

# Conjugated linoleic acid: a novel therapeutic nutrient?

Helen M. Roche<sup>1\*</sup>, Enda Noone, Anne Nugent and Michael J. Gibney

*Unit of Nutrition, Dept of Clinical Medicine, Trinity Health Sciences Centre,  
St James's Hospital, Dublin 8, Ireland*

Conjugated linoleic acid (CLA) refers to a group of fatty acid isomers of linoleic acid. Recent research shows that CLA affects body composition, lipoprotein metabolism, inflammation and carcinogenesis. Therefore, CLA may have potential as a therapeutic nutrient with respect to many common diseases, including obesity, atherosclerosis, chronic inflammatory diseases and cancer. Animal studies show that CLA is a potent anti-adipogenic nutrient, reducing adipose tissue mass and increasing lean mass. However, the effect of CLA on body composition in human subjects has been less spectacular. Several studies have demonstrated that CLA significantly improves plasma cholesterol and triacylglycerol metabolism in a number of animal models. These studies also showed that CLA inhibits the progression and pathogenesis of atherosclerosis. Whilst CLA has also been shown to improve triacylglycerol metabolism in human subjects, it has not been determined whether CLA affects atherogenesis. Animal models show that CLA-rich diets modulate the inflammatory response and preliminary trials with human subjects show that CLA affects the cell-mediated immune response. The molecular basis of the health effects of CLA has not been elucidated, but it is probable that CLA mediates its effect in a number of ways including altered eicosanoid or cytokine metabolism and/or by a direct effect of dietary fats on gene transcription. Most of our knowledge is based on *in vitro* and animal studies; the challenge is to define the nature and molecular basis of any health effects of CLA in human subjects.

## Conjugated linoleic acid: Coronary heart disease: Lipid metabolism

### Introduction

Conjugated linoleic acid (CLA) is a collective term referring to the positional and geometric isomers of linoleic acid. Over the last 5 years there has been a large amount of research that has demonstrated that feeding CLA affects body composition, lipoprotein metabolism,

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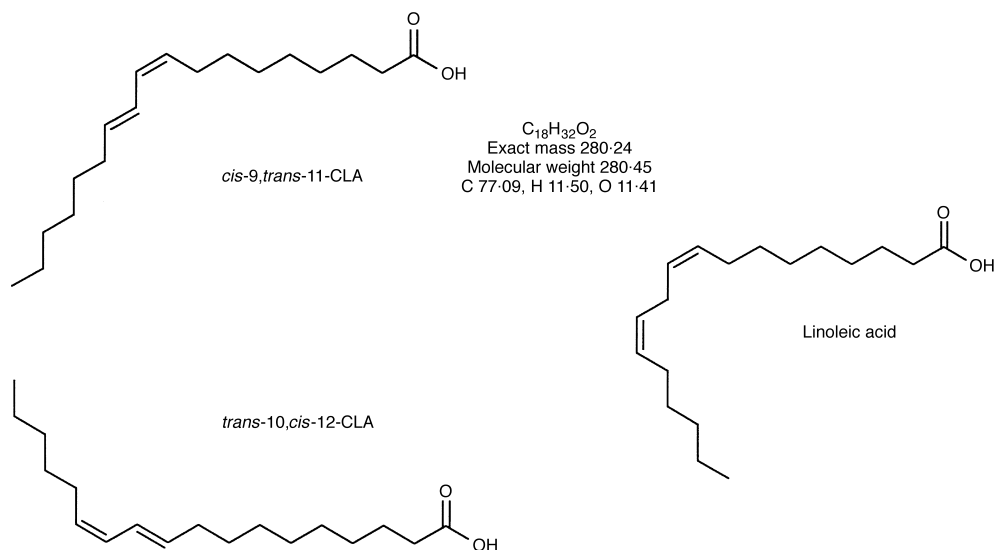
**Abbreviations:** CLA, conjugated linoleic acid; IL, interleukin; LA, linoleic acid; PG, prostaglandin; PPAR, peroxisome proliferator-activated receptors; TAG, triacylglycerol; TNF, tumour necrosis factor.

**\*Corresponding author:** Dr Helen M. Roche, fax +353 1 4542043, email hmroche@tcd.ie

inflammation and carcinogenesis. To date, most of the evidence in relation to the health effects of CLA has been derived from *in vitro* and animal studies, and the challenge is to define the health effects of CLA in human subjects. Furthermore, the majority of studies have used heterogeneous blends of CLA isomers. Recent preliminary research indicates that the individual CLA isomers have distinct cellular and physiological actions. There is a need to define the isomer-specific cellular, physiological and whole-body therapeutic potential and efficacy of the individual CLA isomers. The present review will begin by defining the chemical composition, synthesis and dietary sources of CLA. It will then review the studies that have investigated the effects of CLA on body composition, lipoprotein metabolism and inflammation. This review will not address the anti-mutagenic effects of CLA, as this has been comprehensively reviewed very recently (Kritchevsky, 2000). Whilst the molecular effects of CLA are largely unknown, this review will integrate the information from the studies which have investigated the whole-body biochemical and physiological effects of CLA, with the studies that examined the effects of CLA on gene expression. In conclusion, the review will present evidence that CLA may modulate the risk of many diseases, including obesity, atherosclerosis, chronic inflammatory diseases and cancer. Whilst most of the evidence is based on *in vitro* and animal studies, the review will attempt to define the extent to which these health effects also occur in human subjects.

