Nutrition Research Reviews (2011), **24**, 206–227
doi:10.1017/S0954422411000114

Human health effects of conjugated linoleic acid from milk and supplements

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Abstract

The primary purpose of the present review was to determine if the scientific evidence available for potential human health benefits of conjugated linoleic acid (CLA) is sufficient to support health claims on foods based on milk naturally enriched with *cis*-9, *trans*-11-CLA (*c*9,*t*11-CLA). A search of the scientific literature was conducted and showed that almost all the promising research results that have emerged in relation to cancer, heart health, obesity, diabetes and bone health have been in animal models or *in vitro*. Most human intervention studies have utilised synthetic CLA supplements, usually a 50:50 blend of *c*9,*t*11-CLA and *trans*-10, *cis*-12-CLA (*t*10,*c*12-CLA). Of these studies, the only evidence that is broadly consistent is an effect on body fat and weight reduction. A previous review of the relevant studies found that 3.2 g CLA/d resulted in a modest body fat loss in human subjects of about 0.09 kg/week, but this effect was attributed to the *t*10,*c*12-CLA isomer. There is no evidence of a consistent benefit of *c*9,*t*11-CLA on any health conditions; and in fact both synthetic isomers, particularly *t*10,*c*12-CLA, have been suspected of having pro-diabetic effects in individuals who are already at risk of developing diabetes. Four published intervention studies using naturally enriched CLA products were identified; however, the results were inconclusive. This may be partly due to the differences in the concentration of CLA administered in animal and human studies. In conclusion, further substantiation of the scientific evidence relating to CLA and human health benefits are required before health claims can be confirmed.

Key words: Conjugated linoleic acid: *Cis*-9, *trans*-11-conjugated linoleic acid: Milk

Introduction

The primary purpose of the present review was to determine if the level of scientific evidence available for potential human health benefits of conjugated linoleic acid (CLA) is sufficient to support health claims on foods based on naturally CLA-enriched milk. Health claims on foods in Europe must now be selected from community lists of approved claims or be the subject of a scientific dossier to gain approval[1]. In order to gain approval, the scientific evidence must be based on human studies, with human intervention studies accorded a higher weighting[1]. Cows' milk contains predominantly the *cis*-9, *trans*-11 isomer of CLA (*c*9,*t*11-CLA). Naturally CLA-enriched milk is defined for the purpose of the present review as milk obtained from grass-feeding regimens that have proven to result in higher levels of *c*9,*t*11-CLA than do conventional feeding regimens (see below).

'Conjugated linoleic acid' is a term used to describe a mixture of positional and geometric isomers of linoleic acid containing conjugated double bonds. It is a group of naturally occurring fatty acids synthesised as intermediates in the biohydrogenation of linoleic and linolenic acid in the rumen of animals, and thus is predominantly found in dairy products and ruminant meat[2]. It can also be synthesised by industrial partial hydrogenation or alkali-isomerisation of linoleic acid[3]. CLA includes twenty-eight possible isomers, with two of these – *cis*-9, *trans*-11-CLA (*c*9,*t*11-CLA) and *trans*-10, *cis*-12-CLA (*t*10,*c*12-CLA) – being known to possess biological activity[4]. Commercially available CLA supplements usually contain *c*9,*t*11-CLA and *t*10,*c*12-CLA at a ratio of approximately 1:1. The majority of CLA in the human diet occurs as *c*9,*t*11-CLA, with this isomer accounting for 85–90% of the total CLA content in dairy products[5].

CLA was first discovered in 1932, by scientists at the University of Reading (UK) who were investigating seasonal variation in the vitamin content of milk[6]. Interest in the potential health benefits of CLA was later sparked by the

Abbreviations: BFM, body fat mass; CLA, conjugated linoleic acid; CRP, C-reactive protein; *c*9,*t*11-CLA, *cis*-9, *trans*-11-conjugated linoleic acid; LBM, lean body mass; *t*10,*c*12-CLA, *trans*-10, *cis*-12-conjugated linoleic acid; UHT, ultra-high temperature.

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identification of CLA's anti-carcinogenic activity *in vitro*, in extracts from fried ground beef[7]. Since then, numerous studies and reviews have investigated the potential health benefits of CLA, with purported health benefits including anti-cancer, anti-atherogenic, anti-adipogenic, anti-diabetogenic, anti-inflammatory and effects on bone health, at least *in vitro*.

CLA is present in relatively low quantities (mg) in meat and dairy products[2]. Estimated dietary intakes from 3 d diet records in the USA are 176 mg total CLA per d for men, with slightly lower intakes for women (104 mg/d). However, in the UK, intake of *c*9,*t*11-CLA was estimated to be 97.5 mg/d[8]. Furthermore, this may vary depending on the method used to assess dietary intake[9]. In recent times, there has been a surge of interest in increasing the concentration of CLA in food in order to increase dietary CLA intake. Cows' milk fat is the richest natural source of CLA[10]; therefore, interest has focused on the potential for naturally increasing the *c*9,*t*11-CLA content of milk and dairy products. Levels of CLA in milk fat ranging from 2 to 37 mg/g fat have been recorded and are due to numerous factors[11]. The composition of the animals' diet is a major factor, with cows that graze on fresh pasture having higher concentrations of CLA in their milk fat than those grazing on hay or concentrates[12]. However, cows that are fed the same diet can demonstrate large intra-individual variation in CLA levels, which may be due to differences in metabolism and the rumen microflora responsible for biohydrogenation[10]. Altitude, breed and lactation age can also influence CLA levels[10]. Research in the UK has shown that there is no difference between the content of CLA in milk from organic and conventional farms[13]. Furthermore, it appears that processing of dairy products causes insignificant changes in CLA levels, particularly compared with the large variations in CLA levels due to diet and intra-individual variation[10].

Much research has been carried out on strategies to manipulate the diets of cows to produce CLA-rich milk, which can then be used to make CLA-rich dairy products. Supplementing the diets of cows with plant oils rich in linoleic or linolenic acid (such as sunflower-seed, soyabean or linseed oil) is known to cause an increase in the concentration of *c*9,*t*11-CLA in milk fat[14]. A study which evaluated the characteristics of naturally CLA-enriched ultra-high temperature (UHT) milk, butter and cheese reported that although the sensory profiles of the CLA-enriched products were different from those of control products, subjects did not rate the overall impression and flavour as being different[15]. It has also been shown that consumption of naturally CLA-enriched dairy products for 6 weeks, at similar levels to which conventional dairy products are habitually consumed in the UK, increases *c*9,*t*11-CLA concentration in plasma lipids[16]. Together these data show that it is feasible and acceptable to increase *c*9,*t*11-CLA intake in the human diet by producing naturally CLA-enriched dairy products for consumption.

Methods

The overall purpose of the present review was to examine the current literature in relation to *c*9,*t*11-CLA and human health benefits with the focus on, in particular, milk and dairy products where CLA content has been enhanced by natural feeding regimens. As there are relatively few studies on enhanced dairy products and in order to identify potential opportunities for future research on *c*9,*t*11-CLA, studies on synthetic CLA isomers were also considered but not subject to exhaustive review. Much of the interest in CLA has been provoked by promising results from animal and *in vitro* studies and in order to put this in context, an overview of these studies is provided although this does not represent a complete picture of the large body of literature.

Initially, reviews were identified from PubMed and used to provide an overview of the key areas of interest. Subsequently, Medline, Embase and evidence-based medicine (EBM) reviews (including Cochrane) were searched via OvidSP (Wolters Kluwer, Alphen aan den Rijn, The Netherlands) using the terms 'CLA', 'conjugated linoleic acid' and 'dairy', both separately and together. Thus, for all databases, this yielded:

(a) 535 for 'CLA' (subheading: 'conjugated linoleic acid');
(b) 41 760 for 'dairy' (subheadings: 'dairy products' and 'milk');
(c) Combining both searches above yielded fifty-six papers;
(d) Separate searches with the above databases for '*cis*-9, *trans*-11' yielded 13 525 papers;
(e) Medline was searched for '*cis*-9, *trans*-11'; only 4476 papers were found and the introduction of 'human' reduced the number of papers to 1378. Further specification to linoleic acids, conjugated yielded 348;
(f) Embase (n 9002) – narrowed to 'humans' and 'CLA' (n 120);
(g) EBM reviews (n 37) – narrowed to 'humans' and 'CLA' (n 35).

The searches were merged using a reference manager programme and duplicates removed, with a total of 538, the abstracts were then examined to determine whether the studies were relevant to the present review. A total of sixty-six human studies utilising observational, randomised control trials and crossover designs, published up to July 2011, were included in the present review. References within studies were also checked for completeness. Reviews on animal studies were identified to provide an overview and then key references followed up individually.

Conjugated linoleic acid and cancer

Since the initial identification of CLA from grilled minced beef and its anticarcinogenic effects on skin cancer

https://doi.org/10.1017/S0954422411000114 Published online by Cambridge University Press

tumours in mice[7], the intervening years have provided a cascade of studies and reviews examining the anticarcinogenic properties of CLA. The mechanisms relating to anticarcinogenic properties of CLA are largely unresolved; CLA may act by antioxidant mechanisms, pro-oxidant cytotoxicity, inhibition of nucleotide and protein synthesis, reduction of cell proliferative activity and inhibition of both DNA–adduct formation and carcinogen activation[17–19]. The studies examined in these reviews have identified potential beneficial effects of CLA on colorectal, breast and prostate cancer, with the majority of evidence from animal and *in vitro* studies.

CLA has shown consistent anticarcinogenic effects against several types of experimental cancer[20] including breast cancer[21,22]. A review by Kelley *et al.*[19] examined the literature in terms of the effects of studies where purified isomers of CLA were administered. Results from *in vitro* studies suggest that the effects of the two isomers of CLA vary according to the cancer model examined. In the majority of studies, *c*9,*t*11-CLA did not inhibit tumour growth, whereas *t*10,*c*12-CLA demonstrated inhibitory effects in studies using mouse and human mammary tumour cell lines. The *t*10,*c*12-CLA isomer also inhibited cell growth in colon and gastric cancer cell lines. However, *c*9,*t*11-CLA was more potent than *t*10,*c*12-CLA in colon cell lines where both isomers were examined, though the optimal concentration level varied between studies (50 μmol/l and 200 μmol/l)[23,24]. Subsequent work by Yasui *et al.*[25] also confirmed the chemopreventive effect of *c*9,*t*11-CLA against pre-initiation (dose-dependent) as well as post-initiation stages of colorectal carcinogenesis (doses ≤ 1% of diet).

