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Review article

Dietary fatty acids and human health

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Abstract — A considerable amount of evidence has accumulated to support the view that the very long chain omega 3 fatty acids (eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)) have beneficial cardiovascular and anti-inflammatory properties and that levels of their consumption are insufficient in most Western diets. More recently, attention has been given to the possibility that the precursor omega-3 PUFA, alpha linolenic acid (ALNA), may share some of the beneficial actions of EPA/DHA on human health. Further research into the metabolism and physiological actions of ALNA, and comparisons with EPA/DHA, is needed before conclusions regarding the optimal amounts and types of omega-3 PUFA for human health can be defined. Conjugated linoleic acid (CLA), which arises as a metabolic by-product of rumen hydrogenation and which is found in foods of animal origin, has been proposed to possess potent health promoting properties, but much of this research has been conducted in experimental animals. There is an urgent need for complementary studies in human volunteers, to confirm the putative anti-carcinogenic, anti-atherogenic, anti-lipogenic and immuno-suppressive properties of CLA.

human health / fatty acids / omega-3 PUFA / conjugated linoleic acid

Résumé — **Acides gras alimentaires et santé humaine.** Il a été amplement démontré que les acides gras à très longue chaîne en oméga 3, les acides eicoapentaenoïque (EPA) et decosahexaenoïque (DHA) ont des effets positifs relatifs à l'apparition des maladies cardio-vasculaires, et des propriété anti-inflammatoires. Leur consommation par l'Homme est insuffisante dans la plupart des pays occidentaux. Récemment il est apparu que l'acide alpha linolénique, précurseur des acides gras en oméga 3, pouvait partager avec l'EPA et le DHA certains effets positifs sur la santé humaine. Des recherches complémentaires sur le métabolisme et le rôle physiologique de l'acide alpha linolénique, ainsi que des comparaisons avec l'EPA et le DHA sont nécessaires avant de pouvoir définir les apports recommandés optimaux de chacun de ces acides gras. L'acide linoléique conjugué (CLA) qui provient du métabolisme ruminal des acides gras et qui est présent dans les aliments d'origine animale, a été décrit comme potentiellement favorable à la santé humaine, mais la plupart des expérimentations ont été conduites sur animaux de laboratoire. Il y a une urgence à réaliser des études complémentaires sur des volontaires humains, afin de confirmer les propriétés annoncées du CLA, à savoir des effets anti-carcinogène, anti-athérogène, anti-lipogénique et une modulation de l'immunité.

santé humaine / acides gras / oméga-3 PUFA / acide linoléique conjugué

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1. INTRODUCTION

The epidemic of obesity in most highly developed countries has led to an understandable public health emphasis on low fat, low energy diets and has provided a major stimulus for development of low fat products and fat substitutes. However, recent research suggests that there is a need to consider the quality as well as the quantity of fat in diets of Western populations. Issues of fat quality have previously concentrated on potentially adverse effects of saturated and *trans* unsaturated fatty acids on circulating cholesterol concentrations and these remain important aspects of public health nutrition which will be briefly considered as part of this review. However, there is now much evidence to suggest that specific fatty acids have beneficial effects on human health which could contribute to prevention of many chronic diseases of humans.

2. SATURATED FATTY ACIDS

In most developed countries, dietary guidelines produced since the early 1980s have proposed reductions in total fat and in saturated fatty acid intake as a means of reducing the prevalence of coronary heart disease (CHD). National and international dietary guidelines have recommended that saturated fatty acids should contribute no more than 10% dietary energy [21, 22, 34]. These recommendations have been based on epidemiological studies and numerous controlled intervention studies in volunteers of varying age, sex and genotype which have demonstrated that saturated fatty acids increase blood cholesterol concentrations in a predictable and dose related fashion [44, 60, 79]. Early metabolic studies implicated all saturated fatty acids equally in the elevated cholesterol responses to animal based fats. However, it is now clear that it is mainly lauric, myristic and palmitic fatty acids which are responsible for increasing plasma total and LDL cholesterol concentrations, whilst the other major SFA, stearic acid, has been shown not to increase total cholesterol or LDL-cholesterol concentrations [9]. Difficulties in obtaining clear cut findings for effects of specific fatty acids in many of the earlier studies, reflect limitations in the types of fats and oils which could be used in cooking and food manufacture. This meant that it was rarely possible to bring about changes only in those fatty acid(s) under investigation, thereby confusing the interpretation of the data. However improvements in technology, together with availability of designer fats and oils produced using inter-esterfication, have enabled the production of experimental fats which are acceptable for cooking and spreading and which have rigorously defined fatty acid profiles (e.g. [114]). The high cost of many of the studies, and the demands on the subjects, have in most cases, restricted individual studies to relatively modest numbers. However, most expert bodies and major review articles now apply meta analysis to produce large data sets (e.g. [117]). These advances have enabled a clearer consensus to be reached with respect to the LDL cholesterol raising properties of lauric, myristic and palmitic acids.