### Chemical composition, synthesis and dietary sources of conjugated linoleic acid

CLA is a collective term referring to the positional and geometric conjugated dienoic isomers of linoleic acid (LA) (18:2*n*-6), which are found as minor constituents of the lipid fraction of meat, milk and dairy products (Lin *et al.* 1995). In CLA, the double bonds are conjugated or contiguous, and are not separated by a methylene group as in the case of LA. The two double bonds in CLA can be in C positions 8 and 10, 9 and 11, 10 and 12 or 11 and 13, thus giving rise to the designation of a conjugated diene. Each of the double bonds can be in the *cis* or *trans* configuration and the combination of *cis-trans* double bonds in the molecule accounts for the different geometric isomers. The *cis*-9,*trans*-11 isomer is the principal dietary form of CLA, but lower levels of the other isomers (*trans*-10,*cis*-12, *trans*-9,*trans*-11 and *trans*-10,*trans*-12 isomers) are also present in CLA food sources (Griinari *et al.* 1998). The structure of the *cis*-9,*trans*-11-CLA and *trans*-10,*cis*-12-CLA isomers are illustrated in Fig. 1. To date, most studies have used a blend of CLA isomers, which usually contain 40–45 % of both of these isomers. The *cis*-9,*trans*-11 isomer is produced in the rumen of cattle and other ruminant animals during the microbial biohydrogenation of linoleic and linolenic acids. *Butyrivibrio fibrisolvens* is a Gram-negative bacteria (Brown & Moore, 1960) that isomerises LA to produce CLA (Kepler *et al.* 1966). The *cis*-9,*trans*-11 isomer may be absorbed or biohydrogenated into vaccenic acid (*trans*-11-octadecenoic acid). After absorption, vaccenic acid can be converted into *cis*-9,*trans*-11-CLA by  $\Delta^9$  desaturase. A recent study demonstrated that endogenous synthesis of *cis*-9,*trans*-11-CLA from *trans*-11 octadecenoic acid is the primary source of CLA in milk fat of lactating cows (Griinari *et al.* 1998). The levels and isomer distribution of CLA in beef and milk can be affected by the rumen microbial population and animal feed composition, as recently reviewed elsewhere (MacDonald, 2000). Briefly, milk from cows grazing pasture have higher concentrations of CLA compared with those fed forage and grain. CLA levels in milk are subject to seasonal variation; the concentration of CLA in milk is higher during the summer and lower in spring. The frequency of lactation can also vary milk CLA levels up to 10-fold (0.16–1.60 g CLA/100 g milk fat).



**Fig. 1.** The structure of *cis*-9, *trans*-11-conjugated linoleic acid (CLA), *trans*-10,*cis*-12-CLA and linoleic acid.

*Butyrivibrio fibrisolvens* has been found in the digestive tracts of human subjects (Brown & Moore, 1960) and thus, hypothetically, CLA could be produced in man from LA. However, it has been demonstrated that feeding an LA-enriched diet (16 g/d for 6 weeks) did not increase plasma CLA concentrations in human subjects (Herbel *et al.* 1998). Therefore, man is dependent on dietary sources of CLA (Table 1) or CLA supplements. In human subjects, adipose tissue fatty acid composition is used as a bio-marker of habitual fatty acid intake. Whilst adipose tissue *cis*-9,*trans*-11-CLA levels are low (0.5 (SD 0.1) g/100 g fatty acids), they are correlated with milk-fat intake ( $r$  0.42) (Jiang *et al.* 1999). It has been demonstrated that supplementing the diet of men with cheddar cheese significantly increased (19–27 %) plasma CLA concentrations (Huang *et al.* 1994). Another study showed that a high-dairy-fat diet, rich in CLA (0.31 g/100 g fatty acids) and a *trans*-fatty acid CLA-poor diet (0.04 g/100 g fatty acids) both significantly increased serum CLA levels compared with a stearic fatty acid diet (Salminen *et al.* 1998). This latter effect is very interesting because it suggests that there may be an endogenous pathway

**Table 1.** The principal dietary sources of conjugated linoleic acid\*

Food source	Amount of CLA (g/100 g fat)
Whole milk	0.55
Low-fat milk (2 %)	0.41
Condensed milk	0.70
Butter	0.47
Plain yoghurt	0.48
Low-fat yoghurt	0.44
Frozen yoghurt	0.28
Cheddar cheese	0.41
Processed cheese	0.50
Beef	0.43
Lamb	0.58
Veal	0.27

CLA, conjugated linoleic acid.

\*Data from Chin *et al.* (1992) and Lin *et al.* (1995).

which converts dietary *trans* fatty acids into CLA in man. Indeed, Adlof *et al.* (2000) showed that 11-*trans*-octadecenoate was converted into CLA. Whilst that study was based on data from four subjects and the retrospective nature of the study may account for some denaturation of the stored lipids, it does suggest that CLA may be biosynthesised in human subjects. Maternal dairy product consumption determines the CLA intake of breast-fed infants. Park *et al.* (1999a) demonstrated that a high-fat dairy diet, which provided greater amounts of dietary CLA (291 (SD 75) mg/d), increased human milk *cis*-9,*trans*-11-CLA and total lipid concentration, compared with a low-dairy food, CLA-poor diet (15 (SD 24) mg/d). Whilst the effect of maternal milk CLA concentration on maternal and infant health is unknown, commercial baby milk formulas provide less CLA than breast milk (McGuire *et al.* 1997).

