower/fish oil (SF, 12:1 *n*-6:*n*-3 FA ratio), sunflower/GLA-80/fish oil (SGF; 12:1 *n*-6:*n*-3 FA ratio) or soyabean oil (SOY; 5:1 *n*-6:*n*-3 FA ratio) were used to obtain the various low *n*-6:*n*-3 FA dietary ratios and fed to the four dietary groups (S, SGF, SF and SOY) for 4 weeks before nodule induction. Rats in all dietary groups underwent nodule induction using diethylnitrosamine (DEN) followed by 2-acetylamino-fluorene/partial hepatectomy promotion[42]. Three months after induction, all rats were terminated, livers removed and hepatocyte nodules were harvested with surrounding tissue. Liver tissue from uninduced respective dietary groups was also collected as control tissues. The low *n*-6:*n*-3 FA ratio diets (SF, SGF and SOY diets) mainly affected the phospholipid long-chain PUFA composition in the preneoplastic nodules by increasing the *n*-3 FA content to varying degrees, especially $C_{20}:5n$-3 and $C_{22}:6n$-3, with a concomitant decrease in the *n*-6 long-chain PUFA. The highest *n*-3 PUFA incorporation was achieved with the SF and SGF diets, compared to the SOY diet. Of importance were the different effects exerted on the nodule $C_{20}:4n$-6 level by the three diets. The SOY and SF diets decreased $C_{20}:4n$-6 in PC and PE, whereas the SGF diet did not decrease $C_{20}:4n$-6 to the same extent. Only the SGF diet decreased the $C_{20}:4n$-6 nodule:surrounding ratio in both PC and PE fractions, while the SF diet induced a comparable nodule/surrounding ratio to the S diet. Alteration in the $C_{20}:4n$-6:$C_{20}:5n$-3 ratio and $C_{20}:4n$-6 level by the SGF diet is also suggestive of a decreased PGE_2 synthesis from $C_{20}:4n$-6. Only the SGF diet was associated with a reduction in the development of the nodules which could be related to the interaction between $C_{20}:5n$-3 and $C_{20}:3n$-6 (dihomo-GLA) which appear to synergistically control the metabolism of $C_{20}:4n$-6 and also serve as substrates for less potent prostanoid metabolites, such as PGE_3 and E_1 respectively. This control over $C_{20}:4n$-6 metabolism by the $C_{20}:5n$-3/$C_{18}:3n$-6 (GLA) dietary combination has important implications for the regulation of transcription factors and genes involved in signalling pathways affecting cell proliferation and apoptosis which can influence the development of hepatocyte nodules[41,43].

Interaction between dietary PUFA and food-borne contaminants

Of interest with regard to carcinogenesis is the interplay between dietary FA and toxins present in foods and effect on carcinogenesis. In this regard, a rat hepatocarcinogenesis model was used to investigate the interactive effect of a toxin and dietary fats on the initiation and promotion stages of carcinogenesis[44]. The mycotoxin, fumonisin B_1 (FB_1), acts as a weak, non-genotoxic cancer initiator and expresses strong cancer promoting activity resulting in the induction and promotion of pre-neoplastic hepatic foci in rat liver. The mechanisms responsible involve alteration of the cell membrane structure resulting from changes in lipid metabolism, more specifically phospholipids, sphingolipids and cell membrane FA content[45].

To investigate the effect on cancer initiation, weaned male Fischer F344 rats were fed the AIN 93 diet with different oils as fat sources; either S with an *n*-6:*n*-3 FA ratio of 700:1, or a mixture of SGF to achieve an *n*-6:*n*-3 ratio of 6:1.

Two different initiation/promotion protocols were used: (i) male Fischer F344 rats were treated with dietary FB_1 (250 mg/kg diet) for 6 weeks to induce initiation and promotion ((−) DEN) and (ii) rats were implanted with minipumps releasing DEN (200 mg/kg body weight) over 7 d as initiation ((+) DEN) and dietary FB_1 (250 mg/kg diet) over 6 weeks as cancer promotion treatment. In the (+) DEN study, rats were weaned on the S diet and switched to the SGF diet 15 d after initiation to prevent any dietary effect during initiation. On termination, liver sections were removed and stained for glutathione-S-transferase (placental) positive (GSTP[+]) areas which represent the pre-neoplastic nodules/lesions in the liver.