3. MONOUNSATURATED FATTY ACIDS

Although there is a clear consensus regarding the adverse effects of the major saturated fatty acids on blood cholesterol levels, attempts to reduce levels of consumption of saturated fats within the general population has been relatively unsuccessful because of resistance to low fat diets. Recently there has also been renewed debate as to the best means of achieving low dietary SFA intakes – whether by the use of low-fat, high-carbohydrate diets, or by displacing SFA with polyunsaturated (PUFA) or monounsaturated fatty acids (MUFA). Although the cholesterol-lowering response to PUFA is greater than that to MUFA, there

has been caution in recommending high PUFA diets, because of potentially adverse health effects of their lipoperoxidation products. The potential for reducing SFA through substitution with MUFA has increased in recent years, because improvements in animal feeding technology has made it possible to alter the fatty acid composition of milk and meat fat by altering the fatty acid composition of animal diets. Dairy products produced using this approach have been shown to successfully reduce blood cholesterol levels in human volunteers [90]. Substitution of high MUFA fats for SFA-containing fats in manufactured foods and margarines has also been shown to have beneficial effects on blood cholesterol and other health related outcomes [114]. These data demonstrate the feasibility of reducing population cholesterol levels through strategies involving alteration of fat quality within the agricultural and food manufacturing chains.

Recent studies which show that low fat diets can raise fasting and postprandial triglycerides and reduce the levels of protective HDL cholesterol [77, 101], have renewed interest in the possibility of altering fat quality as a cholesterol-lowering strategy within the population. The increasing strength of evidence showing adverse consequences of raised triglycerides and low HDL in CHD risk, will result in even greater interest in the possibilities for altering the fatty acid composition, as well as fat content, of animal products.

4. *TRANS* FATTY ACIDS

The issue of *trans* fatty acids (TFA) and human health came to prominence in the early 1990s because of epidemiological and experimental studies in humans which supported the possibility of increased risk of CHD in subjects consuming high TFA diets. Because spreads and products manufactured from them provide the major source of TFA in diets of Western populations [32], this issue became highly contentious and ultimately centred around the relative pros and cons of SFA (found in butter), and TFA (found in spreads and margarines). Although manufacturers have since removed much of the TFA from high quality fats, the issue remains contentious and has also raised questions as to possible differences in TFA derived from ruminal metabolism, as compared with those formed from industrial hydrogenation.

4.1. Dietary sources of TFA

TFA are found in ruminant fats (dairy products, beef, lamb) as a result of bacterial action in the rumen, and in shortening and spreads, due to their production during industrial hydrogenation of oils. The distribution of *trans* positional isomers differs somewhat in fats originating from industrial hydrogenation, where a mixture of isomers is found whereas ruminant fermentation results in a predominance of vaccenic acid 18:1 c9,t11 [32]. Although previous studies in the US have suggested intakes of *trans* fatty acids as high as 7–8% dietary energy, more recent data for European intakes suggests much lower figures than this, ranging from the lowest intake in Italy (0.5% total energy) to highest intake in Iceland (2.0% total energy). In the UK, fats, oils and biscuits make the greatest contribution to total *trans* intakes, with dairy and meat products representing the other major contributor [13, 48]. The lower levels of intake reported in recent surveys illustrate the progress that has been made in the reducing the TFA content of margarines and reflects technological changes in margarine manufacture.

Evidence suggesting potentially adverse effects of TFA on risk of CHD arises from two sources; epidemiological studies which have related dietary intake or tissue levels of *trans* fatty acids to subsequent risk of CHD and controlled intervention studies which have evaluated effects on circulating lipoprotein concentrations.