### **Conjugated linoleic acid affects adiposity and energy metabolism: evidence from animal and *in vitro* studies**

This section will present the results of studies which have shown that CLA reduces body-fat accumulation and increases lean body mass in several animal models. Typically, and unless otherwise stated, the diets used in these studies provided a blend of CLA isomers. The predominant isomers provided by these CLA sources were *cis*-9,*trans*-11-CLA and *trans*-9,*cis*-11-CLA (40 g/100 g fatty acids) and *trans*-10,*cis*-12-CLA (40 g/100 g fatty acids). To date, there is relatively little information in relation to the isomer-specific effects of CLA on body composition. The effect of CLA on adipogenesis and body composition is dose dependent. It has been demonstrated that increasing doses of dietary CLA (2.5, 5.0, 7.5, 10.0 g CLA/kg feed) reduced body weight and adipose tissue accumulation in a dose-responsive fashion, which was not associated with reduced food intake in male AKR/J mice (DeLany *et al.* 1999). Furthermore, the anti-obesity effect of CLA is independent of dietary fat content. West *et al.* (1998) showed that CLA supplementation (0.59 mg/kJ) significantly reduced adipose tissue depot weights when fed in combination with both high-fat (45 % energy) and low-fat (15 % energy) diets in male AKR/J mice. It has also been shown that increasing amounts of dietary CLA (2.5 and 5.0 g CLA/kg feed) reduces adipose tissue cell size rather than cell number in a dose-dependent manner in female Sprague–Dawley rats (Azain *et al.* 2000).

There are several mechanisms which may explain the effect of CLA on body composition. A reduction of food intake could play a role. West *et al.* (1998) showed that a CLA-rich diet caused a reduction in food intake, but the reduction was not considered substantial enough to account for the change in weight. In that study, the effect of CLA on body composition was attributable to increased metabolic rate and reduced resting energy expenditure (West *et al.* 1998). In contrast, other studies have demonstrated that a CLA-enriched diet had no significant effect on energy intake (DeLany *et al.* 1999; Azain *et al.* 2000). CLA could reduce adiposity by increasing fat oxidation. Park *et al.* (1997) demonstrated that feeding a diet supplemented with CLA (5.0 g/kg feed) to 6-week-old male ICR mice increased carnitine palmitoyltransferase activity in adipose tissue and skeletal muscle, which would increase fatty acid oxidation. *In vitro* studies show that CLA also inhibits fatty acid synthesis, adipocyte proliferation and differentiation. Stearoyl-Co A desaturase is a key enzyme involved in fatty acid synthesis. It has been demonstrated that the *trans*-10,*cis*-12-CLA decreased stearoyl-CoA desaturase mRNA expression and activity in 3T3-L1 adipocytes (Choi *et al.* 2000). Another study showed that CLA inhibited 3T3-L1 adipocyte proliferation of pre-confluent adipocytes and CLA significantly inhibited adipocyte differentiation in a dose-dependent fashion (Brodie *et al.* 1999).

Recent evidence suggests that *trans*-10,*cis*-12-CLA is the isomer which affects body composition. A CLA-rich diet (1.0 g/kg feed) that provided an isomeric blend of CLA, containing both the *trans*-10,*cis*-12-CLA and the *cis*-9,*trans*-11-CLA (the levels of which were not specified), reduced body-weight gain in male Golden Syrian hamsters (Gavino *et al.* 2000). However, supplementation with an equivalent amount of the *cis*-9,*trans*-11-CLA isomer (0.2 g/kg feed) alone had no effect on weight gain (Gavino *et al.* 2000). Park *et al.* (1999a) showed that a CLA-rich diet (2.5 g CLA/kg feed), which was predominantly *trans*-10,*cis*-12-CLA (92.8 g/100 g fatty acids), significantly reduced body weight in weanling female ICR mice, whereas the diet providing *cis*-9,*trans*-11-CLA had no effect. The latter study also showed that *trans*-10,*cis*-12-CLA significantly reduced 3T3-L1 adipocyte lipoprotein lipase activity and cellular triacylglycerol (TAG) levels in a dose-responsive manner (Park *et al.* 1999a).

Whilst the anti-adipogenic effect of CLA demonstrated in the studies cited earlier would suggest that CLA could be utilised as a nutritional intervention strategy to protect against the global obesity epidemic, a recent study has shown important adverse metabolic effects that were associated with the anti-adipogenic effects of CLA. It was demonstrated that feeding a blend of CLA (10 g CLA/kg feed; 34 g *cis*-9,*trans*-11 and *trans*-9,*cis*-11-CLA/100 g fatty acids, 36 g *trans*-10,*cis*-12-CLA/100 g fatty acids) to C57/BL6 mice (a model of diet-induced obesity) reduced white adipose tissue mass, but this was associated with marked lipodystrophy and insulin resistance (Tsuboyama-Kasaoka *et al.* 2000). The study also showed that CLA induced apoptosis of adipose tissue, an effect which was attributed to increased tumour necrosis factor (TNF)- $\alpha$  and uncoupling protein 2 mRNA expression. The pro-diabetic effect of CLA demonstrated by Tsuboyama-Kasaoka *et al.* (2000) contradicts the findings of Houseknecht *et al.* (1998). The latter study demonstrated that CLA supplementation (15 g CLA/kg feed; 42 g *cis*-9,*trans*-11 and *trans*-9,*cis*-11-CLA/100 g fatty acids, 43.5 g *trans*-10,*cis*-12-CLA/100 g fatty acids) normalised impaired glucose tolerance, prevented hyperinsulinaemia and reduced plasma free fatty acid concentrations in pre-diabetic Zucker rats. The discordance between studies may be explained by the different animal models of obesity and/or a dosage effect. A dose-response study in AKR/J male mice showed that only the highest dose of CLA (10 g CLA/kg feed) was associated with a significant increase in plasma insulin concentrations, although plasma glucose concentrations were not significantly affected (DeLany *et al.* 1999). Clearly, the data in relation to the effects of CLA are mixed and therefore a certain degree of caution should be taken when applying the potential effects of CLA on adipose tissue metabolism to man.