Overall, in studies using animal models of cancer, the purified *c*9,*t*11-CLA isomer reduced tumorigenesis in six studies and showed no effect in two others[19]. Similarly, the *t*10,*c*12-CLA isomer decreased tumorigenesis in six studies, but in contrast increased tumorigenesis in two studies. Interestingly, three studies included in the present review found similar effects on the reduction of mammary tumours when a naturally enriched butter[26] and synthetic isomers of *c*9,*t*11-CLA were fed to rats[27] and mice[28]. Though more recent work suggests that *t*10,*c*12-CLA stimulates mammary tumours in a mouse model, where the gene *erbB2/her2* is over-expressed, application of *c*9,*t*11-CLA showed no apparent effects[29]. The same paper also demonstrated that the reduction in tumours was in the same order of magnitude irrespective of whether the CLA source was natural or synthetic. The authors of this paper suggest that it would be prudent to avoid supplements containing *t*10,*c*12-CLA in those at risk of developing breast cancer in which the *erbB2/her2* gene is over-expressed (observed in 20–30% of human breast cancers), whereas supplements containing *c*9,*t*11-CLA may be safe and efficacious in breast cancer prevention[29]. However, due to the differences in proliferation of tumours by the site of cancer, combining results may not elicit the true effects of CLA as an anti-carcinogenic agent, though *in vitro* and animal studies do demonstrate potential benefits.

The manifestation of cancer is not a practical end-point in human studies, combined with the numerous genetic and environmental risk factors for different cancers. Consequently, the majority of studies relating to CLA and cancer in humans are observational studies, particularly on breast cancer (Table 1). Dietary and serum CLA was shown to be significantly lower in postmenopausal cases of breast cancer compared with controls, thus suggesting a protective effect of CLA[30]. In a continuation of this study, breast adipose concentrations of CLA were not significantly different between cases and controls[31]. Furthermore, there was no association between breast adipose tissue CLA concentration and prognostic factors of breast cancer or occurrence of metastases during a 7.5-year follow-up period[32]. In the Netherlands Cohort Study on Diet and Cancer, intake of milk and milk products and meat products, as major sources of dietary CLA, showed no relationship with breast cancer incidence in postmenopausal patients[33]. This could be attributed to the fact that there were no significant differences in total CLA intake between cancer cases and controls[33]. The null association between breast cancer risk and intake of CLA was also demonstrated in a large epidemiological study in Sweden[34]. In contrast, in the same cohort, women who consumed four or more servings of high-fat dairy foods per d (including whole milk, full-fat cultured milk, cheese, cream, soured cream and butter) had a lower risk of developing colorectal cancer[35]. It has been suggested that a higher intake of *c*9,*t*11-CLA confers a reduced risk of a specific type of breast cancer tumour in premenopausal women. However, further investigation is warranted, as the sample size was small[36].

Recently, one small cross-over study examined colon cancer markers after subjects (*n* 15) consumed milk naturally enriched with *c*9,*t*11-CLA or synthetically enriched with *t*10,*c*12-CLA or normal milk as a control[37]. There were large variations in the responses to supplementation across all three groups (NS), therefore all data were combined and a significant decrease in enzyme activity β-glucosidase, nitroreductase and urease; $P<0{\cdot}01$ between day 0 and day 56 was observed. The authors stated that this was important due to links between enzymic activity and the production of carcinogens. However, it is important to note that the main aim of the study was to examine the effects of CLA-enriched milk on lipid metabolism and body composition[38].

Currently the evidence for the anti-carcinogenic properties of CLA in human subjects is limited to observational studies, with broader epidemiological evidence not specifically focusing on CLA but rather on milk and dairy products. The World Cancer Research Fund & Association for International Cancer Research report reviewed the available evidence on the consumption of milk and links with cancer[39]. The report concluded that milk probably protects against colorectal cancer, whereas there is limited

https://doi.org/10.1017/S0954422411000114 Published online by Cambridge University Press

Table 1. Effect of conjugated linoleic acid (CLA) on cancer in human subjects

Reference	Outcomes examined	Number of subjects	Design	Overall result
Aro *et al.* (2000)[30]	Cancer (breast)	195 Cases; 208 controls, F	Case–control study. Dietary intake of CLA from FFQ	Postmenopausal women who consumed the lowest levels of CLA had a 3.3-fold greater risk of breast cancer than women who consumed the highest levels
Chajes *et al.* (2002)[31]	Cancer (breast)	Cases 241 F; controls 88 F	Case–control study. CLA content of breast adipose tissue	No association between CLA in breast adipose tissue and breast cancer risk
Voorrips *et al.* (2002)[33]	Cancer (breast)	941 Cancer cases, 1598 cancer-free subjects	Prospective study, 6.4-year follow-up. Dietary CLA from FFQ	No evidence of a protective effect of higher CLA intake on breast cancer incidence in postmenopausal women
Chajes *et al.* (2003)[32]	Cancer (breast)	209 F	Prospective study, 7.5-year follow-up. CLA content of breast adipose tissue	No association between CLA in breast adipose tissue and breast cancer risk or death
McCann *et al.* (2004)[36]	Cancer (breast)	Cases 1122 F; controls 2036 F	Case–control study. Dietary intake of CLA and *c*9,*t*11-CLA from FFQ	Very little association between CLA intakes and breast cancer. However, relationship between *c*9,*t*11 intake and premenopausal. women – reduced risk of ER-negative tumours
Larsson *et al.* (2005)[35]	Cancer (colon)	60 708 F	Prospective study, 14.8-year follow-up. Dietary intake of CLA from FFQ	Highest intake of CLA (> 149 mg/d) 29% less likely to develop colorectal cancer compared with women with low intakes (< 73.4 mg/d)
Farnworth *et al.* (2007)[37]	Cancer (colon)	15 M + F	RCT, 8 weeks, cross-over. Daily, 1 litre of control milk (5 mg/g); naturally enriched milk (32 mg *c*9,*t*11-CLA/d); synthetically enriched (*t*10,*c*12-CLA and *c*9,*t*11-CLA 32 mg/g fat)	Inconclusive evidence but reductions in faecal enzyme activity evident, but not attributable to changes in the population of one or more faecal bacteria – long-term effects of reducing these enzymes may be desirable
Larsson *et al.* (2009)[34]	Cancer (breast)	61 M, 433 F	Prospective study, 17.4-year follow-up; dietary intake from FFQ	No evidence of protective effect of CLA on breast cancer development

F, female; *c*9,*t*11, *cis*-9, *trans*-11; ER, oestrogen receptor; M, male; RCT, randomised controlled trial; *t*10,*c*12, *trans*-10, *cis*-12.

evidence suggesting that cheese is a cause of colorectal cancer. There is also limited evidence suggesting that consumption of milk conveys a protective effect against bladder cancer. In contrast, diets high in Ca are a probable cause of prostate cancer, but there is limited evidence suggesting that high consumption of milk and dairy products is a cause of prostate cancer[39]. Currently the evidence available is confusing, with suggestions that the effects are dependent on the site of the cancer due to the complex nature of diet, environment and nutrient interactions. However, a substantial amount of further work is required to fully elucidate the potential anti-carcinogenic properties of CLA in humans.

Conjugated linoleic acid and body composition

The overwhelming increases in the proportion of overweight and obesity in the world have been the focus of much debate and research. Currently two-thirds of the UK adult population are classified as overweight or obese (BMI > 25 kg/m^2)[40]. Obesity is a multifaceted disorder, largely driven by its co-morbidities including type 2 diabetes, insulin resistance and CVD. To date feasible and sustainable approaches to prevent further increases in overweight and obesity, let alone attenuate it, have remained largely elusive. More recently, obesity has been recognised as a state of chronic or low-grade systemic inflammation, due to the abnormal circulating levels of inflammatory molecules, including TNFα, leptin and IL-6, which are secreted by adipose tissue[41].

Studies in animals have shown that feeding CLA at levels of 0.5–1% of the diet reduces body fat in mice, chickens, hamsters, rats and pigs[42]. The most substantial decreases in body fat have been observed in mice, where CLA at levels of 0.5% of the diet has been shown to lower body fat by 40 to 80%[42]. This effect is thought to be attributable to the *t*10,*c*12-CLA isomer, as the greatest body fat reductions in mice were observed with a CLA mix with a higher proportion of *t*10,*c*12-CLA than *c*9,*t*11-CLA[43]. Also, *in vitro*, *t*10,*c*12-CLA prohibits TAG accumulation in cultures of differentiating human preadipocytes, whereas *c*9,*t*11-CLA increases TAG content[43]. Evidence suggests that this effect may be due in part to a reduction in lipid uptake by adipocytes due to effects of CLA on stearoyl-CoA desaturase and lipoprotein lipase activity[4].

Promising evidence from animal studies led to an array of human intervention studies being carried out investigating the effect of CLA on body composition in normal

https://doi.org/10.1017/S0954422411000114 Published online by Cambridge University Press

weight, overweight and obese subjects. The majority of these studies used a 50:50 (*c*9,*t*11-CLA and *t*10,*c*12-CLA) CLA mix, and results have been inconsistent. Almost all of these studies have shown no effect on body weight; however, some have reported reduced body fat mass (BFM) following supplementation with CLA, as discussed in detail below.

The body composition studies conducted in normal-weight adults (Table 2) have supplemented with 0.7–5.5 g 50:50 CLA/d, for 4–16 weeks, and of those which measured BFM, some have reported non-significant changes[44,46,47,50,79,175], and others have reported BFM reductions of 4% up to 20%[51–56]. However, it is important to note that in some of the studies that have reported significant BFM reductions in normal-weight adults, subjects were involved in physical training throughout the supplementation periods, which may potentially be a confounder[53–55].