4.2. *Trans* fatty acids and plasma lipoproteins

Controlled intervention studies have demonstrated adverse effects of TFA on plasma total and LDL cholesterol concentrations [78]. There is also some evidence to suggest that TFA have adverse effects on HDL cholesterol concentrations and may increase levels of the potentially atherogenic Lp(a) [82, 86]. Many of the early studies which investigated effects of high TFA intakes used diets in which TFA were fed at extremely high levels and used fat sources which resulted in differences in dietary fatty acids other than those of the *cis* and *trans* MUFA. Levels of TFA studied were much higher than those habitually consumed in Western diets and varied from 10–18% dietary energy [81]. These early studies produced conflicting findings with some showing large increases in LDL, others showing more moderate changes and others no effect on any lipoproteins. However, a recent reanalysis of these data showed a significant and adverse effect of TFA on LDL cholesterol levels, with each 1% increase in dietary energy from TFA leading to a 0.028 $mmol \cdot l^{-1}$ increase in LDL [81]. This conclusion shows a high degree of concordance with findings from recent controlled dietary intervention studies that have used more modest levels of TFA. The work of Katan and co-workers, which has compared effects of TFA with SFA or oleic acid [79], and stearic acid or linoleic acid [118], has been particularly important in clarifying the health consequences of high TFA intakes. The studies confirmed the LDL cholesterol-raising effects of TFA in comparison to oleic or linoleic acid, and suggested that TFA were intermediate between SFA and MUFA in terms of their LDL cholesterol-raising actions [80]. These authors were the first to report adverse effects of TFA on HDL concentrations, which has since proved to be the feature that distinguishes effects of SFA and TFA on lipoprotein concentrations. Both classes of fatty acids raise LDL cholesterol; only TFA also reduce HDL cholesterol. When high TFA diets were compared with high SFA or high MUFA diets, HDL levels were reduced by 0.17 $mmol \cdot l^{-1}$ [81] and similar findings were subsequently reported by Judd et al. [56] and by Lichtenstein et al. [69] and Nestel and co-workers [86]. These latter studies employed modest levels of TFA and fed hydrogenated sources of MUFA rather than the more unusual isomerised source used by Katan's group. The relevance of these findings to free living populations was illustrated by a recent randomised controlled trial which fed varying amounts of TFA using soyabean oil, four types of spreads or butter, each for a period of 5 weeks. LDL cholesterol levels were lowest on the soya-based spreads, moderate on the high TFA spreads and highest on butter. However, compared with butter, the high TFA spread resulted in a potentially adverse increase in the total cholesterol to HDL cholesterol ratio [70]. This response reflected a significant decrease (6%) in HDL concentrations when subjects consumed the high TFA spread than when they consumed the butter.

4.3. Epidemiological studies

There are a number of retrospective and prospective epidemiological studies of dietary TFA and risk of CHD which generally support adverse consequences of high intakes of TFA. The Nurses Health Study evaluated the diets of 85,00 women in 1980, with follow up for new cases of CHD 8 years later. An increased risk of CHD was observed at the highest TFA intakes and this association was not substantially altered following adjustments for known risk factors and potential confounders such as dietary SFA intake [113]. Of particular interest was a sub-analysis in a group of women who had reported no change in their margarine intakes between 1970–1980. In this group, an increased risk of CHD was found for *trans* isomers from vegetable fats but not for those from animal fats. Specific foods

associated with greater risk of CHD included biscuits and cakes, but not dairy products or meat. Other prospective studies which have evaluated dietary TFA have also shown modest positive associations between TFA intakes and risk of CHD [4, 5, 94]. However when the findings of Ascherio et al. [5] were adjusted for possible effects other dietary confounders, the adverse effects of TFA on CHD risk were no longer seen.

A substantial amount of information has been provide by comparison of adipose tissue, plasma and platelet fatty acid compositions from subjects who have recently died or been diagnosed with CHD [3, 46, 96, 103, 108–110]. In only one of these studies has an association between the major dietary *trans* (18:1 t9 or t11), been observed [46]. In one study there were lower levels of 18:1 *trans* in cases compared with controls [96]). In three studies a positive association was observed between adipose tissue 16:1 *trans* composition [103, 108, 109]. Since animal products have been shown to provide the major amount of 16:1, it was concluded that this fatty acid may be acting as a marker of adverse effects of animal product and SFA intakes.

Overall there is modest evidence from experimental studies in humans and epidemiological dietary data, to suggest that elevated levels of TFA intake may increase risk of CHD. However the data suggest these effects may only be apparent at the highest levels of TFA intake. Findings from the Nurses Health Study [113], and from the other studies which have used the same dietary data base [4, 5] are controversial. There has been much criticism of the methodology used to derive the dietary TFA data and also because the association found for manufactured foods (biscuits, cakes), could reflect an association with a range of adverse dietary practices rather than a specific effect of TFA. Failure to observe associations between biomarkers of TFA (specifically 18:1, 9t or 11t) intake and CHD [2], tends to support the latter possibility.

