REVIEWS: CURRENT TOPICS

Biological effects of conjugated linoleic acids in health and disease

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Abstract

Conjugated linoleic acid (CLA) is a mixture of positional and geometric isomers of octadecadienoic acid [linoleic acid (LA), 18:2n-6] commonly found in beef, lamb and dairy products. The most abundant isomer of CLA in nature is the cis-9, trans-11 (c9t11) isomer. Commercially available CLA is usually a 1:1 mixture of c9t11 and trans-10, cis-12 (t10c12) isomers with other isomers as minor components. Conjugated LA isomer mixture and c9t11 and t10c12 isomers alone have been attributed to provide several health benefits that are largely based on animal and in vitro studies. Conjugated LA has been attributed many beneficial effects in prevention of atherosclerosis, different types of cancer, hypertension and also known to improve immune function. More recent literature with availability of purified c9t11 and t10c12 isomers suggests that t10c12 is the sole isomer involved in antiadipogenic role of CLA. Other studies in animals and cell lines suggest that the two isomers may act similarly or antagonistically to alter cellular function and metabolism, and may also act through different signaling pathways. The effect of CLA and individual isomers shows considerable variation between different strains (BALB/C mice vs. C57BL/6 mice) and species (e.g., rats vs. mice). The dramatic effects seen in animal studies have not been reflected in some clinical studies. This review comprehensively discusses the recent studies on the effects of CLA and individual isomers on body composition, cardiovascular disease, bone health, insulin resistance, mediators of inflammatory response and different types of cancer, obtained from both in vitro and animal studies. This review also discusses the latest available information from clinical studies in these areas of research.

Keywords: Conjugated linoleic acid; Bone; Fat mass; Cancer; Insulin resistance; Inflammation; Cardiovascular health

1. Introduction

Conjugated linoleic acid (CLA) refers to a group of polyunsaturated fatty acids (PUFA) that exist as positional and stereoisomers of octadecadienoic acid. There is no methylene group separating the double bonds of CLA as there is in linoleic acid (LA). Instead, conjugated double bonds (i.e., the two double bonds are separated by one single bond) in either cis (c) or trans (t) configuration are present predominantly in positions 8 and 10, 9 and 11, 10 and 12, or 11 and 13. They are found naturally in ruminant food products such as beef, lamb and dairy because of the process of bacterial biohydrogenation of LA in the rumen [1–3]. Conjugated LA was discovered quite accidentally when Pariza and Hargraves [4] were investigating the carcinogenic properties of grilled beef. To their surprise and contrary to their expectations, the fatty acids present in grilled beef exhibited anticarcinogenic rather than procarcinogenic properties. Ever since that discovery, CLA has been reported to have several beneficial effects in health-related disorders using animal models and cell cultures derived from humans and animals. Thus, CLAs have been shown to have antiadipogenic [5–7], anticarcinogenic [8–15], antiatherogenic [16–19], antidiabetogenic [20,21] and anti-inflammatory properties [22–25].

Although there are 28 different CLA isomers, the major isomer in natural foods is the c9t11 isomer accounting for more than 90% CLA intake in the diet [26]. Conjugated LA isomers can be prepared commercially by heating LA under alkaline conditions or by partial hydrogenation of LA [27,28]. Health benefits of CLA have been attributed to mainly two of its isomers: cis-9, trans-11 (c9t11) and trans-10, cis-12 (t10c12). Structures of the parent LA, c9t11 and t10c12 CLA isomers are shown in Fig. 1. The most commonly used CLA is the mixed isomer preparation containing (approximately 40–45%) equal levels of the...
c9t11 and t10c12 isomers. With the advent of technology, enriched or purified c9t11 and t10c12 CLA preparations have become commercially available in recent years, leading to studies examining the effects of these individual isomers in health-related disorders. Most of the studies have used CLA isomer mix, but recent evidence suggests that c9t11 and t10c12 may have myriad effects in different biological systems. Indeed, it has been found that both the isomers exhibit significant biological activities, which often may be similar or opposite. This review focuses on the biological role of CLA and its purified isomers (c9t11 and t10c12) in different models of health-related disorders in cell culture, animals and clinical studies. Unless otherwise mentioned in this review, CLA refers to a mixture containing equal levels of c9t11 and t10c12 isomers.

2. Conjugated LA and body composition

2.1. Animal studies

Park et al. [5] showed for the first time that intake of 0.5% CLA in ICR male and female mice (50% c9t11 and 50% t10c12) results in decreased body fat mass and increased lean body mass. The mechanisms proposed were increased lipolysis, increased fatty acid oxidation or reduced fatty acid uptake in adipocytes. Subsequent studies in different animal models corroborated the findings and showed that CLA containing equal proportions of both isomers decrease fat mass and enhance lean mass [21,29–34]. These studies and others have been discussed in reviews published elsewhere [35–39].

The availability of purified isomers or CLA enriched in either c9t11 or t10c12 isomers prompted new in vivo and in vitro studies, which identified t10c12 isomer to be primarily involved in reduction of fat mass, and not the c9t11 isomer. When hamsters were fed with a hypercholesterolemic diet containing 1% CLA, 0.2% c9t11 CLA or 1% LA, CLA isomer mix-fed animals had the lowest weight gain [40]. Yet, another study showed that intake of t10c12 isomer-enriched diet decreases body fat significantly compared to diet enriched in c9t11 isomer [6]. In a study in Zucker diabetic fatty (ZDF) rats, dietary intake of 1.5% of CLA (47% c9t11+47.9% t10c12) decreased weight gain and fat mass, whereas dietary intake of CLA containing 91% c9t11 had no effect on these parameters, proving that t10c12 is the isomer responsible for loss of fat mass [20]. In vitro studies using purified c9t11 and t10c12 isomers and cultured 3T3-L1 adipocytes provided further supportive evidence that t10c12 was the isomer responsible for the fat-lowering effects of CLA [6,41].