In overweight and obese human subjects (Table 3), 50:50 CLA given at doses of 1.7–6.8 g/d, over periods of 4 to 104 weeks, has resulted in non-significant BFM changes in some instances[57–63], and reductions of 3–15% in other studies[50,57–63,77,78]. The greatest reduction in BFM (14.8%) was observed in a study of patients on blood pressure-lowering medication, who were supplemented with 4.5 g 50:50 CLA/d for 8 weeks[71]. In apparently healthy adults, the greatest reduction in BFM (6%) was observed in the study by Gaullier *et al.*[66]

Table 2. Effect of conjugated linoleic acid (CLA) on body composition in normal-weight human subjects

Reference	Form of CLA	Number of subjects	Design	Overall result
Thom *et al.* (2001)[53]*	CLA mixture	10 M, 10 F	RCT, 12 weeks, 1.8 g CLA/d	No significant change in body weight. Significant decrease in BFM (20%)
Mougios & Vessby (2001)[51]	CLA mixture	13 M, 9 F	8 weeks, 0.7 g CLA/d for weeks 1–4, 1.4 g CLA/d for weeks 5–8	No significant change in body weight. Significant decrease in BFM (skinfolds) with 1.4 g CLA
Smedman *et al.* (2001)[52]	CLA mixture	80 M + F	RCT, 12 weeks, 4.2 g CLA/d	No significant change in body weight. Significant decrease in BFM (4%)
Noone *et al.* (2002)[106]	CLA mixture or 80:20 *c*9,*t*11 and *t*10,*c*12-CLA	18 M, 33 F	8 weeks, 3 g 50:50 CLA/d or 3 g 80:20 CLA/d	No significant change in body weight. BFM not determined
Kreider *et al.* (2002)[175]	CLA mixture (Tonalin)	23 M	RCT, with resistance training, 28 d, 6 g CLA mixture/d	No significant change in body weight or BFM
Belury *et al.* (2003)[74]	CLA mixture	21 M + F	8 weeks, 6 g CLA/d	No significant change in body weight. BFM not determined
Petridou *et al.* (2003)[44]	CLA mixture	16 F	RCT, 6 weeks, 2.1 g CLA/d	No significant change in body weight or BFM
Tricon *et al.* (2004)[79]	*c*9,*t*11- or *t*10,*c*12-CLA	39–49 M	RCT, cross-over, 8 weeks, 0.59, 1.19 or 2.38 g *c*9,*t*11-CLA/d or 0.6, 1.3 or 2.5 g *t*10,*c*12-CLA/d	No significant change in body weight or BFM
Tricon *et al.* (2006)[45]	Supplemented cows' diets to produce milk naturally enriched with *c*9,*t*11-CLA to make products	32 M	RCT, cross-over, 6 weeks, 0.15 or 1.42 g *c*9,*t*11-CLA/d	No significant change in body weight. BFM not determined
Colakoglu *et al.* (2006)[54]*	CLA mixture	44 F	RCT, 6 weeks, 3.6 g CLA/d	No significant change in body weight. Significant decrease in BFM (8%)
Pinkoski *et al.* (2006)[55]*	CLA mixture	42 M, 43 F	RCT, 7 weeks, 5 g CLA/d	No significant change in body weight. Significant decrease in BFM (4%)
Lambert *et al.* (2007)[46]	CLA mixture	62 M + F	RCT, 12 weeks, 3.9 g CLA/d	No significant change in body weight or BFM
Nazare *et al.* (2007)[47]	CLA mixture, added to yoghurt	44 M + F	RCT, 14 weeks, 3.8 g CLA/d	No significant change in body weight or BFM
Raff *et al.* (2009)[56]	CLA mixture or *c*9,*t*11-CLA	75 F	RCT, 16 weeks, 5.5 g CLA/d or 5.5 g *c*9,*t*11-CLA/d	Significant decrease in BFM (4%) and lower-body fat mass (7%) with CLA mixture
Wanders *et al.* (2010)[49]	Foods enriched with CLA-rich oil, 7% of total energy as CLA (78% *c*9,*t*11-CLA and 17% *t*10,*c*12-CLA)	25 M, 36 F	RCT, crossover, 21 d, oleic (control) or industrial *trans*-fatty acids or 26.8 g CLA isomers/d	No significant changes in body weight between diets
Brown *et al.* (2011)[50]	Beef and dairy products rich in CLA from pasture-fed dairy cattle	18 F	RCT, 56 d, 1.17 g CLA/d	No significant changes in body weight, BFM or LBM

CLA mixture, 50:50 *cis*-9, *trans*-11- and *trans*-10, *cis*-12-CLA; M, male; F, female; RCT, randomised controlled trial; BFM, body fat mass; *c*9,*t*11, *cis*-9, *trans*-11; *t*10,*c*12, *trans*-10, *cis*-12; LBM, lean body mass.
* Subjects exercising.

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Table 3. Effect of conjugated linoleic acid (CLA) on body weight or body composition in overweight and obese human subjects

Reference	Form of CLA	Number of subjects	Design	Overall result
Berven *et al.* (2000)[59]	CLA mixture	17 F, 30 M	RCT, 12 weeks, 3.44 g CLA/d	No significant change in body weight or BFM
Blankson *et al.* (2000)[64]	CLA mixture	47 M + F	RCT, 12 weeks, 1.7, 3.4, 5.1 or 6.8 g CLA/d	No significant changes in body composition between groups. Significant reduction in BFM within 3.4 and 6.8 g/d groups (6%), no additional effect with 6.8 g/d over that seen with 3.4 g/d
Risérus *et al.* (2001)[101]	CLA mixture	24 M	RCT, 4 weeks, 4.2 g CLA/d	No significant change in weight. BFM not determined. Significant decrease in SAD (measure of abdominal obesity)
Risérus *et al.* (2002)[60]	CLA mixture or *t*10,*c*12-CLA	57 M	RCT, 12 weeks, 3.4 g CLA or *t*10,*c*12-CLA/d	No significant difference in body composition between the groups. Significant decrease in weight, SAD and BFM within *t*10,*c* 12-CLA group. Significant decrease in SAD and BFM within CLA group
Risérus *et al.* (2004)[78]	*c*9,*t*11-CLA	25 M	RCT, 12 weeks, 3 g *c*9,*t*11-CLA/d	No significant change in body weight between *c*9,*t*11-CLA and placebo. Significant increase in body weight within *c*9,*t*11-CLA group
Gaullier *et al.* (2004)[65]	CLA mixture	31 M, 149 F	RCT, 1 year, 3.6 g CLA-NEFA/d or 3.4 g CLA-TAG/d	Significant reduction in body weight (1%) and BFM (5%). No effect on LBM
Malpuech-Brugère *et al.* (2004)[77]	*c*9,*t*11- or *t*10,*c*12-CLA	82 M + F	RCT, 18 weeks, 1.5 or 3 g *c*9,*t*11-CLA or 1.5 or 3 g *t*10,*c*12-CLA per d	No significant changes in body composition
Desroches *et al.* (2005)[80]	Supplemented cows' diets to produce butter naturally enriched with *c*9,*t*11-CLA	16 M	RCT, cross-over, 4 weeks, 0.24 or 2.5 g *c*9,*t*11-CLA/d	No significant change in body weight. BFM not determined
Gaullier *et al.* (2005)[66]	CLA mixture	24 M, 110 F	Continuation of 2004 study[65], 2 years, 3.4 g CLA-TAG/d	Significant decrease in body weight (2%) and BFM (6%), no safety issues with long-term supplementation
Naumann *et al.* (2006)[102]	*c*9,*t*11- or *t*10,*c*12-CLA in a dairy drink	48 M, 39 F	RCT, 13 weeks, 3 g *c*9,*t*11-CLA or *t*10,*c*12-CLA per d	No significant change in body weight. BFM not determined
Gaullier *et al.* (2007)[67]	CLA mixture (Clarinol)	93 M + F	RCT, 6 months, 3.4 g CLA/d	BFM was significantly decreased at 3 months (1%) and 6 months (3.4%). Most BFM reduction was in legs, LBM increased
Laso *et al.* (2007)[68]	CLA mixture (Tonalin) added to skimmed milk	33 M, 11 F	RCT, 12 weeks, 3 g CLA/d	Significant decrease in BFM (3%) in overweight subjects, but not in obese subjects
Watras *et al.* (2007)[69]	CLA mixture	8 M, 32 F	RCT, 6 months, 3.2 g CLA/d	Significant decrease in BFM (4%) and body weight (1%)
Steck *et al.* (2007)[57]	CLA mixture	13 M, 35 F	RCT, 12 weeks, 3.2 or 6.4 g CLA/d	No significant change in body weight or BFM. Significant increase in LBM within 6.4 g/d group
Syvertsen *et al.* (2007)[58]	CLA mixture (Clarinol)	18 M, 65 F	RCT, 6 months, 3.4 g CLA/d	No significant change in body weight or BFM. Significant decrease in waist circumference in CLA group
Norris *et al.* (2009)[70]	CLA mixture	35 F	RCT, cross-over, 16 weeks, 6.4 g CLA/d	Significant reduction in BMI and BFM, no effect on LBM
Herrmann *et al.* (2009)[61]	*c*9,*t*11-CLA or *t*10,*c*12-CLA or CLA mixture	34 M	RCT, crossover, 4 weeks, 3.4 g CLA/d	No significant change in body weight, BMI, waist circumference or waist:hip ratio
Zhao *et al.* (2009)[71]	CLA mixture	44 M, 36 F (subjects taking blood pressure medication)	RCT, 8 weeks, 4.5 g CLA/d	Significantly lower %BFM and hip circumference. No significant change in body weight, BMI, waist circumference or waist:hip ratio
Racine *et al.* (2010)[73]	CLA mixture in chocolate milk	53 children 6–10 years	RCT, 7 months, 3 g CLA/d	CLA group had significantly less abdominal body fat (%)
Sluijs *et al.* (2010)[62]	*c*9,*t*11-CLA manufactured from safflower-seed oil	167 M, 179 F	RCT, 6 months, 4 g CLA/d	No significant change in body weight, BMI, waist circumference or waist:hip ratio

Table 3. *Continued*

Reference	Form of CLA	Number of subjects	Design	Overall result
MacRedmond *et al.* (2010)[72]	CLA mixture (Tonalin)	13 M, 13 F	RCT, 12 weeks, 4.5 g CLA/d or placebo	CLA group had significantly lower weight and BMI
Brown *et al.* (2011)[50]	Beef and dairy products rich in CLA from pasture-fed dairy cattle	18 F	RCT, 56 d, 1.17 g CLA/d	No significant changes in body weight, BFM or LBM
Joseph *et al.* (2011)[63]	*c*9,*t*11-CLA or CLA mixture added to yoghurt	27 M	RCT, crossover, 8 weeks, 3.5 g CLA/d	No significant change in body weight, BFM, BMI or LBM

CLA mixture, 50:50 *cis*-9, *trans*-11- and *trans*-10, *cis*-12-CLA; F, female; M, male; RCT, randomised controlled trial; BFM, body fat mass; SAD, sagittal abdominal diameter; *t*10,*c*12, *trans*-10, *cis*-12; *c*9,*t*11, *cis*-9, *trans*-11; LBM, lean body mass.

which was of 104 weeks' duration, and supplemented with 3.4 g 50:50 CLA/d. One study in children found that body fat gain was attenuated during prepubertal growth in 6–10-year-olds supplemented with 3.0 g 50:50 CLA/d[73]. However, in a few cases it has been noted that the largest reduction in BFM occurs in the lower body (for example, legs)[56,67]. Furthermore, some studies have reported increases in lean body mass (LBM) with CLA supplementation[57,64,67]. In the study by Blankson *et al.*[64] increased LBM was only observed in the group which significantly increased their level of intensive physical training during the intervention, hence it is possible that the observed effects were, at least partially, due to increased physical activity and not CLA supplementation.