### **The effect of conjugated linoleic acid on energy metabolism and adiposity: evidence from studies with human subjects**

Whilst the animal studies provide some evidence that CLA could be used to protect against obesity, there is little scientific evidence that CLA affects body composition in human subjects. Our group recently completed a study to investigate the effect of CLA supplementation on plasma lipoprotein metabolism and whilst the study did not measure body composition, there was no effect of CLA supplementation (3 g/d for 8 weeks) on body weight (Noone *et al.* 2001). Zambell *et al.* (2000) demonstrated that CLA supplementation (3 g/d for 64 d) had no significant effect on body weight, fat-free mass, fat mass, energy expenditure, fat oxidation or RER in healthy adult women. Medina *et al.* (2000) investigated the effect of the same dose of CLA (3 g/d for 64 d) on leptin metabolism and appetite regulation. CLA supplementation caused a transient decrease in circulating leptin concentrations during the first 7 weeks of the study, with no change in adiposity or appetite. Leptin concentrations reverted to basal levels before the end of

the supplementation period. The authors concluded that CLA did not affect leptin or appetite in a manner that would decrease adiposity.

There are a number of factors which may explain why CLA does not affect body weight or composition in human subjects. First, it may be due to the lower CLA dose provided, in terms of g CLA/kg body weight, compared with that used in the animal studies. Second, insufficient amounts of the *trans*-10,*cis*-12-CLA isomer, which seems to be the anti-adipogenic isomer (Park *et al.* 1999b) may have been provided by the supplements in the human intervention trial. Third, the lack of effect of CLA in human subjects may be due to the absence of brown adipose tissue in adult humans. Brown adipose tissue plays a key role in adaptive thermogenesis (Klingenberg & Huang, 1999) and CLA could up-regulate brown adipose tissue energy expenditure in rodents.

### Conjugated linoleic acid and cardiovascular disease

Animal studies have shown that CLA has many positive effects on cardiovascular risk factors, reducing plasma cholesterol and TAG levels and improving insulin sensitivity. Details relating to the animal studies that determined the effect of CLA-enriched diets on lipoprotein metabolism and atherosclerosis are presented in Table 2. Lee *et al.* (1994) demonstrated that CLA significantly reduced plasma TAG and LDL-cholesterol concentrations and showed that cholesterol deposition in the aorta was 30 % less in the CLA-supplemented rabbits. Nicolosi *et al.* (1997) demonstrated in hypercholesterolaemic hamsters that CLA significantly reduced plasma and VLDL-TAG (–28 %) and cholesterol (–26 %) concentrations, which was also associated with a significant reduction of aortic streak formation (–26 %). Whilst most studies have shown that CLA is anti-atherogenic, Munday *et al.* (1999) showed that CLA promoted fatty streak formation in C57BL/6 mice fed an atherogenic diet. This occurred despite the fact that CLA treatment improved the lipoprotein profile. CLA-fed mice had significantly higher serum HDL-cholesterol:total cholesterol ratio and significantly lower serum TAG concentrations than controls. More recently, Kritchevsky *et al.* (2000) completed an intensive investigation of the dose–response effect of CLA on the progression and regression of atherosclerosis. Although CLA increased plasma lipid concentration compared with the control diet, this study provided new information whereby CLA caused substantial (–30 %) regression of established atherosclerosis. Whilst some of the data is conflicting, the balance of evidence would suggest that CLA can improve plasma lipoprotein metabolism and prevent atherosclerosis in several animal models. It remains to be determined if CLA has a protective effect against atherosclerosis in human subjects.

The data in relation to the isomer-specific hypolipidaemic effects of CLA are conflicting. *In vitro* experiments show that the *trans*-10,*cis*-12-CLA isomer significantly reduced apolipoprotein B secretion from Hep G2 cells (Yotosumoto *et al.* 1999). Since apolipoprotein B is an integral component of the TAG-rich VLDL, this would suggest that *trans*-10,*cis*-12-CLA may reduce plasma and VLDL-TAG concentrations. *In vitro* studies also show that CLA can inhibit fatty acid synthesis by down-regulating hepatic stearoyl-CoA desaturase mRNA expression (Lee *et al.* 2000) and activity (Park *et al.* 2000). The latter study showed that *trans*-10,*cis*-12-CLA, but not the *cis*-9,*trans*-11-CLA isomer, mediated this effect. Since the liver is a key organ for TAG synthesis, inhibition of stearoyl-CoA desaturase expression and activity could be of great importance with respect to fatty acid and TAG metabolism. Some of the data in relation to the isomer specific effects of CLA *in vivo* conflict and do not agree with the results generated from the *in vitro* studies. To date, two studies have investigated the isomer-specific effects of CLA *in vivo*, the details of which are presented in Table 2. De Deckere *et al.* (1999) investigated the relative