Here we summarize recent information on the impact of CLA or its isomers on body composition. Navarro et al. studied the effect of 6 weeks of supplementation of 0.5% LA, c9t11 CLA or t10c12 CLA in atherogenic diet-fed hamsters. Although there was no difference in body weight, fat mass decreased significantly in t10c12-fed hamsters [42]. In a related study, intake of diet containing 0.5% t10c12 CLA for 6 weeks decreased fat mass in atherogenic diet-fed hamsters [43], but failed to prevent insulin resistance (IR) associated with intake of atherogenic diet. Wargent et al. [44] recently showed that intake of t10c12 CLA isomer for 3 weeks in genetically obese mice decreased gain in body weight and white fat pad weight. In a study using wild-type and stearoyl-CoA desaturase 1 (SCD1) null mice, t10c12 CLA decreased fat mass and enhanced mRNA expression of lipogenic enzymes, fatty acid synthase (FAS) and uncoupling protein 2 (UCP-2), suggesting that antiobesity effects of t10c12 CLA is independent of SCD1 gene expression and enzyme activity [45].

Some recent studies have evaluated the impact of the type of dietary fat on the antiadiposity effects of CLA. A recent study showed that CLA isomer mixture (1.5%, 4 weeks) had no effect on adiposity in Sprague–Dawley (SD) rats when given alongside diets rich in either saturated fat (coconut oil) or unsaturated fat [corn oil (CO)] [46]. Another recent study examined the effects of 14 days of CLA mixture intake on body fat in mice previously treated with diets containing soy oil as control, coconut oil [essential fatty acid-deficient (EFAD)] and fish oil for 42 days [47]. Conjugated LA significantly decreased body weight and epididymal fat mass, but not retroperitoneal fat mass in both control and EFAD diet-fed mice. The study suggested that CLA seems to be more effective in lowering fat mass when diet was deficient in essential fatty acids.
(EFA). However, the same group recently reported that coconut oil-fed mice replenished with EFA did not show any significant difference in fat mass when compared to mice fed coconut oil alone, suggesting that effects of CLA may be independent of EFAD [48]. A study in SD rats for 6 weeks evaluated the effects of hydrogenated soyabean oil (SBO) containing high levels of CLA (21%) and found that inguinal, epididymal and retroperitoneal adipose depots were significantly decreased with CLA supplementation [49]. Although CLA content of SBO was high, level of t10c12 CLA was considerably low in the diet formulation. We recently showed that the combination of 0.4% CLA (50:50 c9t11+t10c12) and moderate treadmill exercise for 14 weeks decreased body fat mass and increased lean mass in high-fat diet-fed BALB/C male mice, which was associated with CLA-induced decrease in serum leptin levels, lower leptin mRNA expression in peritoneal fat pads and exercise-induced increase in oxygen consumption and energy expenditure [50]. In contrast, a study in adult male Wistar rats fed with diets containing 1.0% c9t11 and 1.0% t10c12 isomer or 2.0% CLA (1.0% c9t11 and 1.0% t10c12) in combination with moderate physical activity for 6 weeks did not observe any effect on body composition or body weight [51]. In a subsequent study, the same group showed that CLA isomers stimulate adipose tissue lipogenesis without significantly affecting adipose weight in adult Wistar rats [52]. Studies in mature or adult animals have not reproduced the dramatic results seen in growing animals. Furthermore, mice are more sensitive to CLA than rats in loosing fat mass. We recently found that dietary intake of CLA isomer mixture for 10 weeks significantly decreases fat mass, prevented age-related loss of lean mass and maintained higher gastrocnemius and quadriceps muscle weight when compared to CO control in middle-aged C57BL/6 female mice (Bhattacharya et al., unpublished data).

Some of the mechanisms suggested to be involved in fat reduction with CLA intake are increased energy expenditure [31,33,53,54], increased fat oxidation [31,54], decreased adipocyte size [32,55,56], decreased energy intake [31] and inhibition of enzymes involved in fatty acid metabolism and lipogenesis [6,32,57–60]. Uncoupling proteins (UCP-2 and UCP-3) are key regulators of energy expenditure and diet-induced thermogenesis. Uncoupling protein 2 is highly expressed in skeletal muscles and adipose tissues, whereas UCP-3 is expressed primarily in skeletal muscles. Conjugated LA-mediated up-regulation of UCP-2 expression in white adipose tissues has been proposed as one of mechanisms through which it increases energy expenditure [20,61]. Increase in catecholamines could be one more potential mechanism by which CLA increases energy expenditure [62]. Simultaneous studies with purified isomers, both in vitro and in vivo, have consistently shown that t10c12 and not c9t11 is the key CLA isomer involved in fat reduction through decreased adipocyte size [63], increased fat oxidation [64] and inhibition of enzymes involved in lipogenesis [41,57,65]. Nagao et al. [62] recently showed that t10c12 isomer increases oxygen consumption and energy expenditure, more than the c9t11 isomer.