In the FB_1 initiation study ((−) DEN), GSTP[+] lesions were increased in the S/FB_1 group, but no significant increase observed in the SGF and SGF/FB_1-fed group, demonstrating inhibition of FB_1 initiation by the GLA-80 and fish oil combination (Fig. 1a). This could result from decreased hepatotoxicity and increased apoptosis stimuli to remove altered cells (initiated cells) presumably via increased oxidative stress. The reduced FB_1 associated hepatotoxicity by the SGF diet ((−) DEN initiation), as indicated by decreased serum liver function markers (total bilirubin, alanine aminotransferase, aspartate aminotransferase andalkaline phosphatase), could be related to a decreased inflammatory response resultant from a decreased *n*-6:*n*-3 FA ratio, decreased $C_{20}:4n$-6 and increased $C_{20}:3n$-6 and $C_{22}:6n$-3 content. The shift in $C_{20}:4n$-6 between the major phospholipids, indicated by an increased $C_{20}:4n$-6-PC:PE phospholipid ratio, resulting from the SGF diet has implications for cell survival. A higher content of this FA in PC is thought to indicate a shift towards apoptosis, which counteracted the decrease in the ratio due to FB_1 promotion associated with an increase in cell proliferation known to selectively increase pre-neoplastic development[45,46]. Supplementation with *n*-3 FA, whose anti-inflammatory properties are established in animal models[47,48], can alleviate FB_1-induced inflammation and therefore reduce liver toxicity.

In the group with DEN as initiator ((+) DEN), the SGF diet prevented development in the number and size of GSTP[+] lesions, once again implicating an inhibitory effect on cancer initiation via apoptosis due to an increased oxidative stress (Fig. 1b). However, combination of FB_1 and SGF in the diet enhanced tumour
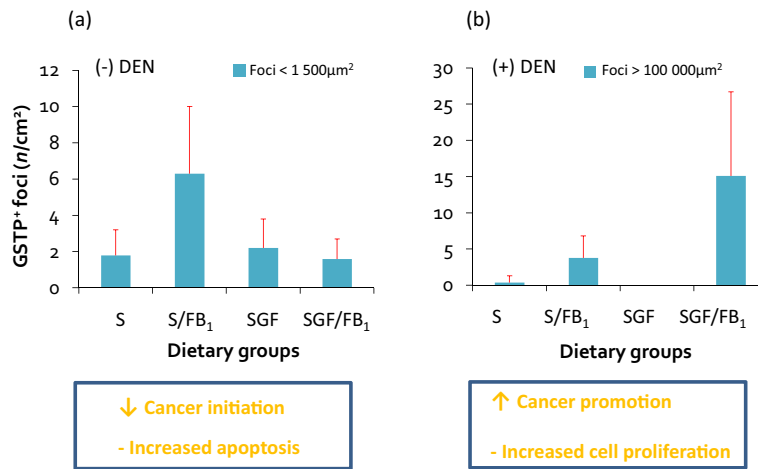
Fig. 1. (colour online) (a) With fumonisin B$_1$ (FB$_1$) as initiator in rat liver, the glutathione-S-transferase placental positive (GSTP+) lesions were higher in the sunflower (S)/FB$_1$ group, but not significantly different in the sunflower/GLA-80/fish oil (SGF) and SGF/FB$_1$-fed group compared with the S-fed group, demonstrating GLA-80 and fish oil inhibition of FB$_1$ initiation[44]. (b) With diethylnitrosamine (DEN) as initiator in rat liver, the SGF diet prevented development in the number and size of GSTP+ lesions. However, the combination of dietary FB$_1$ and SGF enhanced tumour promotion[44].

promotion probably due to the selective resistance of DEN-initiated hepatocytes towards oxidative stress and the apoptotic stimulus. In this scenario of the initiation/promotion model with DEN and FB$_1$, the SGF diet enhanced hepatotoxicity indicated by increases in serum liver markers and stimulated promotion reflected by the increased appearance of GSTP$^+$ lesions. The increased oxidative stress and hepatotoxicity resultant from feeding the SGF diet appeared to create a growth-stimulating environment promoting DEN-initiated foci, i.e. the proliferation stimulus overwhelmed apoptotic effects in the pre-neoplastic lesions thereby increasing the number and size of GSTP$^+$ foci.