Interestingly, in another study, overweight subjects receiving 3.2 g of 50:50 CLA per d over a 6-month period, including the Christmas period, demonstrated a lower rate of weight gain and a 4% reduction in BFM compared with control[69]. A study of subjects with type 2 diabetes supplemented with 6 g of 50:50 CLA per d for 8 weeks found that plasma concentration of *t*10,*c*12-CLA, but not *c*9,*t*11-CLA, was inversely associated with body weight, suggesting that *t*10,*c*12-CLA is the active CLA isomer in relation to weight change[74]. This is in agreement with evidence from animal studies which also points to the *t*10,*c*12-CLA isomer as being the CLA isomer which elicits BFM reductions. A meta-analysis concluded that CLA, at a dose of 3.2 g/d, produces a modest body fat loss in humans of about 0.09 kg/week, with the relationship being linear up to 6 months[75]. This may be partly explained by the isomer- and tissue-specific effects of CLA, whereby *c*9,*t*11-CLA was found to be increased in skeletal muscle and *t*10,*c*12-CLA was incorporated into adipose tissue TAG in a subset of healthy non-obese participants[76].

In addition to studies examining effects of CLA mixes, a number of studies have investigated the effects of individual CLA isomers on body composition. Findings from these studies show that consumption of 0.59–3 g *c*9,*t*11-CLA per d or 0.6–3.4 g *t*10,*c*12-CLA per d does not change body composition[60,77–79].

Currently, only three studies have been carried out which have fed subjects naturally CLA-enriched dairy products and investigated the effects on body composition[45,50,80]. In the study by Desroches *et al.*[80], sixteen normolipidaemic overweight and obese men consumed butter naturally enriched with CLA (*c*9,*t*11-CLA; 2.59 g/d), or non-enriched control butter (0.24 g/d), for 4 weeks each in a cross-over design, and results showed no changes in body composition. Tricon *et al.*[45] fed thirty-two healthy normolipidaemic men either naturally CLA-enriched or control dairy products (UHT full-fat milk, butter and cheese (1.42 *v.* 0.15 g *c*9,*t*11-CLA/d) in a 6-week cross-over study. Similarly, no changes in body weight were observed; however, body composition was not the primary outcome of this study, but rather blood lipid profile. No changes in body composition were

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observed when subjects consumed beef and dairy products naturally enriched with 1.17 g CLA/d for 56 d[50]. Also, with all of these studies it is important to note that the durations (4–8 weeks) were relatively short for investigating effects on body composition.

There are many possible explanations for the lack of reproducibility in studies of CLA's effect on body composition between animals and humans. These include age, sex, genetic predisposition to fat accumulation and differences in experimental design[81]. It is interesting to note that although animal studies have evaluated the effects of CLA on weight gain over time in growing animals, the majority of human studies tend to investigate whether CLA affects weight or fat loss only in adults.

Conjugated linoleic acid, lipid metabolism and atherosclerosis

CVD are the leading cause of mortality globally[82] and so modification of key risk factors such as LDL-cholesterol or blood TAG are key targets (for example, in the UK[83]). The impact of dietary fat and specific fatty acids on blood lipids has been a focus of research at least since Keys *et al.*'s early epidemiological work[84], so it is not surprising that the effect of CLA on blood lipids has been investigated.

Evidence from animal studies in rabbits, hamsters and mice has suggested that CLA has the potential to modulate plasma lipid metabolism and make an impact on the development and regression of cholesterol-induced atherosclerotic plaques[85].

In rabbits, mixed-isomer CLA, fed at levels of 0.1–1% of diet over periods of 13 to 22 weeks, has been shown to reduce cholesterol deposition in the aorta[86] and result in significant regression of established atherosclerotic lesions[87]. Furthermore, mixed-isomer CLA at a lower dose (0.05%) has been shown to be sufficient to decrease lesion development in rabbits[88]. Supplementation with either *c*9,*t*11-CLA or *t*10,*c*12-CLA results in similar reductions in lesion development to that seen with mixed-CLA isomer supplementation[89].

Studies in hamsters that have supplemented with CLA over periods of 6–12 weeks, using different CLA isomers and doses, have shown mixed results, but there is evidence of improvements in lipid profile[90,91]. In addition, there is some indication that CLA in conjunction with a lower-fat diet may reduce atherosclerotic lesions in the hamster[85]. It has been suggested that *t*10,*c*12-CLA may be the protective isomer in relation to lipid profile, as in the study by Gavino *et al.*[91], a CLA mix, but not the *c*9,*t*11-CLA isomer, improved the lipid profile of hamsters.

In mice, studies with supplemental CLA carried out over periods of 4–20 weeks, using different CLA isomers and doses, have also shown mixed results[85]. There has been one promising report of CLA (80:20 blend of *c*9,*t*11-CLA and *t*10,*c*12-CLA) resulting in marked regression of atherosclerotic lesions in apoE mice[92]. In addition, there is some evidence of opposing effects of CLA isomers, with one study in mice showing *c*9,*t*11-CLA decreasing and *t*10,*c*12-CLA increasing atherosclerotic lesion area[93].

Further to the above studies which have supplemented animals' diets with commercial CLA preparations, studies have been carried out to investigate the anti-atherogenic effects of inclusion of dairy foods, and other foods such as eggs, naturally enriched with CLA, into the diets of animals[94–98]. The results of these studies have shown that CLA can improve plasma lipid profile and decrease atherosclerosis-related biomarkers. Overall, at present there is no general consensus as to the effect of CLA supplementation on lipids or atherosclerosis in animals. Furthermore, most animal studies that have suggested protective anti-atherogenic effects have generally provided CLA doses greater than those achievable in the human diet.

Despite much investigation, the precise mechanisms by which CLA affects lipid metabolism and adipose tissue are not fully elucidated. However, it is thought that CLA modulates energy expenditure, apoptosis, fatty acid oxidation, lipolysis and lipogenesis[99]. As discussed in the previous section, the *t*10,*c*12-CLA isomer is thought to exert effects on body composition, partly due to a reduction in lipid uptake by adipocytes due to effects of CLA on stearoyl-CoA desaturase and lipoprotein lipase activity[4].

In humans epidemiological studies on dietary CLA intakes and prevalence of atherosclerosis have not been carried out to date. However, over the past decade, numerous human intervention studies have investigated the effect of CLA on lipids and other markers of atherosclerotic risk (Table 4), the results of which have been highly inconsistent, possibly due to the use of different isomers and varying doses. The majority of these studies have used commercial mixed- or pure-isomer CLA preparations, at levels of 1.7 to 6.8 g/d, over periods of 4 to 13 weeks, and have not shown any overall effect on plasma lipid or lipoprotein concentrations, compared with placebo, in normal-weight and overweight subjects[44,46,50,52,61–64,73,78,100–105]. However, one study did report significant within-group reductions in total cholesterol and LDL-cholesterol with doses of 1.7 and 3.4 g CLA/d[64], but it was stated that the reductions were not clinically important.

Some studies have reported that supplementation with commercial CLA preparations can have a negative effect on the lipid profile. For example, a significant decrease in HDL-cholesterol was observed on supplementing with 3.4 g *t*10,*c*12-CLA per d in obese men with the metabolic syndrome[60], and in healthy subjects who were supplementing their diets with 0.7–1.4 g CLA mix per d[51]. There is some evidence to suggest that CLA (mixtures and individual isomers) can induce lipid peroxidation[78,103]; however, it is not known whether this effect of CLA could be pro-atherogenic in humans.

In contrast, other studies have shown a positive effect of CLA, with 3 g 50:50 CLA mix per d lowering fasting

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Table 4. Effect of conjugated linoleic acid (CLA) on blood lipid concentrations in human subjects