**Table 2.** The effect of conjugated linoleic acid-enriched diets on plasma lipoprotein metabolism and atherosclerosis

Reference	Animal model	Diet details	CLA (g/kg diet)	Study period (d)	TAG	TC	HDL	LDL	Atherosclerosis
Lee <i>et al.</i> (1994)	Rabbits	Fat: 140 g/kg CLA (isomer composition not stated)	5	154	↓	↓	NSD	↓	↓
Nicolosi <i>et al.</i> (1997)	Hamster	Fat: 150 g/kg (35 % energy) Low-CLA (94 % c-9,t-11, t-9, c-11, t-10,c-12)	0.25	77	↓	↓	NSD	↓	↓
		Medium-CLA (94 % c-9,t-11, t-9,c-11, t-10,c-12)	0.5	77	↓	↓	NSD	↓	↓
		High-CLA (94 % c-9,t-11, t-9, c-11, t-10,c-12)	5	77	↑	↓	NSD	↓	
		Fat: 150 g/kg Low-CLA diet (isomer composition not stated)	2.5	105	NSD	NSD	NSD	N/A	↑
Kritchevsky <i>et al.</i> (2000)	Rabbits	High-CLA diet (isomer composition not stated)	5	105	↓	NSD	↑	N/A	
		Fat: 140 g/kg (33 % energy) CLA (43 g c-9,t-11, 44 g t-10, c-12/100 g fatty acids)+2 g	0.1	90	NSD	NSD	NSD	N/A	↓
		Chol/kg diet CLA (43 g c-9,t-11, 44 g t-10, c-12/100 g fatty acids)+2 g	1	90	NSD	NSD	NSD	N/A	↓
		Chol/kg diet CLA (43 g c-9,t-11, 44 g t-10, c-12/100 g fatty acids)+0 g	1	90	↑	↑	N/A	N/A	↓
De Deckere <i>et al.</i> (1999)	Hamster	Fat: 131 g/kg (30 % energy) CLA mix (50 g c-9,t-11, 50 g t-10,c-12/100 g fatty acids)	6	56	↑	NSD	↓	↓	N/A
		CLA c-9,t-11	5.6	56	NSD	NSD	NSD	NSD	
		CLA t-10,c-12	4.9	56	↑	NSD	↓	↓	
		Fat: 14.5 g/kg CLA mix (43 g c-9,t-11, 44 g t-10,c-12/100 g fatty acids)	10	90	↓	↓	NSD	↓	N/A
Gavino <i>et al.</i> (2000)	Hamsters	CLA c-9,t-11	2	90	NSD	NSD	NSD	NSD	N/A

CLA, conjugated linoleic acid; TAG, triacylglycerol; TC, total cholesterol; ↑, ↓, variable significantly increased or reduced compared with the value for control diet; NSD, not significantly different from the control diet; c, *cis*; t, *trans*; N/A, variable not measured; Chol, cholesterol.

**Table 3.** The effect of conjugated linoleic acid-enriched diets on indices of the immune response



efficacy of diets enriched with a CLA blend, the *cis*-9,*trans*-11-CLA isomer and the *trans*-10,*cis*-12-CLA isomer on lipoprotein metabolism. The CLA blend and the *trans*-10,*cis*-12-CLA isomer significantly reduced LDL- and HDL-cholesterol concentrations and increased VLDL-TAG concentrations. Whilst most other studies showed that CLA reduced TAG level, the unique information derived from this study is that *trans*-10,*cis*-12-CLA isomer, rather than the *cis*-9,*trans*-11-CLA isomer, affect lipoprotein metabolism. Another study confirmed that a diet providing *cis*-9,*trans*-11-CLA only had no significant effect on plasma lipoprotein metabolism, whereas plasma cholesterol and TAG concentrations were significantly reduced in hamsters fed a diet enriched with a blend of CLA (containing both *trans*-10,*cis*-12-CLA and *cis*-9,*trans*-11-CLA isomers) (Gavino *et al.* 2000). In contrast, recent findings by our group show that a *cis*-9,*trans*-11-CLA-rich diet significantly reduced postprandial serum TAG concentrations, whilst the *trans*-10,*cis*-12-CLA-rich diet had no significant effect in hypertriacylglycerolaemic male Ob/Ob mice (HM Roche, E Noone, A Nugent and MJ Gibney, unpublished results).

Whilst the data from the animal studies show that CLA can improve plasma lipoprotein metabolism, there are contradictory findings. Moya-Camarena & Belury (1999) completed a comprehensive review of the animal studies and concluded that CLA exerted varying potencies depending on rodent species, strain and sex. These findings are important when extrapolating the rodent data to be relevant to man. There is a paucity of information on the effect of CLA on lipoprotein metabolism in human subjects. Our research group has recently completed the first double-blind placebo-controlled CLA supplementation trial in human subjects to investigate the effect of CLA on lipoprotein metabolism (Noone *et al.* 2001). The effect of dietary supplementation (3 g/d for 8 weeks) with two isomeric blends of CLA providing different proportions (50:50 or 80:20, w/w) of the *cis*-9,*trans*-11 and *trans*-10,*cis*-12-CLA isomers in normolipaemic subjects. The 50:50 isomeric blend of CLA significantly reduced plasma TAG concentrations (−20 %). Plasma TAG concentration has been identified as an independent risk factor for future CHD (Stampfer *et al.* 1996) and an important contributor to atherothrombosis (Roche & Gibney, 2000). Therefore, the improvement in TAG-rich lipoprotein metabolism may also be associated with an anti-atherogenic effect of CLA in human subjects, as has been shown in several models of diet-induced atherogenesis.

### Conjugated linoleic acid and inflammation

A number of *in vitro* and animal studies show that CLA has the ability to modulate the immune response. An *in vitro* dose–response study demonstrated that increasing levels (ranging from  $1.78 \times 10^{-5}$  M to  $7.14 \times 10^{-5}$  M) of a blend of CLA isomers (46 g *cis*-9,*trans*-11-CLA, *trans*-9,*cis*-11-CLA/100 g fatty acids, 50 g *trans*-10,*cis*-12-CLA/100 g fatty acids) enhanced porcine lymphocyte proliferation in response to the T-cell mitogen phytohaemagglutinin, inhibited concanavalin A-induced interleukin (IL) 2 production and suppressed the phagocytic activity of murine macrophages (Chew *et al.* 1997). Details of the animal studies that investigated the effect of CLA on indices of the inflammatory response *in vivo* are presented in Table 3. Miller *et al.* (1994) showed that 5 g CLA/kg diet fed to mice for 2 weeks enhanced phytohaemagglutinin-stimulated lymphocyte proliferation. A dose-response study demonstrated that supplementation of Balb/c mice with CLA for 3 weeks significantly enhanced phytohaemagglutinin-induced blastogenesis and concanavalin A-induced IL-2 production, but this effect was not evident after 6 weeks supplementation (Wong *et al.* 1997). That study also showed that CLA had no significant effect on lymphocyte cytotoxicity or mammary tumour incidence and latency. Turek *et al.* (1998) showed that a CLA-enriched diet significantly reduced basal macrophage