5. OMEGA-3 POLYUNSATURATED FATTY ACIDS

In recent years there has been considerable interest in the beneficial physiological effects of the long chain (LC) omega-3 polyunsaturated fatty acids (PUFA), EPA and DHA. These fatty acids are present in the diets of most developed countries in very small amounts due to low consumption of fish and its products. It has been suggested that the typical Western diet, may not supply the appropriate balance of omega-6 and omega-3 PUFA [104] and this imbalance could contribute to a greater risk of CHD via a more pro-thrombotic and pro-inflammatory state. Other chronic disorders which have been suggested to be linked with lack of omega-3 PUFA include hypertension, inflammatory and immune disorders, depression and neurological dysfunction. The recognition of the importance of DHA in neuronal development in the foetus and the new-born has also highlighted the vital role of this class of fatty acids in infant as well as adult nutrition [97].

5.1. Pathways of omega-6 and omega-3 metabolism

Linoleic acid (LA) is the precursor fatty acid of the bioactive omega-6 PUFA, arachidonic acid; alpha linolenic acid (ALNA) is the precursor of the bioactive omega-3 PUFAs, eicosapentaenoic and docosahexaenoic acids. Both precursors are converted to their long chain metabolites by a series of desaturation and elongation steps and share common enzymes for these metabolic transformations (Fig. 1). Conversion of ALNA to EPA and DHA is low in humans and may be further suppressed due to inhibition of the delta-6-desaturase enzyme, by high intakes of linoleic acid. AA and EPA/DHA are the substrates for the formation of two families of eicosanoids. The eicosanoids formed from AA have generally greater potency than those formed from EPA, and as a consequence, their actions

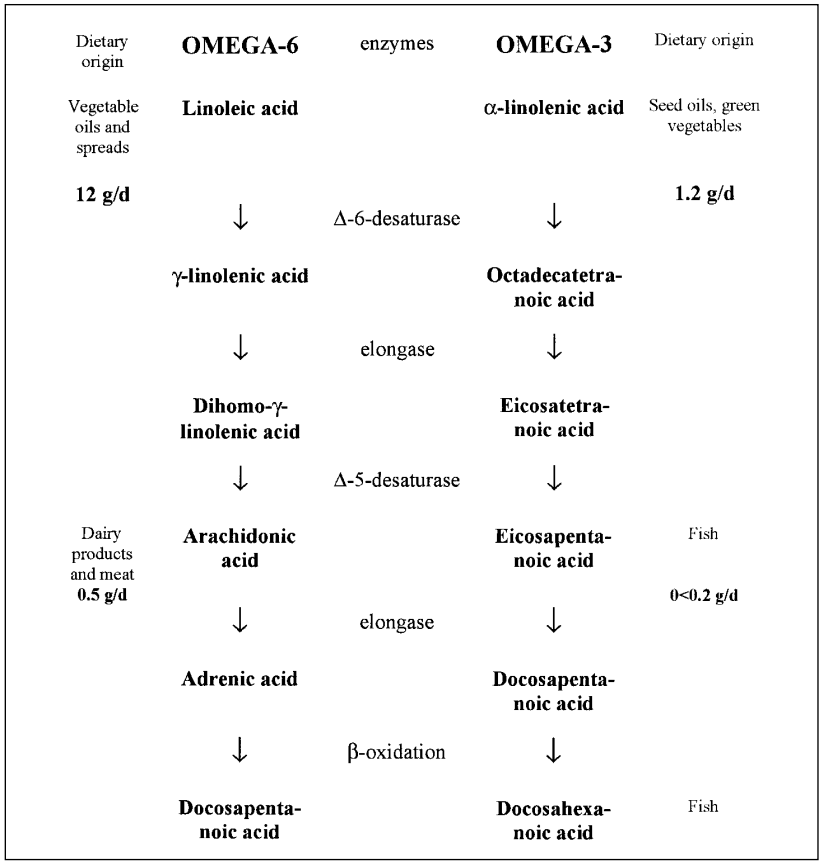

Figure 1. Pathways of omega-6 and omega-3 metabolism showing chain elongation and desaturation steps which share common enzyme systems. Major sources of precursor and long chain omega-6 and omega-3 PUFAs in the diet are indicated.

on vascular, immune and inflammatory systems differ markedly. The ability of EPA/DHA to displace AA from cell membranes when fed at increasing levels in the diet may therefore result in a shift towards the less potent eicosanoids from the omega 3 family. However it is also likely that cellular processes other than those involving altered eicosanoid status, e.g. gene expression of regulatory proteins, are also influenced differently according to the type of PUFA present within the cell (or within the phospholipid components of cell membranes). Recent studies have identified a family of receptors, the peroxisome proliferator activating receptors (PPARS), which act as nuclear transcription factors. Since PUFAs and their metabolites appear to act as ligands for this receptor family, PPARS may provide the cellular locus through which fatty acids can regulate the gene expression of key proteins of lipid metabolism.