Reference	Form of CLA	Number of subjects	Design	Overall result
Blankson *et al.* (2000)[64]	CLA mixture	47 M + F	RCT, 12 weeks, 1.7, 3.4, 5.1 or 6.8 g CLA/d	No significant effect on HDL, LDL or tChol between groups
Mougios *et al.* (2001)[51]	CLA mixture	13 M, 9 F	8 weeks, 0.7 g CLA/d for weeks 1–4, 1.4 g CLA/d for weeks 5–8	Significant decrease in HDL
Risérus *et al.* (2001)[101]	CLA mixture	24 M	RCT, 4 weeks, 4.2 g CLA/d	No significant effect on cholesterol or TAG
Benito *et al.* (2001)[100]	CLA mixture	17 F	RCT, 13 weeks, 3.9 g CLA/d	No significant effect on HDL, LDL, tChol or TAG
Smedman & Vessby (2001)[52]	CLA mixture	50 M + F	RCT, 12 weeks, 4.2 g CLA/d	No significant effect on apoA-1, apoB, HDL, LDL, NEFA, tChol, TAG or VLDL
Noone *et al.* (2002)[106]	CLA mixture or 80:20 *c*9,*t*11 and *t*10,*c*12-CLA	18 M, 33 F	8 weeks, 3 g 50:50 CLA or 3 g 80:20 CLA per d	50:50 CLA decreased TAG, 80:20 CLA decreased VLDL
Risérus *et al.* (2002)[60]	CLA mixture or *t*10,*c*12-CLA	57 M	RCT, 12 weeks, 3.4 g CLA or *t*10,*c*12-CLA per d	Significant decrease in HDL with both CLA mix and *t*10,*c*12-CLA
Petridou *et al.* (2003)[44]	CLA mixture	16 F	RCT, 6 weeks, 2.1 g CLA/d	No significant effect on tChol, TAG or HDL
Moloney *et al.* (2004)[107]	CLA mixture	32 M + F	RCT, 8 weeks, 3 g CLA/d	Significant increase in HDL, and decrease in LDL:HDL
Tricon *et al.* (2004)[134]	*c*9,*t*11- or *t*10,*c*12-CLA	39–49 M	RCT, cross-over, 8 weeks, 0.59, 1.19 or 2.38 g *c*9,*t*11-CLA/d or 0.6, 1.3 or 2.5 g *t*10,*c*12-CLA/d	*t*10,*c*12-CLA increases LDL:HDL and total cholesterol:HDL, but *c*9,*t*11-CLA decreases these
Risérus *et al.* (2004)[78]	*c*9,*t*11-CLA	25 M	RCT, 12 weeks, 3 g *c*9,*t*11-CLA/d	No significant effect on HDL, LDL, tChol, TAG or VLDL. Significant increase in lipid peroxidation (8-iso-$PGF_{2\alpha}$ and 15-*keto*-dihydro-$PGF_{2\alpha}$)
Naumann *et al.* (2006)[102]	*c*9,*t*11- or *t*10,*c*12-CLA in a dairy drink	48 M, 39 F	RCT, 13 weeks, 3 g *c*9,*t*11-CLA or *t*10,*c*12-CLA per d	No significant effect on TAG, HDL or LDL
Lambert *et al.* (2007)[46]	CLA mixture	62 M + F	RCT, 12 weeks, 3.9 g CLA/d	No CLA specific effects. tChol and LDL significantly decreased in both groups and HDL decreased in women
Raff *et al.* (2008)[103]	Foods baked with butter synthetically enriched with CLA mixture	38 M	RCT, 5 weeks, 4.6 g CLA/d	No significant effect on HDL, LDL, tChol or TAG. Significant increase in lipid peroxidation (8-iso-$PGF_{2\alpha}$)
Turpeinen *et al.* (2008)[104]	*c*9,*t*11-CLA	12 M, 28 F	RCT, 12 weeks, 2 g CLA/d	No significant changes in plasma lipids
Herrmann *et al.* (2009)[61]	*c*9,*t*11-CLA or *t*10,*c*12-CLA or CLA mixture	34 M	RCT, crossover, 4 weeks, 3.4 g CLA/d	No significant changes in tChol, HDL, LDL, TAG or blood pressure
Zhao *et al.* (2009)[71]	CLA mixture	44 M, 36 F (subjects taking blood pressure medication)	RCT, 8 weeks, 4.5 g CLA/d	Significant effects on HDL, LDL:HDL, blood pressure. No significant effect on tChol, LDL, TAG or VLDL
Racine *et al.* (2010)[73]	CLA mixture in chocolate milk	53 children aged 6–10 years	RCT, 7 months, 3 g CLA/d	No significant changes in LDL. CLA group had significant decreases in HDL
Sluijs *et al.* (2010)[62]	*c*9,*t*11-CLA manufactured from safflower-seed oil	167 M, 179 F	RCT, 6 months, 4 g CLA/d or placebo	No significant effect on HDL, LDL, TAG, tChol or blood pressure
Wanders *et al.* (2010)[49]*	Foods enriched with CLA-rich oil, 7% of total energy as CLA (78% *c*9,*t*11-CLA and 17% *t*10,*c*12-CLA)	25 M, 36 F	RCT, crossover, 21 d, oleic (control) or industrial *trans*-fatty acids or 26.8 g CLA isomers/d	Significantly higher tChol, HDL, LDL, apoB relative to control diet. No significant difference in TAG

Table 4. *Continued*

Reference	Form of CLA	Number of subjects	Design	Overall result
Engberink *et al.* (2011)[105]*	Foods enriched with CLA-rich oil, 7% of total energy as CLA (78% *c*9,*t*11-CLA and 17% *t*10,*c*12-CLA)	25 M, 36 F	RCT, crossover, 21 d, oleic (control) or industrial *trans*-fatty acids or 26.8 g CLA isomers/d	No significant effect on blood pressure
Brown *et al.* (2011)[50]	Beef and dairy products rich in CLA from pasture-fed dairy cattle	18 F	RCT, 56 d, 1.17 g CLA/d	No significant changes in tChol, TAG, HDL, LDL, VLDL or IDL
Joseph *et al.* (2011)[63]	*c*9,*t*11-CLA or CLA mixture added to yoghurt	27 M	RCT, crossover, 8 weeks, 3.5 g CLA/d	No significant effect on tChol, TAG, HDL, LDL, VLDL or oxidised LDL

CLA mixture, 50:50 *cis*-9, *trans*-11- and *trans*-10, *cis*-12-CLA; M, male; F, female; RCT, randomised controlled trial; tChol, total cholesterol; *c*9,*t*11, *cis*-9, *trans*-11; *t*10,*c*12, *trans*-10, *cis*-12.
*Same study with results reported over two papers.

TAG, and 3 g 80:20 CLA mix per d decreasing VLDL, in healthy subjects[106]. Furthermore, 3 g 50:50 CLA per d was shown to significantly increase HDL-cholesterol and significantly decrease LDL:HDL-cholesterol in patients with type 2 diabetes[107]. Consumption of foods enriched with 26.8 g CLA/d led to a significant positive effect on HDL concentration and a significant lowering of LDL-cholesterol[49]. Interestingly, Tricon *et al.*[79] observed divergent responses in plasma lipids with CLA supplementation, with *t*10,*c*12-CLA (0.6–2.5 g/d) increasing LDL:HDL-cholesterol and total:HDL-cholesterol and *c*9,*t*11-CLA (0.59–2.38 g/d) decreasing these ratios, with no dose-dependent effect observed. Elevated cholesterol ratios of LDL:HDL and total:HDL-cholesterol are independent risk factors for CHD[108,109].

Recently, the effects of consuming dairy products, naturally rich in CLA or naturally enriched with CLA, on lipids in human subjects have been examined in four studies[45,50,80,110]. Three of these studies manipulated cows' diets to produce dairy products naturally enriched with CLA[44,50,80]. In the study by Desroches *et al.*[80], normolipidaemic overweight and obese men consumed butter naturally enriched with CLA (*c*9,*t*11-CLA; 2.59 g/d), or non-enriched control butter (0.24 g/d), for 4 weeks. Results showed plasma lipid subfraction levels (VLDL, LDL and HDL) were not significantly different between the two treatments; however, consumption of the non-enriched butter resulted in a significantly greater reduction of total cholesterol, total:HDL-cholesterol and LDL:HDL-cholesterol compared with the CLA-enriched butter, a result which was contradictory to the hypothesis. Tricon *et al.*[45] fed healthy normolipidaemic men either naturally CLA-enriched or control dairy products (UHT full-fat milk, butter and cheese (1.42 *v.* 0.15 g *c*9,*t*11-CLA per d)) in a 6-week cross-over study. Overall, lipid subfractions were not affected; however, a small but significant increase in LDL:HDL-cholesterol was observed. These results were similar to findings by Brown *et al.*[50] where consumption of beef and dairy products rich in CLA (1.17 g CLA/d) for 56 d did not alter blood lipid profile.

A small, cross-over study in ten healthy subjects found that consumption of cheese made from naturally CLA-rich sheep's milk (0.25 g *c*9,*t*11-CLA per d) for 10 weeks had no effect on plasma lipids, as compared with consumption of a regular cows' cheese[110]. The daily intake of CLA was confirmed as being 0.25 g *c*9,*t*11-CLA in correspondence with the author. It is important to note that using cows' milk cheese as a control was not ideal, due to the fact that it has a very different fatty acid profile compared with sheep's cheese. Overall these studies have shown no significant effect of treatment with dairy products naturally rich in CLA or naturally enriched with CLA on plasma lipids.

Dairy products which are naturally enriched in CLA are also higher in *trans*-vaccenic acid (*trans*-18 : 1), lower in SFA content, and slightly higher in *n*-3 PUFA content

https://doi.org/10.1017/S0954422411000114 Published online by Cambridge University Press

than conventional dairy products, due to the feeding strategies employed for enrichment[15]. It has been suggested that consuming *trans*-fatty acids impairs the lipid profile by lowering HDL-cholesterol and raising LDL-cholesterol levels[111]. Whether the content of *trans*-vaccenic acid in naturally CLA-enriched dairy products could counteract the potential benefit of CLA on the lipid profile unclear. The current evidence examining the intake of *trans*-fatty acids from animal sources and associations with CHD presents a confusing picture, particularly given the higher than typically consumed levels of *trans*-fatty acids used within studies[112–116]. However, it is unclear whether the partial conversion of *trans*-vaccenic to *c*9,*t*11-CLA in human intestines, liver and adipose tissue promotes adverse or beneficial effects on lipid profile[113,117].

The reason for the inconsistent and mostly neutral results in relation to the effects of CLA on lipids in human studies compared with animal studies is unclear. However, it is important to note that while animal studies examined the effect of using CLA to supplement hyperlipidaemic animals that were eating atherogenic diets, human studies examined the effect of supplementing diets of normolipidaemic subjects with CLA. Furthermore, it is conceivable that the anti-atherosclerotic effects of CLA observed in animal studies may be due to mechanisms other than effects on lipids, for instance anti-inflammatory effects, as atherosclerosis is an inflammatory disease.

Conjugated linoleic acid, inflammation and immune effects

Inflammation underlies a wide range of conditions. For example, as noted above, obesity is now recognised as a state of chronic or low-grade systemic inflammation, due to the abnormal circulating levels of inflammatory molecules, including TNFα, leptin and IL-6, which are secreted by adipose tissue[41]. In addition, inflammation is central to atherosclerosis[118] and the metabolic syndrome[119].

In vitro studies have shown that CLA has anti-inflammatory effects. CLA (CLA mix, or *c*9,*t*11-CLA or *t*10,*c*12-CLA) is associated with a lower mRNA expression of the inflammatory mediators cyclo-oxygenase-2, TNFα, and inducible NO synthase, and decreases production of induced PGE_2, NO, IL-6 and IL-1β in mouse macrophage cells[120]. The *c*9,*t*11-CLA isomer inhibits induced eosinophil activation, decreases transcription of TNFα, IL-6 and IL-12 in Caco-2 cells and enhances IL-10 production in murine dendritic cells[121–123]. Furthermore, both *c*9,*t*11-CLA and *t*10,*c*12-CLA reduce PGE_2 and thromboxane B_2 concentrations in human macrophages[124].

Animal studies have been carried out to determine if CLA exerts anti-inflammatory effects *in vivo*; however, results to date have been inconsistent. Three animal studies have found a CLA mix to be anti-inflammatory[125–127]. Obese rats fed 1.5% CLA mix for 8 weeks were found to have less adipose TNFα mRNA expression; however, other markers of inflammation did not change[127]. Butz *et al.*[126] reported that mice fed 0.5% CLA mix for 3 weeks had less plasma TNFα compared with mice on a control diet. In pigs fed 2% CLA mix for 14 d, a decrease in induced elevation and mRNA expression of pro-inflammatory cytokines (IL-6 and TNF-α), and an increase in an anti-inflammatory cytokine (IL-10) were observed. Furthermore, a molecular aspect of the same study determined *t*10,*c*12-CLA to be the main isomer to which the anti-inflammatory effect can be attributed[125]. However, in contrast to these findings, two studies have established *t*10,*c*12-CLA to have pro-inflammatory effects, where mice fed 0.5% *t*10,*c*12-CLA for 14 d showed induced pro-inflammatory cytokine transcripts in white adipose tissue[128], and short-term supplementation with *t*10,*c*12-CLA in mice also increased pro-inflammatory cytokine gene expression in a study[129].