**Table 3.** The effect of conjugated linoleic acid-enriched diets on indices of the immune response

Reference	Animal model	Intervention details	CLA dose (g/kg diet)	Study period (d)	Lymphocyte proliferation	Cytokine response	Other
Miller <i>et al.</i> (1994)	Mice	Control diet: 2.5 g fat/kg diet (linoleic acid, without CLA) CLA diet (25 % linoleic acid + 5 % CLA)	5	15	↑	N/A	N/A
Wong <i>et al.</i> (1997)	Balb/c mice	Control diet: 5 g fat (safflower oil instead of CLA) kg diet Low-CLA (35 g c-9,t-11, t-9, c-11, 39 g t-10,c-12/100 g fatty acids) Medium-CLA (35 g c-9,t-11, t-9,c-11, 39 g t-10,c-12/100 g fatty acids) Medium-CLA (35 g c-9,t-11, t-9, c-11, 39 g t-10, c-12/100 g fatty acids) Control diet: 70 g fat/kg diet (soyabean oil instead of CLA) CLA diet (40 g c-9,t-11, 40 g t-10,c-12/100 g fatty acids)	1 3 9	21 and 42	NSD ↑ ↑	NSD ↑ Con-A IL-2 ↑ Con-A IL-2	NSD: LC NSD: LC NSD: LC
Turek <i>et al.</i> (1998)	Sprague-Dawley rats	Control diet: 50 g fat/kg diet (soyabean oil instead of CLA) CLA mix (43 g c-9,t-11, t-9, c-11, 45 g t-10,c-12/100 g fatty acids)	10	42	N/A	↓ TNF $\alpha$ IL-6 NSD: IL-1	NSD: PGE $_2$
Hayek <i>et al.</i> (1999)	C57BL/6NCrIBR	Control diet: 50 g fat/kg diet (soyabean oil instead of CLA) CLA mix (43 g c-9,t-11, t-9, c-11, 45 g t-10,c-12/100 g fatty acids)	10	56	↑	↑ Con A IL-2 NSD: IL-1	NSD: NKC NSD: PGE $_2$ NSD: DTH

CLA, conjugated linoleic acid; ↑, ↓, variable significantly increased or reduced compared with the value for control diet; c, *cis*; t, *trans*; NSD, not significantly different from the control diet; LC, lymphocyte cytotoxicity; Con A, concanavalin A; IL, interleukin; N/A, variable not measured; TNF, tumour necrosis factor; PG, prostaglandin; NKC, natural killer cell activity; DTH, delayed-type hypersensitivity.

TNF- $\alpha$  and lipopolysaccharide-stimulated IL-6 production in male Sprague–Dawley rats, but had no significant effect on macrophage IL-1 production or hepatic prostaglandin (PG) E<sub>2</sub> production. Hayek *et al.* (1999) showed that feeding mice with a CLA-enriched diet significantly increased splenocyte proliferation and concanavalin A-induced IL-2 production in young and old C57BL/6NCrIBR mice. The latter study showed that CLA-enriched diet had no significant effect on natural killer cell activity, splenocyte IL-1 and PGE<sub>2</sub> production or delayed-type hypersensitivity. As yet there have been no studies of the isomer-specific effects of CLA on indices of the inflammatory response in animal models.

There is very little information in relation to the effects of CLA supplementation on the inflammatory response in human subjects. Our group has recently completed the first double-blind placebo-controlled trial which investigated the effect of CLA supplementation on indices of the inflammatory response in healthy human volunteers (Nugent *et al.* 2001). Healthy volunteers were supplemented (3 g/d for 8 weeks) with two isomeric blends of CLA providing different proportions (50:50 or 80:20, w/w) of the *cis*-9,*trans*-11 and *trans*-10,*cis*-12-CLA isomers. It was demonstrated that the *cis*-9,*trans*-11-CLA–*trans*-10,*cis*-12-CLA isomer blend (80:20, w/w) significantly enhanced peripheral blood lymphocyte proliferation in response to the T-cell mitogen phytohaemagglutinin, whereas treatment with the *cis*-9,*trans*-11-CLA–*trans*-10,*cis*-12-CLA isomer blend (50:50, w/w) significantly decreased concanavalin A-induced blastogenesis. These results suggest that the different CLA isomers may have different effects on cell-mediated immune response. The supplement providing more of the *cis*-9,*trans*-11-CLA isomer promoted the cell-mediated immune response, whereas the supplement which had a greater amount of the *trans*-10,*cis*-12-CLA isomer attenuated the immune response. Whilst the study clearly shows that CLA has the ability to modulate the inflammatory response in human subjects, the molecular basis and clinical implications of the pro- and anti-inflammatory potential of the individual CLA isomers has yet to be determined.