2.2. Clinical studies

There have been few studies that have examined the effects of CLA or its isomers in humans. However, most of the studies have not reflected the dramatic findings obtained in animal studies. One of the first studies in healthy adult women examined the effects of 3 g/day intake of CLA for 64 days on fat-free mass, fat mass and percentage fat mass compared to sunflower oil (SFO) placebo. Other parameters like energy expenditure, fat oxidation and respiratory exchange ratio were also measured. The study reported no differences in body composition or any of the parameters examined [66]. In sedentary young women, intake of 2.1 g CLA/day for 45 days did not induce any changes in body composition. Body fat was measured by measurement of skinfold thickness at 10 different sites in this study [67]. However, two studies from Norway in healthy exercising humans (CLA, 1.8 g/day) and in overweight and obese humans (CLA, 1.7, 3.4, 5.1, and 6.8 g/day) for 12 weeks showed that CLA can decrease fat mass without significantly affecting body weight [68,69]. Results of the first study in athletes were encouraging considering that a much lower dose of CLA (1.8 g/day) produced significant results compared to the second study where the authors concluded that a dose of 3.4 g/day of CLA was enough to cause reduction of body fat. Another study in 2001 evaluated the effects of intake of 4.2 g CLA/day for 4 weeks on changes in adipose tissue and cardiovascular risk factors in middle-aged obese men with signs of metabolic syndrome [70]. None of the factors were altered except sagittal abdominal diameter (as a measure of abdominal obesity), which decreased with CLA intake. The study concluded that CLA might decrease only abdominal fat mass without affecting overall obesity and cardiovascular risk factors. In the same year, a study from Greece reported that CLA administered first at 0.7 g/day for 4 weeks and thereafter at 1.4 g/day for the next 4 weeks decreased fat mass in healthy volunteers [71]. However, another study evaluating the effects of CLA supplementation (6 g/day, 28 days) during resistance training on body composition found no effect on total body mass, fat mass, fat-free mass or percentage of body fat [72]. A 12 weeks, intervention study with t10c12 isomer found decreased abdominal fat mass and body weight but at the same level as control diet in obese individuals with signs of metabolic syndrome [73].

In some other human studies, CLA was given to the subjects in the form of dairy products. A study in middle-aged overweight men and women evaluated the effects of purified CLA isomers (1.5 and 3.0 g/day for 18 weeks; supplemented as a drinkable dairy product) on body composition with SFO as control [74]. The study did not find any difference in fat mass or lean mass. Effects of intake of modified butter fat high in CLA content for 4 weeks (4.22 g/100 g butter fat) was
compared recently with control butter low in CLA (0.38 g/100 g butter fat) on body composition in overweight and obese men [75]. The study again found no differences in accumulation of abdominal or subcutaneous adipose tissue between the two experimental diets.

In an interesting study from Netherlands, 13-week intervention with 1.8 or 3.6 g/day CLA in overweight humans supplemented prior to this with a very-low-calorie diet (which induced weight loss) increased resting metabolic rate and lean mass without affecting body weight regain [76]. Other studies by Blankson et al. [69] and Thom et al. [68] had previously showed a net decrease in body fat greater than net decrease in body weight, suggesting that lean mass may have increased in the subjects. Volunteers in both these studies were either on intensive training programs or exercised for 90 min, three times per week. These studies seem to suggest that exercise could enhance the fat-lowering effects of CLA and also help improve lean mass in humans.

So far, there have been reports of only two long-term CLA intervention studies in healthy overweight humans by the same study group [77,78]. In the first study, subjects (male and female; average body mass index, 25–30 kg/m²) were followed for 12 months. Conjugated LA was supplemented in the form of either CLA–free fatty acid (CLA-FFA) or CLA–triacylglycerol (CLA-TG), with olive oil as placebo. Both CLA-FFA and CLA-TG decreased body fat mass significantly. Conjugated LA-FFA-fed subjects had higher lean body mass compared to placebo. In the second study, subjects were supplemented with 3.4 g CLA-TG per day for another 12 months and decrease in fat mass was maintained over a period of 24 months, suggesting that CLA may help maintain initial reductions in body fat mass and weight with long-term intervention.

In summary, some short- and long-term studies in healthy and obese, sedentary and exercised humans have indicated the beneficial effects of CLA in reducing fat mass without significantly affecting body weight. However, the dramatic effects seen in animal studies, especially in mice, have not been reflected in human studies. This may be partly because CLA dosage used in human studies is much lower than those used in animal studies. Moreover, most animal studies have been in young growing mice or rats, whereas studies in humans were mostly in mature volunteers. As noted earlier, the effects of CLA on body composition in mature animals have not been as dramatic as those seen in young animals. The findings are summarized in Table 1. More human trials in large groups of subjects, both in adolescents and adults, are urgently needed before CLA isomer mixture or individual isomers can be recommended for improving body composition, especially in reducing fat mass in humans. In our opinion, combination of CLA and moderate exercise such as walking or treadmill may be an ideal and safe therapeutic approach for humans to decrease body weight and fat mass, and improve lean mass. Both short- and long-term clinical studies need to be undertaken in different age-group subjects in the near future.