Human intervention studies have investigated the effect of CLA (both commercial preparations and naturally CLA-enriched dairy products) on various biomarkers of inflammation (Table 5). Results to date have been mixed, with most studies either showing an increase in inflammatory markers, or no change. Three studies that have supplemented subjects with a CLA mixture at doses of 4.2 to 6.4 g/d, over periods of 12 to 16 weeks, have found increases in plasma levels of C-reactive protein (CRP)[57,130,131]. There were no significant effects on inflammatory markers including CRP and a range of interleukins when subjects were supplemented with 4 to 4.5 g CLA mixture/d[132,133]. Two studies with CLA added to foods showed no effect on plasma CRP levels; however, the duration of these trials was relatively short (5 and 8 weeks)[63,103]. Furthermore, two crossover studies that provided *c*9,*t*11-CLA at doses of 4 g/d[62] or 0.6–2.4 g/d and 0.6–2.5 g/d *t*10,*c*12-CLA[134], for 6 months and 8 weeks respectively, observed no change in plasma CRP concentrations.

Supplementation with *t*10,*c*12-CLA at doses of 3–3.4 g/d for 12–13 weeks has produced inconsistent results. A study in obese men with the metabolic syndrome found increased plasma CRP levels; on the other hand, a study in overweight men and women demonstrated no effect on plasma CRP, or on other markers of inflammation[135,136]. In the case of *c*9,*t*11-CLA, supplementation with similar doses (3 g) for similar durations (12–13 weeks) has also resulted in contrasting results, with one study reporting increased excretion of a pro-inflammatory marker (15-keto-dihydro-PGF2α) in obese subjects[78], and another study reporting no effect on a range of pro-inflammatory markers in overweight subjects[135].

As described in the previous section, the effect of feeding subjects dairy products which are naturally enriched in *c*9,*t*11-CLA (due to the manipulation of diets of cows) has been investigated in two studies to date[45,80]. In these studies, daily doses of 1.4–2.6 g *c*9,*t*11-CLA were fed for durations of 4–6 weeks, and no changes in plasma CRP

https://doi.org/10.1017/S0954422411000114 Published online by Cambridge University Press

Table 5. Effect of conjugated linoleic acid (CLA) on inflammation and other immune indices in human subjects

Reference	Form of CLA	Number of subjects	Design	Overall result
Kelley *et al.* (2000)[137]	CLA mixture	17 F	9 weeks, 3.9 g CLA/d	No significant effects on any immune indices (number of circulating white blood cells, granulocytes, monocytes, lymphocytes, and their subsets, lymphocytes proliferation in response to phytohemagglutinin, and influenza vaccine, serum influenza antibody titers, and DTH response)
Risérus *et al.* (2002)[60]	CLA mixture or *t*10,*c*12-CLA	57 M	RCT, 12 weeks, 3.4 g CLA or *t*10,*c*12-CLA per d	*t*10,*c*12-CLA significantly increased CRP
Risérus *et al.* (2004)[78]	*c*9,*t*11-CLA	25 M	RCT, 12 weeks, 3 g *c*9,*t*11-CLA/d	*c*9,*t*11-CLA significantly increased a pro-inflammatory marker (15-keto-dihydro-$PGF_{2\alpha}$)
Tricon *et al.* (2004)[134]	*c*9,*t*11- or *t*10,*c*12-CLA	39–49 M	RCT, cross-over, 8 weeks, 0.59, 1.19 or 2.38 g *c*9,*t*11-CLA/d or 0.6, 1.3 or 2.5 g *t*10,*c*12-CLA/d	Both isomers decreased mitogen-induced T lymphocyte activation. No significant effect on lymphocytes or CRP
Smedman *et al.* (2005)[130]	CLA mixture	50 M + F	RCT, 12 weeks, 4.2 g CLA/d	Significant increase in CRP. No significant change in TNFα, TNF receptors or VCAM-1
Desroches *et al.* (2005)[80]	Supplemented cows' diets to produce butter naturally enriched with *c*9,*t*11-CLA	16 M	RCT, cross-over, 4 weeks, 0.24 or 2.5 g *c*9,*t*11-CLA/d	No significant effect on CRP
Ramakers *et al.* (2005)[135]	*c*9,*t*11-CLA or *t*10,*c*12-CLA	38 M + F	RCT, 13 weeks, 3 g *c*9,*t*11-CLA or *t*10,*c*12-CLA per d	No significant change in CRP, IL-6, IL-8 and TNFα
Song *et al.* (2005)[139]	CLA mixture	8 M, 20 F	RCT, 12 weeks, 3 g CLA/d	Significant decrease in pro-inflammatory cytokines TNFα and IL-1β. Significant increase in anti-inflammatory cytokine IL-10
Nugent *et al.* (2005)[138]	*c*9,*t*11- or *t*10,*c*12-CLA blends	20 M, 35 F	RCT, 8 weeks, 2 g 50:50 CLA/d or 1.8 g 80:20 CLA/d	*Ex vivo*: no significant effect on PBMC IL-4 production. *In vivo*: no significant effect on ICAM-1, PGE_2, LTB_4
Tricon *et al.* (2006)[45]	Supplemented cows' diets to produce milk naturally enriched with *c*9,*t*11-CLA to make products	32 M	RCT, cross-over, 6 weeks, 0.15 or 1.42 g *c*9,*t*11-CLA/d	No significant effect on IL-6, VCAM-1, CRP, E-selectin
Mullen *et al.* (2007)[140]	CLA mixture	30 M	RCT, 8 weeks, 2.2 g CLA/d	No significant change in CRP, IL-6 fibrinogen
Steck *et al.* (2007)[57]	CLA mixture	13 M, 35 F	RCT, 12 weeks, 3.2 or 6.4 g CLA/d	6.4 g CLA significantly increased CRP and IL-6
Tholstrup *et al.* (2008)[131]	CLA mixture or *c*9,*t*11-CLA, added to oil	75 F	RCT, 16 weeks, 5.5 g CLA mix/d or 5.5 g *c*9,*t*11-CLA/d	CLA mix, compared with *c*9,*t*11-CLA, significantly increased CRP and fibrinogen. PAI-1, VCAM-1, ICAM-1,MCP-1, IL-6 and TNFα were unaffected
Raff *et al.* (2008)[103]	Foods baked with butter synthetically enriched with CLA mixture	38 M	RCT, 5 weeks, 4.6 g CLA/d	No significant effect on inflammatory markers (CRP, PAI-1, FVII-C)
Turpeinen *et al.* (2008)[104]	*c*9,*t*11-CLA	12 M, 28 F	RCT, 12 weeks, 2 g CLA/d	Significant effects on 8-iso-$PGF_{2\alpha}$, 15-keto-dihydro-$PGF_{2\alpha}$, EDN, GM-CSF, IFN-γ, TNFα and sneezing
Sofi *et al.* (2009)[110]	Sheep's cheese naturally rich in *c*9,*t*11-CLA	6 F, 4 M	Cross-over, 10 weeks, 0.25 g *c*9,*t*11-CLA/d	Significant decrease in cytokines; IL-6, IL-8 and TNFα. Significant decrease in platelet aggregation
Zhao *et al.* (2009)[71]	CLA mixture	44 M, 36 F (subjects taking blood pressure medication)	RCT, 8 weeks, 4.5 g CLA/d	Significant effects on adiponectin, leptin. No significant effect on ACE activity
Sluijs *et al.* (2010)[62]	*c*9,*t*11-CLA manufactured from safflower-seed oil	167 M, 179 F	RCT, 6 months, 4 g CLA/d or placebo	No significant effect on CRP

Table 5. *Continued*

Reference	Form of CLA	Number of subjects	Design	Overall result
MacRedmond *et al.* (2010)[132]	CLA mixture (Tonalin)	13 M, 13 F	RCT, 12 weeks, 4.5 g CLA/d or placebo	No significant effect on adiponectin, leptin, IL-6, IL-8, IL-5, IFN-γ, TNF-α, PAI-1, MCP-1, HGF, ECP
Joseph *et al.* (2011)[63]	*c*9,*t*11-CLA or CLA mixture added to yoghurt	27 M	RCT, crossover, 8 weeks, 3.5 g CLA/d	No significant effect on CRP, IL-6, TNF-α or adiponectin
Stickford *et al.* (2011)[133]	CLA mixture (Tonalin)	3 M, 3 F	8 weeks, 4.8 g/d	No significant effect on urinary markers of airway inflammation (LTC_4-E_4 and 9α,11β-$PGF_{2\alpha}$)

CLA mixture, 50:50 *cis*-9, *trans*-11- and *trans*-10, *cis*-12-CLA; F, female; DTH, delayed-type hypersensitivity; *t*10,*c*12, *trans*-10, *cis*-12; M, male; RCT, randomised controlled trial; *c*9,*t*11, *cis*-9, *trans*-11; CRP, C-reactive protein; VCAM, circulating vascular adhesion molecule; PBMC, peripheral blood mononuclear cell; ICAM, intercellular adhesion molecule; LT, leucotriene; E-selectin, endothelial leucocyte adhesion molecule; PAI, plasminogen activator inhibitor; MCP, monocyte chemoattractant protein; FVII-C, factor VII coagulant; EDN, eosinophil-derived neurotoxin; GM-CSF, granulocyte macrophage colony-stimulating factor; IFN, interferon; ACE, angiotensin-converting enzyme; ECP, eosinophil cationic protein; LTC_4-E_4, cysteinyl 4-series leukotrienes.

concentrations and other inflammatory markers were observed. In contrast, a study by Sofi *et al.*[110] found that consumption of sheep cheese, naturally rich in CLA (0.25 g *c*9,*t*11-CLA per d), for 10 weeks decreased circulating levels of the pro-inflammatory cytokines IL-6, IL-8 and TNFα, compared with consumption of a control cows' cheese. However, as noted above, this study was small, poorly controlled and may not have been adequately powered for the multiple variables measured.