Therefore, cancer initiation and promotion can be selectively altered by dietary FA which, depending on their interactive roles, provides important information regarding the dietary modulation of food-borne hepatocarcinogenesis.

Lipid profile and tumour cell survival

Alterations in the lipid profile of cancerous cells and membrane FA content can affect cell survival, dependent on type of FA and n-6:n-3 ratio. These alterations will influence oxidative status, inflammation, cell signalling, thereby disrupting the protective environment of a cancer cell, promoting a shift from cell proliferation and survival to cell removal and/or cell death.

The characteristic lipid profile associated with the growth and development of neoplastic lesions are highlighted as a driving force sustaining the altered growth characteristics of cancerous lesions together with a diet high in n-6 PUFA playing an underlying stimulating role. Therefore modulation by dietary PUFA and alteration in membrane cholesterol, phospholipid and

PUFA metabolism resulting in a shift in C$_{20}$:4n-6 phospholipid distribution and cellular oxidative status has implications towards cancer cell survival.

The type of FA to affect these alterations in cell survival is influenced by factors such as competitive incorporation into cell membranes, desaturase enzyme competition, eicosanoid metabolite synthesis and conversion efficiency from C$_{18}$ to C$_{20}$ and C$_{22}$ carbon PUFA. The conversion from C$_{18}$:3n-3 (α-linolenic acid) to C$_{20}$:5n-3 has been demonstrated to range between 8 and 20 %, while conversion from C$_{18}$:3n-3 to C$_{22}$:6n-3 is even lower, i.e. 0·5–9 %[49–51]. The interaction between certain PUFA types and families, such as C$_{20}$:5n-3 and C$_{20}$:3n-6, can determine the effect produced on cell survival and neoplastic development.

Points to consider

However, many questions still need to be resolved with regard to which specific dietary factors are most closely linked to cancer prevention and the mechanisms by which food components exert their anti-cancer effects. A compounding effect is inter-individual variations in susceptibility arising from common polymorphisms in genes governing the metabolism of exogenous substances which can modify the carcinogenic or anti-carcinogenic effects of food components and, thus, add an extra level of difficulty to the interpretation of studies. Further complications with regard to cancer prevention are the lack of dose recommendations to date. However, a generalised guide can be deduced from guidelines for other diseases in which PUFA have demonstrated a beneficial component, such as the recommendations from the American Heart Association for

patients without CHD of 'at least two (preferable oily) fish meals per week' or approximately 500 mg EPA + DHA/d; while for patients with CHD an EPA + DHA intake of 1 g, preferably from oily fish is recommended[52].

Conclusion

The increasing number of clinical and experimental studies on the *n*-3 FA reflects the growing awareness and importance of these components in our diet. The *n*-3 FA, in a specific ratio with *n*-6 FA, is proving to be not only important for normal growth and development but also for the prevention and treatment of certain diseases. In this regard, the wide influence range that dietary PUFA may exert on cells, structurally and functionally, is important. Structurally, PUFA may exert alterations in cell behaviour by their incorporation into membranes affecting cholesterol content and receptor activity. Functionally, as substrates for signalling metabolites, PUFA may affect cellular processes such as proliferation and apoptosis by influencing cellular signalling. However, much remains to be learned about the actions of PUFA in health and disease and many questions remain unanswered, such as what constitutes an optimum range of intake for *n*-3 FA? Does this range remain the same in different background diets with different amounts of *n*-6 and *n*-3 FA? Is the optimum range a reflection of absolute requirements or optimum FA ratios? How are requirements affected by different levels and types of dietary fat? These and many other questions motivate research to improve our fundamental understanding of biochemistry, health promotion and disease prevention with regard to FA.

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