5.2. Eicosapentaenoic and docosahexaenoic acids

Evidence for anti-atherogenic, anti-thrombotic, anti-inflammatory and immuno-

suppressive actions of long chain omega-3 PUFA from fish and fish oils are available from a range of epidemiological and experimental studies in man [10, 25, 64]. That these findings reflect the specific biochemical properties of EPA/DHA, is supported by experimental studies which demonstrate beneficial effects of EPA and DHA (usually provided in the form of a capsule supplement), on platelet aggregation and blood clotting, fasting and postprandial triglyceride levels and on immune function and inflammatory response [10, 26]. In some studies, beneficial effects of supplemental EPA and DHA intake on blood pressure have been reported [35] but these findings are variable [10, 66]. In animal studies, EPA and DHA have been shown to reduce the susceptibility to ventricular fibrillation [17]. Epidemiological data supports potent cardioprotective actions of EPA/DHA [107]. Intervention studies in populations at risk of CHD support the conclusion that low levels of daily EPA/DHA are sufficient to protect against CHD [12, 37, 106], and there is some data to support the view that this reflects anti-arrythmic effects of dietary omega-3 PUFA [20, 106]. The strength of the data has led to a widespread consensus that there should be modest increases in omega-3 PUFA intake (either as ALNA and/or EPA/DHA) and this has provided the basis for the formal recommendations made by a number of expert bodies [11, 22, 26, 34].

5.3 Approaches to increasing omega-3 PUFA intake

In general, effects of EPA/DHA on surrogate markers of cardiovascular function, including platelet aggregation, blood clotting time, and in vitro immune and inflammatory response, are observed at levels of intake of approximately 1 g per day. This is approximately five times the current level of intake seen in many northern European and North American diets [36]. These data illustrate that only Spain and Portugal are achieving levels of intakes found to be beneficial in the short-term experimental studies described above.

Although it is feasible to achieve levels of omega-3 PUFA intake by modest increases in fish consumption, many people find oily fish unpalatable and a large proportion of the UK population do not eat fish [38]. Supplemental oils and capsules provide an alternative approach but this can be expensive and may not be suitable for all age groups. Recent innovations in the food industry have provided an alternative approach through the availability of highly refined fish oils which can be used in fats and spreads [76] and microencapsulated omega-3, a dry product which can be incorporated into a wide range of different foods particularly bakery products and milk powders [45, 63, 88]. A recent study which employed an LC omega-3 spread together with a range of products enriched with microencapsulated fish oils (milk shake, orange drink, pasta, bread, biscuits, cakes) was able to achieve a daily intake of 1.4 g EPA/DHA [72]. However considerable progress has been made in enriching the omega-3 PUFA content of animal products such as eggs [43] and milk [6, 41] and of the carcass meat of both non-ruminant [33] and ruminant animals [30, 115], through diet modification. This offers a particularly attractive approach to increasing dietary intakes of omega-3 PUFA, since fatty acids provided through this route are thought to be more stable to the effects of processing than are fats and oils added to foods in manufacturing.

6. ALPHA LINOLENIC ACID

6.1. Metabolic effects of alpha linolenic acid

Consideration of the optimal balance of omega-6 and omega-3 PUFA has led to greater interest in the potential health benefits of ALNA itself and the advantages of increasing omega-3 PUFA via this route

rather than direct addition of the LC omega-3 PUFA. Previous disinterest in the potential health effects of dietary ALNA has been due to data suggesting that the level of conversion of ALNA to EPA/DHA was too low to be of any biological significance [16]. However, contrary to early reports there is now clear evidence that dietary ALNA can increase tissue levels of EPA [24, 73, 99] and that, in some studies, this is associated with altered platelet aggregation and bleeding tendency [1, 50, 95, 112]. There is strong evidence from animal studies, with limited though supportive data from human studies, to suggest that ALNA can act as a powerful modulator of eicosanoid metabolism [23, 49, 75]. However it is important to note that ALNA and the long chain omega-3 PUFA have different effects on both membrane fatty acid compositions and on levels of eicosanoid precursors. ALNA supplementation can significantly increase the level of ALNA and EPA in plasma and cells [1, 15, 65, 68, 111, 112, 116] but ALNA supplementation does not increase (and may even decrease) DHA and in some studies does not reduce AA levels [74, 99]. One study has demonstrated that ALNA and long-chain omega-3 PUFA supplementation have equivalent effects on haemostatic factors [31], however other studies have failed to observe significant effects of dietary ALNA on haemostatic variables [61, 68]. In addition, unlike EPA and DHA, ALNA supplementation has no effect on plasma TAG concentrations [31, 100] except at very high levels of dietary intake. Nevertheless there is some data to support modulatory effects of ALNA on eicosanoids, since ALNA has been shown to reduce blood pressure in hypertensives [105] and some data is available to suggest that ALNA can inhibit lymphocyte proliferation in animals [14] and in healthy adults [57, 58] and more recently has been shown to suppress the production of TNF and IL-1 in subjects fed ALNA enriched diets [15]. The work of Nestel and co-workers is also of interest since they have shown marked improvements in in vivo measures of arterial compliance in subjects receiving ALNA enriched diets [87].