### Table 1

<table>
<thead>
<tr>
<th>Model</th>
<th>Significant findings</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>Rats, mice and pigs</td>
<td>↑ lean mass, ↓ fat mass</td>
<td>[5,21,29,32–34]</td>
</tr>
<tr>
<td>Mice</td>
<td>t10c12-enriched diet, ↓ fat mass compared to c9t11</td>
<td>[6]</td>
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<td></td>
<td>↓ body weight and epididymal fat mass in essential fatty acid-deficient diet-fed mice</td>
<td>[47]</td>
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<tr>
<td>ZDF rats</td>
<td>CLA ↓ weight gain, fat mass; ↔ enriched c9t11</td>
<td>[20]</td>
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<tr>
<td>Atherogenic diet fed hamsters</td>
<td>CLA ↓ weight gain compared to c9t11</td>
<td>[40]</td>
</tr>
<tr>
<td></td>
<td>Fat mass ↓ t10c12 ↔ c9t11; no effect on body weight</td>
<td>[42]</td>
</tr>
<tr>
<td></td>
<td>t10c12 ↓ fat mass</td>
<td>[43]</td>
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<tr>
<td>SCD1 null mice</td>
<td>↓ obesity with t10c12 independent of SCD1 expression</td>
<td>[45]</td>
</tr>
<tr>
<td>Exercised high-fat diet-fed mice</td>
<td>↓ body weight, fat mass ↑ lean mass</td>
<td>[50]</td>
</tr>
<tr>
<td>Exercised adult rats</td>
<td>↔ body weight, body composition, adipose weight</td>
<td>[51,52]</td>
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<tr>
<td>Sedentary young women</td>
<td>45 days; 2.1 g CLA/day ↔ body fat</td>
<td>[67]</td>
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<tr>
<td>Healthy adult women</td>
<td>↔ 64 days; 3 g/day CLA — body fat, percentage fat, fat-free mass</td>
<td>[66]</td>
</tr>
<tr>
<td>Healthy exercising humans</td>
<td>12 weeks; 1.8 g/day CLA ↓ fat mass ↔ body weight ↓ lean mass</td>
<td>[68]</td>
</tr>
<tr>
<td></td>
<td>↔ 6 g CLA/day; 28 days — body mass, fat mass, fat-free mass, percent body fat</td>
<td>[72]</td>
</tr>
<tr>
<td>Overweight/obese humans</td>
<td>12 weeks different doses ↓ fat mass ↔ body weight ↑ lean mass</td>
<td>[69]</td>
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<tr>
<td></td>
<td>13 weeks ↑ lean mass</td>
<td>[76]</td>
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<tr>
<td></td>
<td>12 months CLA-TG and CLA-FFA ↑ lean mass</td>
<td>[78]</td>
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<td></td>
<td>24 months 3.4 g CLA-TG/day ↓ fat mass</td>
<td>[77]</td>
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<tr>
<td></td>
<td>4 weeks; 4.2 g CLA/day ↓ abdominal fat mass ↔ overall fat mass</td>
<td>[70]</td>
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<td></td>
<td>18 weeks ↔ fat mass, lean mass</td>
<td>[74]</td>
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<tr>
<td></td>
<td>4 weeks ↔ abdominal, subcutaneous adipose tissue (CLA supplemented as butter fat)</td>
<td>[75]</td>
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<tr>
<td></td>
<td>12 weeks; t10c12 ↔ body fat, abdominal fat, body weight (same as control)</td>
<td>[73]</td>
</tr>
<tr>
<td>Healthy volunteers</td>
<td>8 weeks ↓ fat mass (4 weeks 0.7 g/day; 4 weeks 1.4 g/day)</td>
<td>[71]</td>
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↓, decreased; ↑, increased; ↔, no effect.
3. Conjugated LA and cardiovascular health

3.1. Animal studies

Initial study in rabbits when fed with 14% fat (high fat) and 0.1% cholesterol as atherogenic diet, and either a control diet or a diet containing CLA isomer mix (0.5 g/day) for 22 weeks showed the aortas of CLA-fed rabbits had lower atherosclerosis [16]. One percent CLA has also been shown to cause 30% regression of established atherosclerosis in rabbits rendered atherosclerotic by the intake of diet containing 0.1–0.2% cholesterol for 90 days [18]. In a follow-up study by the same group, CLA mixture and individual isomers at 90% purity, fed at 1% of the diet, significantly inhibited atherosclerosis in rabbits fed with 0.2% cholesterol for 90 days. However, there were no differences between the CLA groups [79]. As little as 0.05% CLA was shown to reduce severity of atherosclerotic lesions in rabbits; this increased inversely with CLA content in diet [80], which is a particularly interesting observation considering the low CLA content in the diet, which may be attainable in human diets.

In hamsters fed with 10% saturated fat and 0.12% cholesterol, 11 weeks of treatment with CLA (0.06%, 0.11% or 1.1%) decreased atherosclerosis, but the results were similar to that obtained for LA [17]. Conjugated LA isomer mix was shown to be more effective compared to LA control in preventing atherosclerotic lesions in hamsters fed with 20% saturated fat and 0.12% cholesterol [81]. In a recent study, c9t11 and t10c12 CLA (1%) showed decreased fatty lesions compared to LA in Syrian hamsters fed with atherogenic high-fat, high-cholesterol diet, although the results were not statistically significant [82]. Another recent study showed that hamsters fed with 20% butter fat for 12 weeks showed significantly lower aortic lipid deposition when the diet was supplemented with 1% c9t11 CLA [83]. These studies suggest that in hamsters, CLA or the individual isomers may be more effective against atherosclerosis when diet is high in saturated fat content.

Surprisingly, in the C57BL/6 mouse atherosclerosis model, CLA increased the development of aortic fatty streaks [84]. In contrast, structured lipids containing 0.6% CLA decreased aortic fatty streaks formation in C57BL/6 mice compared to olive oil- and lard-supplemented diets [85]. In athero-sclerosis-prone ApoE knockout mice fed with 1% cholesterol, intake of 80:20 (c9t11:t10c12) CLA isomer blend (1%) not only prevented progression, but completely abolished atherosclerosis compared to 1% saturated fat control [86].