Some studies have investigated other immune effects in addition to inflammation. A study where the diets of young women were supplemented with a CLA mixture at 3.9 g/d for 9 weeks found that no indices of immune status were affected (such as the number of circulating leucocytes; granulocytes; monocytes; lymphocytes and their subsets; lymphocytes proliferation in response to phytohaemagglutinin and influenza vaccine; and serum influenza antibody titres)[137]. However, the sample size was small, at seventeen. In a larger study, with fifty-five subjects, Nugent *et al.*[138] found that either a 50:50 CLA mixture or an 80:20 CLA mixture at about 2 g/d had minimal effects on lymphocytes and cytokines, and had no additional benefit on immune function compared with linoleic acid. CLA supplementation has also been linked to reduced symptoms of birch pollen allergy[104] and improved airway hyper-responsiveness in asthmatics[132]. However, a second study in asthmatics found no attenuation of airway inflammation or bronchoconstrictive response[133].

However, Song *et al.*[139] found that supplementing twenty-eight males and females with 3 g CLA 50:50 for 12 weeks had beneficial effects on immune function as it decreased pro-inflammatory cytokines (TNFα and IL-1β) and increased an anti-inflammatory cytokine (IL-10). Furthermore, Tricon *et al.*[134] found that supplementing men with 0.6 to about 2.5 g of either *c*9,*t*11-CLA or *t*10,*c*12-CLA per d decreased mitogen-induced T lymphocyte activation dose-dependently (however, lymphocytes and cytokines were unaffected). Mullen *et al.*[140] showed that 2.2 g CLA 50:50 per d for 8 weeks decreased stimulated peripheral blood mononuclear cell IL-2 secretion, but did not affect other markers including plasma levels of IL-6, CRP, fibrinogen or TNFα, in thirty men.

Overall, studies investigating the effect of CLA (both supplements and naturally CLA-enriched products) on immune indices and inflammation provide inconsistent results.

Conjugated linoleic acid, insulin resistance and diabetes

In addition to the potential anti-atherogenic, anti-obesity and anti-inflammatory properties of CLA, the effects on diabetes have also been examined. As previously stated, increases in overweight and obesity have been concurrent with increases in type 2 diabetes, which is characterised by insulin resistance and occurs as a result of excess adipose

https://doi.org/10.1017/S0954422411000114 Published online by Cambridge University Press

Table 6. Effect of conjugated linoleic acid (CLA) on insulin resistance in human subjects

Reference	Form of CLA	Number of subjects	Design	Overall result
Noone *et al.* (2002)[106]	CLA mixture or 80:20 *c*9,*t*11 and *t*10,*c*12-CLA	18 M, 33 F	RCT with OGTT, 8 weeks, 3 g 50:50 CLA or 80:20 CLA per d	No effect on insulin or glucose
Risérus *et al.* (2002)[60]	CLA mixture or *t*10,*c*12-CLA	57 M	RCT, 12 weeks, 3.4 g CLA or *t*10,*c*12-CLA per d	*t*10,*c*12-CLA increased insulin resistance and glycaemia
Risérus *et al.* (2004)[78]	*c*9,*t*11-CLA	25 M	RCT, 12 weeks, 3 g *c*9,*t*11-CLA/d	*c*9,*t*11-CLA increased insulin compared with placebo in obese men
Eyjolfson *et al.* (2004)[155]	CLA mixture	4 M, 12 F	RCT, 8 weeks, 4 g CLA/d, OGTT at 0, 4 and 8 weeks	Improvements for insulin resistance, corresponding decrease in fasting insulin, though large variations in response
Gaullier *et al.* (2004)[65]	CLA mixture	31 M, 149 F	RCT with OGTT, 1 year, 3.6 g CLA-NEFA/d or 3.4 g CLA-TAG/d	No effects on glucose or insulin
Moloney *et al.* (2004)[107]	CLA mixture	32 M + F	RCT with EGC, 8 weeks, 3 g CLA/d	Negative effect on glucose and insulin in type 2 diabetics
Gaullier *et al.* (2005)[66]	CLA mixture	24 M, 110 F	Continuation of 2004 study[65], with OGTT, 2 years, 3.4 g CLA-TAG/d	No effects on glucose or HbA1c. Original CLA-TAG group significant increase in insulin between 12 and 24 months, though authors contend that not diabetogenic
Naumann *et al.* (2006)[102]	*c*9,*t*11- or *t*10,*c*12-CLA in a dairy drink	48 M, 39 F	RCT, 13 weeks, 3 g *c*9,*t*11-CLA or *t*10,*c*12-CLA per d	No change in glucose or insulin
Tricon *et al.* (2006)[45]	Supplemented cows' diets to produce milk naturally enriched with *c*9,*t*11-CLA to make products	32 M	RCT, cross-over, 6 weeks, 0.15 or 1.42 g *c*9,*t*11-CLA/d	No effects on insulin or glucose
Gaullier *et al.* (2007)[67]	CLA mixture (Clarinol)	93 M + F	RCT with OGTT, 6 months, 3.4 g CLA/d	No effects on insulin or glucose
Laso *et al.* (2007)[68]	CLA mixture (Tonalin) added to skimmed milk	33 M, 11 F	RCT, 12 weeks, 3 g CLA/d	No change in insulin resistance
Syvertsen *et al.* (2007)[58]	CLA mixture (Clarinol)	18 M, 65 F (of these, 41 completed substudy using euglycaemic insulin clamp)	RCT, 6 months, 3.4 g CLA/d	No effects on insulin resistance in main study or subsample
Tarnopolsky *et al.* (2007)[154]	CLA mixture	19 M, 20 F	RCT with OGTT, with and without resistance training, 6 months, 6 g CLA/d plus 5 g creatine/d or placebo plus creatine	No effects on glucose or insulin
Raff *et al.* (2008)[103]	Foods baked with butter synthetically enriched with CLA mixture	38 M	RCT, 5 weeks, 4.6 g CLA/d	No effect on insulin or glucose
Raff *et al.* (2009)[56]	CLA mixture or *c*9,*t*11-CLA	75 F	RCT, 16 weeks, 5.5 g CLA/d or 5.5 g *c*9,*t*11-CLA/d (same study as Tholstrup *et al.* (2008)[131])	No effect on glucose or insulin, HOMA-IR. However, women with the highest waist circumference (3rd tertile, 94–109 cm) had higher fasting insulin in the CLA-mix group than in control and c9,t11groups – post hoc analysis
Turpeinen *et al.* (2008)[104]	*c*9,*t*11-CLA	12 M, 28 F	RCT, 12 weeks, 2 g CLA/d	No significant effect on glucose, insulin, HOMA-IR or QUICKI
Ahren *et al.* (2009)[153]	CLA mixture (Clarinol)	Younger 12 lean, 10 obese; older 16 lean, 11 obese M	RCT with EGC, cross-over, 12 weeks, 3 g CLA/d plus 3 g *n*-3 long-chain PUFA/d	No effects in young lean or obese or older lean adults. Obese older adults estimated insulin resistance was increased with supplementation
Norris *et al.* (2009)[70]	CLA mixture	35 F	RCT, cross-over, 16 weeks, 6.4 g CLA/d	No effect on glucose or insulin
Herrmann *et al.* (2009)[61]	*c*9,*t*11-CLA or *t*10,*c*12-CLA or CLA mixture	34 M	RCT, crossover, 4 weeks, 3.4 g CLA/d	No significant changes in glucose or HOMA-IR

Table 6. *Continued*

Reference	Form of CLA	Number of subjects	Design	Overall result
Zhao *et al.* (2009)[71]	CLA mixture	44 M, 36 F (subjects taking blood pressure medication)	RCT, 8 weeks, 4.5 g CLA/d	No significant effect on glucose, insulin, insulin sensitivity or HOMA-IR
Racine *et al.* (2010)[73]	CLA mixture in chocolate milk	53 children aged 6–10 years	RCT, 7 months, 3 g CLA/d	No significant effect on glucose, insulin or HOMA-IR
Sluijs *et al.* (2010)[62]	*c9*, *t*11-CLA manufactured from safflower-seed oil	167 M, 179 F	RCT, 6 months, 4 g CLA/d or placebo	No significant effect on glucose, insulin or HOMA-IR
MacRedmond *et al.* (2010)[132]	CLA mixture (Tonalin)	13 M, 13 F	RCT, 12 weeks, 4.5 g CLA/d or placebo	No significant effect on insulin
Brown *et al.* (2011)[50]	Beef and dairy products rich in CLA from pasture-fed dairy cattle	18 F	RCT with OGTT, 56 d, 1.17 g CLA/d	No significant changes in glucose, insulin or glucagon
Joseph *et al.* (2011)[63]	*c9*,*t*11-CLA or CLA mixture added to yoghurt	27 M	RCT, crossover, 8 weeks, 3.5 g CLA/d	No significant effect on HOMA-IR

c9,*t*11, *cis*-9, *trans*-11; *t*10,*c*12, *trans*-10, *cis*-12; M, male; F, female; RCT, randomised controlled trial; OGTT, oral glucose tolerance test; CLA mixture, 50:50 *cis*-9, *trans*-11- and *trans*-10, *cis*-12-CLA; EGC, hyperinsulinaemic-euglycaemic clamp; HOMA-IR, homeostasis model assessment of insulin resistance; QUICKI, quantitative insulin sensitivity check index.

tissue. A 5% reduction in body weight has been shown to decrease insulin resistance in overweight and obese subjects[43,141,142]. Therefore the observed modest reductions in body weight with CLA mixtures at 3 g/d may also improve insulin resistance.

Overall, the results from both animal and *in vitro* work are conflicting, with the effects of CLA on insulin resistance examined in addition to other outcomes (atherogenic and obesogenic properties). The vast majority of studies have examined the effects of CLA isomer mixtures, though some results do suggest isomeric differences[143]. In a mouse model, feeding a diet rich in *t*10,*c*12-CLA induced insulin resistance whereas *c*9,*t*11-CLA improved lipid metabolism without impairing insulin action[144] by possible mediation of the pro-inflammatory state[145]. Similarly, studies with male Zucker diabetic fatty (ZDF) rats feeding a 50:50 blend of CLA reduced glucose and insulin concentrations[146], although the diet with 91% *c*9,*t*11-CLA showed no effect[147]. In contrast, in another mouse model of diabetes, a blend of CLA isomers induced marked lipodystrophic insulin resistance and glucose tolerance[148,149]. In the same strain of young and ageing mice, supplementation with the individual isomers or a CLA mix demonstrated divergent responses[148,149]. Supplementation, with *c*9,*t*11-CLA elicited no effects on indices of insulin resistance, plasma insulin and glucose, whereas supplementation with *t*10,*c*12-CLA or a CLA mix increased plasma glucose, insulin and homeostasis model assessment of insulin resistance (HOMA-IR). However, during an intravenous glucose tolerance test, mice supplemented with *c*9,*t*11-CLA eliminated glucose faster than the control, *t*10,*c*12-CLA- or CLA mix-fed mice[150]. These data highlight the importance of not just measuring plasma glucose and insulin, as true effects may only be apparent when more robust measures of insulin resistance are used.