Despite equivocal data with respect to beneficial effects of ALNA on markers of human health, interest in the health benefits of dietary ALNA has been sustained by findings from a secondary intervention study which showed markedly reduced mortality from cardiac events in subjects who were following a healthy 'Mediterranean' diet which included 2 g per day of ALNA in the form of a canola oil-based margarine. The authors attributed their remarkable findings to the anti-thrombotic or anti-arrythmic actions of ALNA [27]. Whilst it remains unclear whether marked benefits of this diet were indeed attributable to effects of ALNA, the data show concordance with those of Burr and co-workers who showed significant reductions in rates of secondary CHD in subjects consuming very modest amounts of EPA/DHA [12]. Evidence of cardioprotective effects of dietary ALNA is also provided by findings from a recent large prospective study in women in the USA [47].

Caution is needed with respect to excessive enthusiasm for the putative protective effects of ALNA because studies to date are either insufficient, inconclusive or observe effects only at very high levels of intake. Further long-term studies are required, using moderate doses of ALNA, before firm conclusions can be drawn with respect to the comparable effects of ALNA and EPA/DHA on markers of human health and disease outcomes.

6.2. Approaches to increasing ALNA intakes

Flaxseed oil provides the richest potential source of ALNA (55% total fatty acids) for use in foods, although rapeseed (canola) oil is also relatively high in ALNA (15% total fatty acids). Although ALNA shows a lesser susceptibility to undergo oxidation than do

the LC omega-3 PUFA, oxidation is nevertheless an important issue particularly in the processing of flaxseed oil which contains very large amounts of the PUFA. Microencapsulation is also being used to expand the range of products, which may be manufactured, using flaxseed [91]. The use of rapeseed in food processing has increased dramatically in recent years, since this oil has provided a cheap and stable form of fat for use in spread and food manufacture, and is widely used in the large scale production of foods for the catering industry. The feeding of rape or linseed oils to ruminant and non-ruminant animals has been shown to enhance tissue levels of ALNA, and in some studies, enhanced levels of EPA have been demonstrated [15, 42, 54, 59, 84], thereby illustrating the potential use of animal product manipulation as a means of increasing dietary levels of the bioactive forms of omega- PUFA.

7. CONJUGATED LINOLEIC ACID

Conjugated linoleic acid is a collective term for a mixture of geometric and positional isomers of octadecanoic acid (18:2) in which double bonds are conjugated rather than methylene separated as they are in linoleic acid (18:2,c9,c12). The CLA found in dairy products, which represents the major source in the human diet, is present primarily as 18:2,c9,t11. Conjugated fatty acids are formed as partial hydrogenation products during ruminal hydrogention, by the action of specific bacterial isomerases, and industrial hydrogenation by the action of hydrogen in the presence of a catalyst. The latter reaction is essentially similar to autoxidation-mediated conjugated reactions where free radical reactions lead to shifting and recombination of double bonds adjacent to the site of free radical attack. Although 18:2,c9,t11 is the major CLA formed in the rumen from linoleic acid in the diet, other CLAs can be produced when ALNA or TFA are present in the animal diet [98]. CLA synthesis by ruminants can be disproportionate to dietary precursors suggesting that unknown rumen or metabolic factors influence synthesis [28]. Recent studies have shown that the intestinal bacteria of rodents are capable of converting unsaturated fatty acids to conjugated linoleic acids [19], although it is not known whether this capacity is also present in human gut microflora.