## The molecular effects of conjugated linoleic acid

### *Cytokines and eicosanoid metabolism*

CLA has many potent effects which have implications with respect to cardiovascular disease, obesity, diabetes and cancer. In order to fully understand the health effects of CLA, it is essential that the molecular effects of the individual CLA isomers are elucidated. Research to date suggests a number of potential molecular mechanisms; CLA may mediate its effect via the production of cytokines, eicosanoid metabolism or a direct effect on gene expression. The animal studies detailed in Table 3 provide strong evidence that CLA modulates the inflammatory response. Cytokines are potent bioactive compounds that regulate the inflammatory response, but they also affect a number of other biological processes. For example, TNF- $\alpha$  is a pro-inflammatory cytokine that has a plethora of biological functions. TNF- $\alpha$  not only modulates the inflammatory response, but it is also a key mediator in other pathological processes including cachexia (Cahlin *et al.* 2000), cancer (Mayo & Baldwin, 2000), atherosclerosis (Niemann-Jonsson *et al.* 2000), obesity (Ronnema *et al.* 2000), fatty acid metabolism and insulin sensitivity (Hotamisligil, 1999). Turek *et al.* (1998) demonstrated that CLA reduced TNF- $\alpha$  expression (Table 3). It has also been demonstrated that mice fed CLA were protected against TNF- $\alpha$ -induced cachexia (Pariza *et al.* 1999). Therefore, it is possible that the whole-body effects of CLA may be partly mediated by TNF- $\alpha$ . Whilst it is an attractive hypothesis that CLA may partly mediate its effects in a cytokine-dependent manner, it is probably over-

simplistic. All cytokines, in particular TNF- $\alpha$ , are very complex compounds that are regulated by numerous cell-signalling pathways and have a plethora of biological functions.

Since fatty acids are the precursors of eicosanoids, it is possible that some of the effects of CLA may be mediated by the eicosanoids or other lipid-derived cell signals. Eicosanoids affect a range of biological functions, including lipid metabolism, cytokine synthesis and action. Accordingly, the molecular basis of the health effects of CLA may be related to eicosanoid metabolism. Sebedio *et al.* (1997) demonstrated that both *cis*-9,*trans*-11-CLA and *trans*-10,*cis*-12-CLA are elongated and desaturated, in a manner similar to the conversion of linoleic acid into arachidonic acid, the principal eicosanoid precursor. Therefore, it is possible that CLA-derived eicosanoids could mediate the effects of CLA *in vivo*. Alternatively, CLA could alter the synthesis and/or action of the eicosanoids. Basu *et al.* (2000) demonstrated that CLA supplementation (4.2 g CLA isomer mix/d for 3 months) increased urinary 8-iso-PGF<sub>2 $\alpha$</sub>  and 15-keto-dihydro-PGF<sub>2 $\alpha$</sub>  excretion. 8-iso-PGF<sub>2 $\alpha$</sub>  is formed by free radical-catalysed oxidation of arachidonic acid and 15-keto-dihydro-PGF<sub>2 $\alpha$</sub> , the stable metabolite of PGF<sub>2 $\alpha$</sub>  is a PG derived from arachidonic acid via the cyclooxygenase pathway. Whilst the biological actions of altered eicosanoid production following CLA supplementation are unknown, it is probable that altered eicosanoid metabolism accounts for some of the health effects associated with CLA.

#### *Nutrient regulation of gene expression: peroxisome proliferator-activated receptors and lipid metabolism*

CLA may have a direct effect on gene transcription. Peroxisome proliferator-activated receptors (PPAR) are members of the nuclear receptor superfamily of transcription factors which are activated by a number of compounds, including polyunsaturated fatty acids (Kersten *et al.* 2000). The PPAR regulate the expression of many genes which play a role in cellular proliferation, apoptosis, inflammatory response and lipid metabolism (Vanden Heuvel, 1999). Two studies have shown that CLA is a PPAR ligand (Houseknecht *et al.* 1998; Moya-Camarena *et al.* 1999). Therefore, some of the health effects of CLA may be mediated by the PPAR. There are three mammalian subtypes of PPAR ( $\alpha$ ,  $\delta$ ,  $\gamma$ ) (Kersten *et al.* 2000). PPAR $\alpha$  is primarily expressed in the liver, monocytes and T lymphocytes. PPAR $\gamma$  is primarily expressed in adipose tissue, and to a lesser extent in the immune system and the colon. PPAR $\delta$  is ubiquitously expressed.

CLA could mediate its beneficial effects on lipoprotein metabolism via activation of PPAR $\alpha$  (Moya-Camarena *et al.* 1999). In the liver, PPAR $\alpha$  regulates the expression of the genes involved in hepatic lipid metabolism (acyl-CoA oxidase, cytochrome P450 4A1, fatty acid transport protein, acyl-CoA synthetase and lipoprotein lipase). Pharmacological PPAR $\alpha$  ligands (e.g. fibrates) regulate lipid homeostasis and improve plasma TAG metabolism (Fruchard *et al.* 1999). Several CLA isomers are high-affinity ligands and activators of hepatic PPAR $\alpha$ . Incubation of FaO hepatoma cells with CLA increased the transcription of many genes involved in lipid metabolism which have specific PPAR response elements (Moya-Camarena *et al.* 1999). In that study it was demonstrated that the *cis*-9,*trans*-11-CLA isomer was the most potent PPAR $\alpha$  ligand, followed by the *trans*-10,*cis*-12-CLA isomer. In addition, feeding CLA-enriched diets increased hepatic expression of the PPAR $\alpha$  responsive genes *in vivo*. Female SENCAR mice fed increasing amounts of dietary CLA (5, 10 or 15 g/kg diet) induced PPAR $\alpha$ -responsive gene expression, such as acyl-CoA oxidase, cytochrome P450 4A1 and fatty acid transport protein (Belury *et al.* 1997). The CLA diets increased acyl-CoA oxidase mRNA and protein expression in a dose-dependent fashion. Therefore, we can conclude that in the liver

CLA modulates the expression of PPAR $\alpha$ -responsive genes, which may partly explain the molecular basis of the beneficial effect of CLA on plasma TAG metabolism.