One group has used a proteomics approach for eliciting the interactions between CLA isomers and diseases in an animal model[151]. Proteomic techniques measure changes in the protein complement of a biological system and enable modelling of biological processes in response to dietary interventions[150]. In a study with apoE mice consuming 7% *c*9,*t*11-CLA or *t*10,*c*12-CLA or control (linoleic acid), results suggested that *c*9,*t*11-CLA exerted anti-diabetic effects due to altered expression of markers, whereas *t*10,*c*12-CLA asserted pro-diabetic effects[60,78,152]. Overall, this study suggests that *c*9,*t*11-CLA potentially contributes to a less severe inflammatory response or protection against the development of atherosclerosis. However, conducting a trial in human subjects would be prohibitively expensive and require a rigorously controlled protocol in order to examine the effects of CLA supplementation on protein structure and function.

Currently the anti-diabetic properties of CLA in human subjects (Table 6) cannot be fully determined, as few studies are undertaken using rigorous measures of insulin resistance such as the hyperinsulinaemic–euglycaemic

clamp[107,153] or the oral glucose tolerance test[50,56,58,65–67,106,154,155]. Indeed the majority of results on the anti-diabetic properties of CLA relate to studies where only fasting plasma or serum glucose or insulin have been measured, are not the main focus of the study and typically have small sample sizes. Given these limitations, it is perhaps not surprising that the overall results show no effects of CLA supplementation[56,61,62,65–67,70,71,104,106,132,154] or consumption of CLA-enriched products[45,63,73,102,103] on glucose and insulin. However, supplementing with a CLA mixture has shown beneficial effects on insulin resistance in healthy male subjects[155] and type 2 diabetic subjects[74]. In contrast, a negative effect on insulin resistance was reported in type 2 diabetic patients; however, this may have been due to the bias in the glucose tolerance between the supplementation and placebo groups and may not have been due to CLA supplementation[144].

A recent study also found increased insulin resistance in older obese subjects, but no effects of combined CLA–*n*-3 supplementation in lean or obese younger subjects or older lean subjects[153]. Supplementation with the individual isomers, *c*9,*t*11-CLA or *t*10,*c*12-CLA increased insulin resistance (+15%) in obese men with the metabolic syndrome[60,78], whereas a CLA isomer mixture did not affect insulin resistance[60]. Furthermore, lipid peroxidation increased relative to placebo when the individual isomers were administered, but the differences did not remain significant when adjusted for changes in lipid peroxidation[60,78]. The authors of these papers suggest that irrespective of the CLA isomer, CLA-induced lipid peroxidation may mediate insulin resistance. However, further work is required, particularly studies where the hyperinsulinaemic–euglycaemic clamp is utilised[152,156]. The conflicting responses to increased CLA intake in both human and animal studies do not currently imply compelling anti-diabetic properties of CLA. Thus, studies should be designed that provide rigorous measures of insulin resistance in subjects of varying age groups and weight status[157].

Conjugated linoleic acid and bone health

Bone is a complex tissue system whereby the skeleton is continually renewed through the resorption (breakdown) of existing bone and the formation of new bone (remodelling). Peak bone mass in humans usually occurs late in the second or early in the third decade of life with a progressive decline in bone mineral density starting in the fourth decade of life for both men and women[158]. Bone modelling (children and young adults) or remodelling (adults) is influenced by many factors including nutritional status, hormones and mechanical loading. One of the consequences of low bone turnover or remodelling is the development of osteoporosis, particularly in white, postmenopausal women. In the UK, the costs of osteoporosis to the National Health Service are estimated at

Table 7. Effect of conjugated linoleic acid (CLA) on bone health in human subjects

Reference	Form of CLA	Number of subjects	Design	Overall result
Kreider *et al.* (2002)[175]	CLA mixture (Tonalin)	23 M	RCT, with resistance training, 28 d, 6 g CLA mixture/d	No change on BMD
Gaullier *et al.* (2004)[65]	CLA mixture	31 M, 149 F	RCT, 1 year, 3.6 g CLA-NEFA/d or 3.4 g CLA-TAG/d	No effects on bone resorption or formation
Brownbill *et al.* (2005)[173]	Dietary intake	136 F	Observational study. Dietary intake of CLA from 3 d diet record	CLA intake significant predictor (4%) of Ward's triangle BMD in multiple regression. Subjects with above median intake of CLA had higher BMD of forearm
Doyle *et al.* (2005)[174]	CLA mixture	60 M	RCT, 8 weeks, 3 g CLA/d	No effects on markers of bone formation or resorption
Gaullier *et al.* (2005)[66]	CLA mixture	24 M, 110 F	Continuation of 2004 study[65], 2 years, 3.4 g CLA-TAG/d	No effects on bone resorption or formation
Pinkoski *et al.* (2006)[55]	CLA mixture (Tonalin)	42 M, 43 F	RCT, with resistance training, 7 weeks, 5 g CLA/d	Positive decrease in bone resorption markers
Gaullier *et al.* (2007)[67]	CLA mixture (Clarinol)	118 M and F	RCT, 6 months, 3.4 g CLA/d	No effects on BMD
Tarnopolsky *et al.* (2007)[154]	CLA mixture	19 M, 20 F	RCT, with and without resistance training, 6 months, 6 g CLA/d plus 5 g creatine/d or placebo plus creatine	No effects on total BMD, hip BMD or femur BMD
Racine *et al.* (2010)[73]	CLA in chocolate milk	53 children aged 6–10 years	RCT, 7 months, 3 g CLA/d	BMC accrual in CLA group significantly less than control group
Brown *et al.* (2011)[50]	Beef and dairy products rich in CLA from pasture-fed dairy cattle	18 F	RCT with OGTT, 56 d, 1.17 g CLA/d	No significant changes in BMC or bone area (cm^2)

CLA mixture, 50:50 *cis*-9, *trans*-11- and *trans*-10, *cis*-12-CLA; M, male; RCT, randomised controlled trial; BMD, bone mineral density; F, female; BMC, bone mineral content; OGTT, oral glucose tolerance test.

https://doi.org/10.1017/S0954422411000114 Published online by Cambridge University Press

£2.3 billion per year or £6 million per d, with almost 3 million individuals diagnosed with osteoporosis[159]. Thus, strategies that attenuate decreases in bone mass are of great importance, with much of research focused on Ca, vitamin D, protein and vitamin K intakes[160]. However, other nutrients, including CLA, have been the focus of research due to influences on bone mass and metabolism[18,161–163].

The majority of work on CLA and bone metabolism has been conducted using human cells and animal models, particularly those reflecting postmenopausal women. Supplementation studies have demonstrated decreased PGE_2 production in rats, but results were dependent on the CLA concentration levels[164–168]. PGE_2 is an important factor in the regulation of bone metabolism, including bone formation as well as bone resorption[158]. PGE_2 production increases in postmenopausal bone loss due to oestrogen deficiency[158]. CLA may also stimulate Ca absorption, thus making more Ca available for bone formation[164,169]. Recently, Park *et al.*[170] reanalysed previous studies in mice and showed that extra Ca (0.66%) in the diet improved CLA effects on bone mass in male, but not female mice. A recent review concluded that based on the current evidence from *in vitro* and animal studies the addition of CLA, overall, improves bone strength and density[161]. However, the majority of studies currently published were conducted using CLA isomer mixtures. Only two studies have examined the differences between the *c*9,*t*11 and *t*10,*c*12 isomers and found no direct effects on bone, but rather attenuation of parathyroid hormone concentration[171,172].

Whilst there are numerous publications examining the effects of CLA and bone formation in cell and animal models, studies in human subjects are lacking (Table 7). Data from an observational study showed that in postmenopausal women dietary intake of CLA was a weak but significant predictor of Ward's triangle bone mineral density[173]. The same study also found that subjects with above median intake of CLA had higher bone mineral density of the forearm. In contrast, supplementation with a CLA mix (3.0–3.4 g/d) did not affect bone formation or resorption in healthy lean, overweight, obese men and women[65–67,174]. A further two studies in young and elderly subjects who completed resistance training in addition to CLA supplementation (6 g/d) also demonstrated no change in bone mineral density and bone mass[154,175]. Brown *et al.*[50] reported no change in bone mineral content when subjects consumed a CLA-enriched diet, although the study duration was only 56 d, an insufficient length of time for observing changes in bone mineral content. In children, significantly less bone mineral content accretion occurred in the CLA supplemented after 7 months[73]; however, the reasons are not fully elucidated. Currently, the only human study to demonstrate a positive association between CLA supplementation(5 g/d) and bone found a decrease in bone resorption markers and increase in LBM[55]. However, this study did not identify whether the increases in LBM were due to increased muscle or bone mass and whether it was an artifact of the 7-week resistance training programme. Since there are relatively few human studies (four out of seven studies where bone was not the primary outcome examined), the lack of consistency in protocols, measurement of bone metabolites and small sample sizes hinder a clear conclusion between the effects of CLA and bone.

Overall conclusions

The overall evidence from the studies examined here demonstrates a lack of definitive and reproducible results, particularly in relation to the consumption of naturally enriched CLA products, as the number of published studies is low relative to the number on synthetic supplements. The majority of randomised controlled trials are conducted with CLA supplements, with varying mixtures of isomers and dosage levels. However, the evidence from animal studies is promising, but extrapolation from animal to human studies is difficult due to the differences in the amount of CLA used. For example, in animal studies the observed benefits of CLA on bone are between 0.1–1% CLA of total weight of diet[173]. For men consuming on average 3.0 kg food and beverages per d, this is equivalent to 3–30 g CLA/d; for women consuming about 2.2 kg food and beverages per d, this equates to 2.2–22 g CLA/d[176]. In addition, given the differences in study protocols, relatively small sample sizes and other methodological issues (including measurement of dietary CLA intakes[8] and accurate measurement of body composition), it is not surprising that there is a lack of consensus on what health claims could be applicable to CLA, either natural or synthetic products. Current submissions on CLA health claims to the European Food Safety Authority (EFSA) include seven for body weight/LBM, two on immune function, two on antioxidant properties and one relating to insulin. The present review suggests that the only possible candidate would be in relation to the synthetic *t*10,*c*12-CLA isomer and reductions in body fat.

Acknowledgements

The present review was prepared as part of a project funded by Scottish Enterprise (Glasgow, UK). Nino Binns Consulting provides consultancy in nutrition and food regulation to a variety of commercial clients.

T. A. M. and E. M. K. drafted the review. J. M. W. W., N. B. and M. B. E. L. commented on the review.

There are no conflicts of interest.

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