7.1. Sources in foods

The major sources of CLA in the human diet are meat and dairy products, particularly cheese. Unlike the situation with TFA, margarines and oils make only a very slight contribution to total CLA intakes [32]. In dairy products the amount of CLA present varies according to the breed, feeding conditions and subsequent processing. In milk fat the CLA content has been shown to vary from 0.24 to 2.81% from winter to summer, reflecting the effect of pasture feeding on linoleic acid intake and subsequent CLA accumulation in the rumen [18]. In theory cheese production offers a range of processing conditions (temperature, whey protein, ripening time) which may influence the final CLA content since some result in enhanced production and others increased breakdown of CLA. However studies suggest the major determinant of the CLA content of various natural cheeses is the CLA content of the raw materials. Similarly, although the processing conditions and cooking conditions used for meat should be expected to influence the final product, only high temperature grilling was found to markedly influence the CLA content of beef. Overall it seems that feeding, rather than processing, conditions, determine the CLA content of foods [32].

7.2. Dietary intake levels

The ability to quantify the CLA content of the human diet has previously been limited by lack of accurate data on the CLA

content of major food sources. Recent data on the CLA content of animal food products produced under varying conditions have become available allowing estimates of human intakes to be made [18]. Whilst earlier studies have suggested intakes of CLA to be in the region of 0.5–1.5 $g \cdot d^{-1}$ [32], recent studies suggest much lower intakes than this. A Swedish study which measured intakes using a 7 day weighed intakes combined with 7 repeat 24 hour recall interviews conducted over 7 consecutive months, showed intakes in the region of 0.16 $g \cdot d^{-1}$ [55]. This study also measured adipose tissue CLA content, which was shown to significantly correlate with both total dietary CLA content and milk intake. Of interest was the comparison of adipose tissue fatty acid content with previous reports made in the early 1960s. Although data was not available for adipose tissue CLA content in the 1960s, values for 14:1 and 16:1 were available and these enable comparison with current tissue levels. These data showed amounts of 14:1 and 16:1 to be twice as high in the 1960s than today. Since these authors have shown a strong correlation between the CLA and 14:1 and 16:1 contents of human adipose, they concluded that this would indicate a marked reduction in CLA intake in the past 30 years consistent with the reduced consumption of total animal fat.

7.3. Potential health benefits of conjugated linoleic acid

Interest in the levels of CLA in the human diet have increased in recent years because of accumulating evidence, largely based on animal studies, which suggest potential health benefits of CLA [7, 52]. Anti-carcinogenic properties of CLA have been reported against rodent mammary and colon cancer models, as well as in vitro models of human melanoma, colorectal and breast cancer. Potentially beneficial actions on body composition and on immune function have also been reported. Less consistent have been reports of putative hypocholesterolaemic, anti-atherogenic actions of CLA.

7.4. Anti-carcinogenic actions of CLA

Early work showed potent anti-carcinogenic actions of CLA using the mouse skin cancer and fore-stomach models [40, 52] which showed that CLA given prior to administration of DMBA (skin) or benzopyrene (fore-stomach) reduced subsequent tumour development by 50%. More recent work has concentrated on effects of CLA against mammary tumour development in the rat which confirmed a dose dependent reduction in tumour yield when CLA was given prior to carcinogen treatment [51]. A dosage equivalent to 0.04 $g \cdot kg^{-1}$ body weight was shown to reduce tumour incidence by 36%. The observation that only the 18:2c9,t11 isomer was incorporated into epithelial cell phospholipids, despite the feeding of a mixed profile of CLAs, suggest a highly specific effect of this isomer [53]. The data obtained from the rat mammary model have been strengthened by similar observations using the colon cancer rodent model [71] and by studies which show direct inhibitory action on MCF-7 human breast cancer cells in culture [29, 102]. The mechanism by which CLA inhibits tumour development in a range of animal and in vitro models is not yet understood, although perturbation of the eicosanoid-dependent cell signalling systems, antioxidant effects, and disturbance of the receptor mediated actions of oestrogen have all been suggested. The recent demonstration that CLAs act as agonists for PPARS a family of receptors which directly alter nuclear transcription for regulatory proteins involved in lipid metabolism [8], offers another line of investigation. Although the inhibitory effects of CLA against carcinogens has been suggested as the most important property of CLA, recent studies have shown that CLA given during weaning can reduce subsequent tumour development, both

following carcinogen administration and in the absence of carcinogen [53]. This effect appears to be due to inhibition of proliferative activity of epithelial cells during the sensitive stage of maturation of the mammary gland, suggesting that exposure to CLA during early development may reduce subsequent risk of mammary tumour development.