Adipose tissue PPAR $\gamma$  plays a key role in regulating the expression of the genes that control adipogenesis, lipid metabolism and insulin sensitivity (Lowell, 1999). Pharmacological PPAR $\gamma$  agonists (thiazolidiones) improve TAG and glucose metabolism (Olefsky, 2000). *In vitro*, CLA induces a dose-dependent transactivation of PPAR $\gamma$  in CV-1 cells (Houseknecht *et al.* 1998). The interaction between PPAR $\gamma$  and CLA *in vivo* and its effect on adipose tissue lipid metabolism is complex. Houseknecht *et al.* (1998) demonstrated that feeding a CLA-rich diet (15 g CLA/kg diet; 42 g *cis*-9,*trans*-11 and *trans*-9,*cis*-11-CLA/100 g fatty acids, 43.5 g *trans*-10,*cis*-12-CLA/100 g fatty acids) induced PPAR $\gamma$ -responsive gene expression (activator protein 2), normalised impaired glucose tolerance, prevented hyperinsulinaemia and reduced plasma free fatty acid concentrations in pre-diabetic Zucker rats. These results suggest that CLA could improve lipoprotein metabolism by up-regulating the PPAR $\gamma$ -responsive gene expression (lipoprotein lipase, activator protein 2, acyl-CoA synthetase, fatty acid transport protein), which would promote the removal of plasma TAG for storage in adipose tissue. In contrast, Tsuboyama-Kasaoka *et al.* (2000) have shown that CLA (10 g CLA/kg feed; 34 g *cis*-9,*trans*-11 and *trans*-9,*cis*-11-CLA/100 g fatty acids, 36 g *trans*-10,*cis*-12-CLA/100 g fatty acids) reduced PPAR $\gamma$  adipose tissue mRNA expression, induced insulin resistance and hyperlipidaemia. The results of a number of *in vitro* studies also conflict. In 3T3-L1 preadipocytes CLA inhibited PPAR $\gamma$ 2 and activator protein 2 mRNA expression (Brodie *et al.* 1999). In contrast, Choi *et al.* (2000) showed no significant effect of CLA on 3T3-L1 adipocyte PPAR $\gamma$  and activator protein 2 mRNA expression. Park *et al.* (1997) showed that while 5  $\mu$ M-CLA increased 3T3-L1 adipocyte lipoprotein lipase activity by 40 %, concentrations greater than 20  $\mu$ M inhibited lipoprotein lipase activity by 40 %. Clearly the effect, if any, of CLA on adipose tissue PPAR $\gamma$  gene expression needs to be clarified. The disparity between the studies may reflect different concentrations and blends of CLA isomers, or in the case of the animal studies the different experimental models that were used by the different groups.

*Nutrient regulation of gene expression: peroxisome proliferator-activated receptors and immune response*

PPAR $\alpha$  and PPAR $\gamma$  are widely expressed in the immune system, but the full nature of their effect on the inflammatory response is unknown (Kersten *et al.* 2000). Hypothetically, the immuno-modulatory effects of CLA could be mediated via PPAR, since it is known that CLA is a ligand for both PPAR $\alpha$  and PPAR $\gamma$ . This hypothesis is supported by pharmacological studies which show that pharmacological PPAR ligands modulate the inflammatory response. PPAR $\alpha$  agonists (e.g. fibrates) inhibit peripheral blood mononuclear cell IL-1 $\alpha$  and IL-6, TNF- $\alpha$  protein and mRNA expression (Jiang *et al.* 1998). Staels *et al.* (1998) showed that PPAR $\alpha$  activators attenuate IL-1, IL-6 and cyclooxygenase-2 expression in human aortic smooth muscle cells, which would suggest that fibrates could attenuate the inflammatory component of atherosclerosis. *In vitro* studies showed that PPAR $\gamma$  ligands up-regulate macrophage CD36 expression and promote foam cell formation (Tontonoz *et al.* 1998). Therefore, there was concern that PPAR $\gamma$  ligands could accelerate atherosclerosis. However, Li *et al.* (2000) showed that PPAR $\gamma$  ligands inhibited the development of atherosclerosis in LDL-receptor deficient mice, despite the fact that the PPAR $\gamma$  ligand up-regulated macrophage CD36 expression. PPAR $\alpha$  and PPAR $\gamma$  agonists inhibit NF- $\kappa$ B signalling (Staels *et al.* 1998; Su *et al.* 1999), which down-regulates inflammatory cytokine expression (Meager, 1999). Whilst we know that CLA modulates

the immune response (Table 3), it remains to be determined whether CLA mediates this effect via the PPAR.

## Conclusion

The CLA isomers are a very interesting group of fatty acids, which may protect against CHD, chronic inflammatory diseases and cancer. Nevertheless, most of our understanding of the health effects of CLA are based on *in vitro* and animal studies. Whilst the preliminary information from the studies with human subjects show that CLA may have a number of positive health effects, there is an urgent need to extend our understanding of the effects of CLA supplementation in man. It is very difficult to extrapolate data from the animal studies to predict the dose of CLA which would be required to have an effect in human subjects. The animal studies have used a relatively high CLA dose (5–10 g CLA/kg diet) over a short period of time (4–8 weeks). An adult human subject could easily consume 1 kg food/d, which at the CLA supplementation level used in the animal studies (5–10 g CLA/kg diet) would be equivalent to 5–10 g CLA/d for human subjects. This represents a very high dose of any fatty acid, which probably could only be achieved using CLA supplements. Also within the context of human nutrition, it is important to consider the long-term effects of a nutrient, rather than the acute effects associated with a high dose of CLA. Therefore, we need to determine the long-term dose–response health effect of CLA in human subjects. We should also investigate the effects of CLA in different disease states (CHD, chronic inflammatory diseases and cancer) which could benefit from the potential therapeutic effects of CLA. It is also vital that we determine the efficacy of the individual CLA isomers *in vivo*. Finally, it is essential that we investigate and understand the molecular effect of CLA in order to have a true understanding of the mechanistic basis of the health effects of CLA.

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