Unlike their effect on atherosclerotic lesions, there have been considerable variations in the effects of CLA or its isomers on atherogenic risk factors. In hypercholesterolemic hamsters, CLA reduced total cholesterol (TC), triglycerides (TGs) and non-high-density lipoproteins without affecting high-density lipoprotein cholesterol (HDL-C) compared to LA [17]. In another study, CLA mix and t10c12 isomer, not c9t11, decreased low-density lipoprotein cholesterol (LDL-C) and HDL-C, but increased very low-density-lipoprotein cholesterol (VLDL-C); however, there was no effect of c9t11 [87]. This study suggested that t10c12 may be the active isomer influencing lipid profiles. Yet another study in high-fat-fed hamsters showed that 1% CLA isomer mix lowers plasma TG and TC without effecting HDL-C compared to 0.2% c9t11 CLA or LA, again suggesting that t10c12 may be the active isomer [40]. In contrast, a recent study in male hamsters fed with cholesterol-rich diet (0.6 g/kg), 0.5% c9t11 CLA improved HDL-C and HDL-C/LDL-C ratio [88]. Similar to the findings above, another study showed that c9t11 at 1% of the diet significantly reduced non-HDL-C/HDL-C ratio in hamsters [83]. In a study in SD rats, intake of hydrogenated SBO containing 21% CLA for 6 weeks decreased TG, TC and HDL-C levels, whereas it increased the HDL-C/TC ratio [49].

In rabbits, CLA mix was first shown to decrease TC and TG, but a subsequent study showed that it increased both cardiovascular risk factors [16,18]. In SD rats, 3 g/100 g CLA mix lowered VLDL-C without effecting LDL-C and HDL-C compared to SFO control [89]. Another study in rats showed that 3% and 5% CLA isomer mix decreased LDL-C and HDL-C [90]. In C57BL/6 mice fed with CLA or structured lipids containing 0.6% CLA, improved HDL-C, and decreased TC and TG levels were noted in serum compared to those in the respective controls [84,85].

The underlying mechanisms involved in the antiatherosclerotic and lipid-lowering effects of CLA or individual isomers have not been adequately addressed in both in vitro and in vivo studies. Some of the proposed mechanisms include their role on peroxisome proliferator-activated receptors (PPARs), sterol regulatory element-binding proteins (SREBPs) and SCD. Peroxisome proliferator-activated receptors are ligand-activated nuclear receptors regulating the expression of genes that control lipid and glucose homeostasis, thus modulating the major metabolic disorders predisposing to atherosclerosis [91]. Moreover, PPARs exert additional anti-inflammatory and lipid-modulating effects in the arterial wall, therefore being interesting molecular targets for the treatment of atherosclerosis [92]. Peroxisome proliferator-activated receptor α regulates the expression of genes involved in fatty acid oxidation and energy homeostasis. Natural ligands of the PPARα include fatty acids and their derivatives (eicosanoids, 8S-HETE) and leukotriene B4. Peroxisome proliferator-activated receptor γ is expressed primarily in adipose tissue and induces expression of genes that promote lipid storage including lipoprotein lipase that is critical in the removal of TG-rich lipoproteins [93]. Studies with pure isomers suggest that c9t11 isomer is more effective than t10c12 isomer in modulating key modulators of lipid metabolism. Although both c9t11 and t10c12 isomers are ligands for PPAR-activated receptor PPARα, results suggest that c9t11 isomer is the more potent activator of the two [94]. However, the recent finding that CLA-fed PPARα null mice have lower plasma TG levels suggests that lipid-lowering effects of CLA may be independent of PPARα [95]. Sterol regulatory element-binding protein 1 isoforms regulate fatty

acid and TG synthesis [96]. Studies suggest that liver SREBP-1c expression is dependent on the nuclear hormone receptor liver X receptor (LXR). C9t11 isomer was shown to down-regulate mRNA expression of LXR-α and SREBP-1c, whereas the t10c12 isomer had no effect [97]. The results suggested that c9t11 isomer positively influences lipid metabolism by reduced synthesis and cleavage of hepatic SREBP-1, which in turn is regulated by hepatic LXRα expression. Sterol regulatory element-binding protein 1c enhances the transcription of the genes required for fatty acid synthesis and fatty acid elongation including FAS and SCD [98]. Stearoyl-CoA desaturase gene expression is highly regulated and may be influenced by dietary lipids, hormones, peroxisomal proliferators, etc. [99]. Stearoyl-CoA desaturase knockout mice have lower synthesis of TG and cholesterol esters [100]. The SCD1-deficient animals also produce low levels of VLDL, suggesting that the rate of VLDL production might itself be influenced by SCD1 activity [100,101]. Both isomers have been shown to inhibit SCD activity in breast tumor cell lines [102], but in HepG2 cells and 3T3-L1 cells, t10c12 isomer showed lower SCD activity and expression compared to the c9t11 isomer [41,103]. Thus, inhibition of SCD1 activity could be one of the mechanisms involved in the lipid-lowering effect of CLA.

Hypertension is also a common pathological state associated with an increased risk of cardiovascular diseases. Nagao et al. have consistently shown that CLA (50:50) or the t10c12 isomer, but not c9t11 isomer, decreases blood pressure and hypertension in various rat models prone to develop obesity, diabetes and obesity together, or hypertension [62,104,105].

3.2. Clinical studies

Compared to studies in animal models, there have been very few human studies that have evaluated the effects of CLA or individual isomers on risk factors for cardiovascular health. Moreover, there have been considerable variations between different studies also. Benito et al. [106] reported that there was no change in plasma lipid or lipoprotein levels after intake of 3.9 g CLA/day containing 11.4% c9t11 isomer and 14.7% t10c12 isomer. Smedman and Vessby [107] showed that intake of 4.2 g CLA/day for 12 weeks did not affect serum lipid or lipoprotein concentrations. No effect on serum TG, TC, and HDL-C was again reported by Petridou et al. [67] when they studied the effect of supplementation of 2.1 g CLA/day for 45 days in nonobese young sedentary women. In contrast, Mougios et al. [71] showed that CLA supplementation (0.7 g/day for 4 weeks) not only decreased serum TC and TG but also decreased HDL-C.