Whilst animal and cell culture studies support the possibility of remarkable anticarcinogenic actions of CLA, whether such effects are present in humans and whether variations in human dietary intakes of CLA (and dairy products) can explain variations in susceptibility to cancer at different sites remains to be determined. The data are consistent with reports of protective effects of dairy products against human breast cancer, obtained from a recent prospective study [62], although the number of cases (n = 88) involved were limited. The dose levels shown to produce 36% inhibition in animal models (0.04 $g \cdot kg^{-1}$) would equate with human intakes in the region of 3 $g \cdot d^{-1}$ (Tab. I). Although earlier estimates supported the possibility of intakes in the region of 0.5–1.5 $g \cdot g^{-1}$, more recent estimates provide much lower figures than this. However it should be noted that intakes of CLA have almost certainly fallen since the early 1960s as a consequence of reduced animal product consumption. Should the beneficial effects of CLA on health be proven, then this decline in intakes may become a matter of concern and one that might be addressed through attempts to increase levels of CLA in milk, dairy products and carcass meats.

7.5. Effects on body composition

The other action of CLA which has promoted much interest are recent reports of modulatory effects of these fatty acids on body weight and body fat accumulation [19]. Studies in rats and mice have shown that feeding CLA at levels in the region of 0.5% total diet produced small reductions in body weight gain, but marked reductions in body fat gain in growing animals [92, 93]. Similar effects on milk fat content have been observed when CLA is fed to cattle [39]. With respect to effects on body fat, the authors suggested these effects may be attributable to inhibitory actions of CLA on adipose tissue lipoprotein lipase activity, with concomitant stimulation of hormone sensitive lipase and carnitine palmitoyl transferase. The consequence of these would be stimulation of fatty acid β oxidation, inhibition of adipose tissue TG accumulation and stimulation of adipose tissue TG breakdown, thereby contributing to a resistance to body fat accumulation. Other reported actions of CLA in modulating the catabolic effects of immune stimulation [83], appear to be consistent with the suggestion that CLAs share many of the actions of omega-3 PUFA and support modulation of eicosanoid metabolism as a mechanisms for explaining at least some of the effects of this group of fatty acids. Whilst the actions of CLA in inhibiting body fat accumulation have received considerable attention arising from increasing concern for marked increases in obesity in western societies, care should be taken in extrapolating these

Table I. Human dietary equivalents of conjugated linoleic acid levels shown to produce protective effects in animals.

Action	Active dose level in animals	Equivalent diet level in humans
Anti-carcinogen	0.04 $g \cdot kg^{-1}$ body weight	3.0–3.5 $g \cdot d^{-1}$
Anti-lipogenic	0.05% diet	15–20 $g \cdot d^{-1}$
Anti-atherogenic (early lesions)	5 $g \cdot kg^{-1}$	400 $g \cdot d^{-1}$

findings to man until more information is available. Effects observed on body composition and on immune response, have been reported at dietary intake levels of 0.5% total diet. This equates with dietary intakes in man in the region of 15–20 g·d^{-1}, considerably higher than the human intakes that have been reported in recent studies.

7.6. Anti-atherogenic effects

Although anti-atherogenic properties of CLA are frequently quoted to support the view of beneficial health properties of this fatty acid, there is remarkably little information in the literature to support this view. Two studies, one in rabbits and one in hamsters, have shown that CLA fed at levels of 5 g·kg^{-1} (rabbits [67]), and at varying levels (0.06, 0.11 and 1.1%) in hamsters [89], protects against arterial accumulation of lipids. No dose response was observed in the hamster study and only early atherosclerotic lesions were studied in both cases. A more recent study in mice, fed an atherogenic diet with addition of either 2.5 or 5.0 g CLA·kg^{-1}, suggested greater lipid accumulation in CLA fed animals [85]. At the present time these data make it difficult to conclude anything with respect to possible effects of CLA against atherogenesis in humans.

In general terms whilst there is good evidence for anti-carcinogenic actions of CLA from animal and cell culture studies, other reported health benefits of CLA are less well substantiated. A major difficulty lies in extrapolating levels of intakes in animal studies with levels of exposure reported in human diets (Tab. I). The certainty of data from human diet studies and variations that exist across different populations is not yet known. Clearly human studies are required including epidemiological studies that can evaluate disease incidence in relation to variations in dietary exposure. However such studies will be fraught with difficulty, especially in relation to the anti-atherogenic properties of CLA, where protective effects of CLA may be difficult to identify against the potentially confounding effects of other fatty acids of animal origin which have opposite effects. Until further experimental studies are conducted in humans to support the interesting data emerging from animal studies it would be premature to consider significant enrichment of human diets with CLA, either through manufacturing, or animal product manipulation.

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