Intake of 3 g of CLA isomer mix per day (c9t11 50%+t10c12 50%) decreased TG levels, whereas c9t11 80%+t10c12 20% decreased VLDL-C in nonobese subjects. However, there was no effect on LDL-C and HDL-C [108]. In a recent study in healthy men, supplementation of CLA enriched in either c9t11 or t10c12 showed that mean plasma TG, LDL-C/HDL-C and TC/HDL-C increased with t10c12 CLA and decreased with c9t11 CLA [109]. Intake of 3 g/day of CLA (50:50 c9t11 and t10c12) for 8 weeks increased HDL-C and reduced LDL-C/HDL-C ratio in type 2 diabetic patients without affecting other inflammatory markers of CVD like C-reactive protein and interleukin (IL) 6 [110]. In a study in obese men with signs of metabolic syndrome, intake of 3.4 g/day CLA mix or t10c12 CLA for 12 weeks decreased HDL-C [73]. However, in a follow-up study in obese men, c9t11 CLA did not induce any change in serum lipids or lipoproteins compared to olive oil control [111]. A recent study evaluating the safety and efficacy of CLA (supplemented in TG form) for 24 months in overweight humans showed decreased plasma TC and LDL-C with unchanged HDL-C and TG levels [77]. In another recent study in overweight subjects with LDL phenotype B, intake of c9t11 and t10c12 CLA isomers (3 g/day) as a drinkable dairy product for 13 weeks had no effect on serum levels of LDL-C, HDL-C and TG [112].

In summary, animal studies have consistently indicated that intake of CLA either prevents or reduces the growth of preformed atherosclerotic lesions. However, one of the outcomes that emerge from these studies is that beneficial effects of CLA or its isomers on cardiovascular risk factors like plasma lipids and lipoproteins are inconsistent and do not necessarily correlate with beneficial effects on incidence of atherosclerosis. More studies are necessary with different ratios of isomers and also purified isomers to clearly establish their antihyperlipidemic and antiatherosclerotic activities. There is a complete lack of information on CLA and its impact on atherosclerosis during long-term intervention studies in animals, which needs to be pursued in the near future. The results from clinical studies have not reflected the beneficial effects seen with CLA intake in some animal studies. Data from human studies are mainly on the plasma levels of atherogenic lipids and lipoproteins. Antiatherosclerotic or plaque regression studies that have been fairly consistent in animal studies have not been reported so far in humans for obvious reasons. Lack of consistent data in human studies could partly be because human trials have differed in terms of the type of controls used, diet control, metabolic state of the subjects, dosage and period of CLA supplementation. However, recent observation that CLA decreased some atherogenic risk factors in healthy overweight volunteers in a 24-month study is encouraging. The cardiovascular effects of CLA are summarized in Table 2. More long-term studies are urgently needed in different populations before CLA or purified CLA isomers can be recommended for improving cardiovascular health in humans.

4. Conjugated LA and carcinogenesis

4.1. Conjugated LA and gastrointestinal and colon cancer

Information on the beneficial effects of CLA on gastrointestinal and colon cancer has been derived mainly from animal and in vitro studies.
Healthy volunteers

A. Lipid and lipoproteins

Model
Atherosclerosis
Lipids and lipoproteins
Hypertension
Abdominal obese men
Type 2 diabetic patients
Normolipidemic subjects
Healthy volunteers
Overweight with LDL phenotype B
Young healthy women

Significant findings
CLA and 90% enriched isomers — rabbits
++ low-fat diet ↓ high-fat diet — hamsters
++ c9t11 and t10c12 — atherogenic diet — hamsters
↓ c9t11 — butter fat — hamsters
↑ CLA — C57BL/6 mice
80:20 c9t11: t10c12 ↓ ApoE−/− mice
↓ TC, TG, lipoproteins ↔ HDL-C — hypercholesterolemic hamsters
↑ TC, TG ↔ HDL-C — high-fat diet — hamsters
c9t11 ↓ non-HDL-C/
HDL-C ↑ HDL-C,
HDL-C/LDL-C — hamsters
Soyabean oil containing
CLA ↓ TG, TC, HDL ↓ C HDL-C/cholesterol — rats
↑ HDL-C/TC ↓ TG — C57BL/6 mice
t10c12 ↓ serum and hepatic TG ↔ c9t11
↓ TC, TG — rabbits
↑ TC, TG — rabbits
↓ blood pressure and hypertension
t10c12 ↓ blood pressure and hypertension ↔ c9t11
12 weeks CLA, t10c12 ↓ HDL-C
12 weeks c9t11 ↔ lipids, lipoproteins
↓ LDL-C/HDL-C ↑ HDL-C
50:50 ↓ TG 80:20 ↓ VLDL-C
++ lipids, lipoproteins
++ TG, cholesterol, HDL-C
↑ TG, LDL-C/HDL-C, total/HDL-C with enriched
t10c12 ↓ enriched c9t11
24 months cholesterol, HDL-C ↔ HDL-C, TG
++ 13 weeks c9t11 and t10c12 — serum lipids
++ 45 days — serum lipids

References
[16–18,79,80]
[17,81]
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[16]
[18]
[104,105]
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4.1.2. Animal studies

As early as in 1990, a study showed that CLA inhibited the initiation of mouse forestomach tumorigenesis induced by benzo(a)pyrene (BAP) and found CLA to be a more potent antioxidant than α-tocopherol or butylated hydroxytoluene [8]. A subsequent study evaluating the effects of 98% pure c9t11 and t10c12 CLA isomers in BAP-induced forestomach neoplasia indicated that individual isomers have stronger inhibitory effects compared to a mixture of CLA isomers [120]. Conjugated LA showed protective effects against colon carcinogenesis induced by 2-amino-3-methylimidazoquinoline in F344 rats [11]. In a recent study, CLA at 1% of the diet, for 30 weeks, reduced 1,2-dimethylhydrazine (DMH)-induced tumor incidence in the colon of SD rats, possibly through induction of apoptosis [121]. The same study showed decreased mucosal levels of PGE2, thromboxane B2 and arachidonic acid indicating the involvement of eicosanoids in decreasing the tumor incidence. In the same year, another study examined the effects of CLA on the promotion of colon carcinogenesis induced by a single dose of azoxymethane [122]. Sprague–Dawley rats fed with 1% CLA for 12 weeks did not show any difference in aberrant crypt foci or average crypt multiplicity when compared to control. The study concluded that elevated levels of insulin in CLA-fed mice may have countered the colon-carcinogenesis inhibitory effects of CLA. One group examined the dose-dependent inhibitory effects of CLA on mammary and colon carcinogenesis induced by treatment with 7,12-dimethylbenzanthracene and DMH in SD rats and found that dietary level of 1%
CLA as the optimal dose for suppression of carcinogenesis in both target organs [123]. Another study examined the role of purified isomers fed at 1% of the diet on Min mouse model of intestinal carcinogenesis and found that t10c12 isomer promoted carcinogenesis instead of inhibiting it. The authors suggested that activation of NF-κB pathway and cyclin D1 could be involved in the cancer-promoting effect of t10c12 isomer [124]. The same study also showed that t10c12 significantly elevated 8-iso-prostaglandin PGF$_{2\alpha}$ levels, which is a marker for lipid peroxidation. A study examining the effects of CLA on DMH-induced colon carcinogenesis in SD rats showed decreased PGE$_2$, thromboxane B$_2$ and increased apoptotic index values, indicating that beneficial effects of CLA may be mediated through modification of signal transduction in colonic mucosal cells [125]. A recent study in BALB/c nu/nu mice, inoculated with MKN28 (human gastric cancer cells) and Colo320 (human colon cancer cells) in their peritoneal cavity, showed decreased metastatic foci in peritoneal cavity with CLA intake, indicating that CLA inhibited metastasis of human gastric and colon cancer cells [126]. Another recent study confirmed the findings of the previous studies and showed that 1% CLA decreased colon cancer in rats by decreasing PGE$_2$ levels and increasing Bax/Bcl-2 ratio [127].

4.1.3. Clinical studies

There has been just one human study that suggests that intakes of high-fat dairy foods and CLA may reduce the risk of colorectal cancer [128]. In the Swedish Mammography Cohort study, for each daily increment of two servings of high-fat dairy foods, the risk of colorectal cancer decreased by 13% and the risk of distal colon cancer decreased by 34%. The study concluded that the observed protective effect of high-fat dairy foods may only be partly attributed to CLA intake.

In summary, both in vivo and in vitro studies suggest that CLA (both isomers in equal proportion) and particularly the t10c12 isomer alone can prevent GIC and colon cancer. However, lack of studies in humans makes it difficult to recommend CLA as a therapy for GIC and colon cancer at this time.

4.2. Conjugated LA and breast cancer

4.2.1. In vitro studies

Initial study in MCF-7 breast cancer cells showed that CLA was growth inhibitory in culture, but was more cytotoxic to MCF-7 cells [129]. That CLA has cell cycle inhibitory effects on estrogen receptor-positive MCF-7 cells compared to LA, whereas there was no effect on estrogen receptor-negative MDA-MB-231 cells, was shown in another study [130]. Conjugated LA inhibited growth and thymidine incorporation of MCF-7 cells, whereas LA was found to be stimulatory [131]. The addition of CLA along with a lipoxigenase inhibitor resulted in synergistic growth suppression, suggesting that effects of CLA may be mediated through lipoxigenase inhibition. Conjugated LA was shown to be cytotoxic and to induce lipid peroxidation in MCF-7 cell line irrespective of the fact that it induced activities of antioxidant enzymes [132]. The same group indicated that milk-fat TG-bound CLA, consisting primarily of c9t11 isomer, was cytotoxic toward MCF-7 cells, although it significantly decreased cancer cells [133]. Another study by the same group indicated that growth-suppressing effects of CLA isomers in MCF-7 cells might be related to changes in arachidonic acid distribution and an altered PG profile [134]. One study showed that CLA-induced growth inhibition in MCF-7 cell line was not mediated through phospholipase C-, protein kinase C- or prostaglandin E$_2$-dependent signal-transduction pathways [135]. Conjugated LA significantly inhibited proliferation of MCF-7, MDA-MB-231 and MCF-10a mammary cells in another study. The study suggested that CLA exerts proapoptotic effects through both p53-dependent and independent pathways, depending on the cell type [136]. Yet another study showed that CLA or its isomers might influence essential fatty acid metabolism, leading to reduction of PGE$_2$ and tumor growth [137]. The two CLA isomers inhibit proliferation through separate mechanisms in MCF-7 cells; this was reported by another study, which showed that t10c12 inhibited cell proliferation when induced by insulin and estrogen, but not epidermal growth factor (EGF). None of these factors affected c9t11-mediated inhibition of cell proliferation [138]. A recent study showed that CLA isomers down-regulate estrogen receptor $\alpha$ expression at both mRNA and protein levels, and decrease binding of nuclear protein to a canonical estrogen response element. The study concluded that CLA isomers have significant antiestrogenic properties in MCF-7 cells, which, in part, may account for their antitumor activity in breast cancer cells [139]. In a study in estrogen-unresponsive MDA-MB-231 breast cell line, CLA reduced levels of antiapoptotic protein Bcl-x and up-regulated proapoptotic protein Bak. The study concluded that CLA triggers apoptosis through mechanisms that may involve the mitochondrial pathway [140]. In a recent study, decreased cell proliferation by CLA in MCF-7 cells was shown to be associated with increased nuclear localization of phosphorylated, activated p53 protein and decreased nuclear localization of the transcription factor FKHR/Ser256 [141]. When MCF-7 cancer cells were co-cultured with human breast stromal cells in the presence of c9t11 and t10c12 CLA isomers, both isomers decreased VEGF-A mRNA expression and protein levels in MCF-7 cancer cells [142]. However, t10c12 CLA appeared to be the more active isomer of the two.

4.2.2. Animal studies

There have been very few animal studies documenting the effects of CLA or its isomers in decreasing the risk of breast cancer. Conjugated LA was found to be an effective agent in inhibiting the development of mammary tumors.
induced by dimethylbenz(a)anthracene (DMA). Rats were fed with diet supplemented with 0.5%, 1%, or 1.5% CLA from 2 weeks prior to carcinogen administration and continued until the end of the experiment [10]. The mammary adenocarcinomas decreased by as much as 60%. The final tumor incidence and cumulative tumor weight were also lowered in rats fed with CLA diets. An interesting study evaluated the effects of CLA in inhibition of DMA-induced mammary cancer, when animals were fed with diets containing increasing levels of fat and differed in terms of type of fat ingested. The results suggested that protective effect of CLA was not influenced by the level or type of fat [144]. In another study, intake of 1% CLA for 14 weeks in severe combined immunodeficient (SCID) mice significantly inhibited growth of human breast adenocarcinoma cells (MDA-MB468) and prevented metastases to lungs, peripheral blood and bone marrow, further supporting the role of CLA in tumor suppression [145]. Another group demonstrated that CLA had a significant inhibitory effect on latency, metastasis and pulmonary tumor burden of transplantable murine mammary tumors in mice fed with 20% fat diets [145]. Recent studies by Ip et al. have indicated that CLA may prevent breast cancer through its antiangiogenic activity. This has been discussed in a separate section in this review.

4.2.3. Clinical studies

There are few studies in humans that have tried to establish an association between CLA with lowered risk of breast cancer. Two reports in the 2002 (Netherlands Cohort Study and Tours, France) indicated that CLA content in diet or adipose tissue from patients was not associated with lowered risk of breast cancer [146,147]. In a separate study, Chajes et al. [148] evaluated the CLA content of breast adipose tissue in a cohort of patients with already localized breast cancer and tried to find an association between CLA and risk of metastasis in these patients. Although the results from the study were inconclusive, a hypothesis was extended that higher intake of CLA may have a protective effect on metastasis. The results from a previous study from 1992 to 1995 in pre- and postmenopausal women with breast cancer failed to clearly establish an association between dietary CLA or serum CLA and risk of breast cancer, although it suggested that CLA-rich foods may have some beneficial effects [149]. In a recent study in breast cancer patients, although no association was established between CLA intake and risk of breast cancer, marginal association was shown between CLA and tumor biology in premenopausal women but not in postmenopausal women [150].

In conclusion, although results from animal and cell line studies indicate the beneficial effects of CLA in breast cancer, few studies in humans reported so far have not clearly established an association between dietary CLA and decreased risk of breast cancer. Since none of the human studies discussed here has actually used CLA supplementation, well-controlled and designed long-term human intervention studies are required with purified CLA supplements to fully understand the potential risks and benefits associated with CLA intake on breast cancer in women.

4.3. Conjugated LA and prostate cancer

4.3.1. In vitro studies

Two studies evaluated the effectiveness of CLA isomers on PC-3 prostate carcinoma cell line in vitro [151,152]. Both the studies showed that the isomers differed in their antiproliferative activity and that t10c12 was more potent compared to the c9t11 isomer. The first study showed that t10c12 induced caspase-dependent apoptosis. In the second study, t10c12 decreased bcl-2 expression and increased p21 (WAF/Cip1) expression, whereas c9t11 induced changes in eicosanoids production by influencing 5-lipoxygenase and cyclooxygenase expression. The study concluded that while the effects of t10c12 are mediated through modulation of apoptosis and cell cycle control, c9t11 mediates its effects through alternation in AA metabolism. A recent study in LNCaP prostate cells indicated that CLA isomers are antiproliferative and might have variable effects on protein kinase C isoforms; that, in part, may explain their antitumorogenic activity [153]. In summary, in vitro data suggest that both c9t11 and t10c12 isomers may have beneficial effects against prostate cancer, which may be mediated through different pathways.

4.3.2. Animal studies

Dietary intake of 1% CLA for 14 weeks decreased local tumor load and lung metastases in an in vivo study in SCID mice injected with DU-145 human prostatic carcinoma cells compared to mice given diets supplemented with LA or regular diet [154]. In contrast, CLA did not inhibit the in vivo growth and development of prostate tumor cells grown in Copenhagen mice inoculated with R-3327-AT-1 tumor cells. Moreover, CLA significantly increased tumor volume over controls [155].

There have been very few in vivo and in vitro experiments to form a definite conclusion regarding the beneficial effects of CLA on prostate cancer, unlike breast cancer where there is a wealth of information. Moreover, to our knowledge, there have been no published reports on the intake of CLA and risk of prostate cancer in men.

4.4. Conjugated LA and angiogenesis

Masso-Welch et al. showed for the first time that CLA could inhibit angiogenesis in vivo in implanted rat breast tumors. In this study, CD2/F(1) mice were given angiogenic challenge after treating them with 1% and 2% CLA for 6 weeks [156,157]. Pellets collected from CLA-fed mice showed fewer infiltrating cells with collapsed lumen and no RBCs compared to control mice. Conjugated LA-fed mice had lower serum and mammary gland levels of vascular
endothelial growth factor (VEGF). In the same study, c9t11 and t10c12 CLA isomers inhibited angiogenesis in vitro dose dependently. It was suggested that antiangiogenic effects of CLA could be mediated, in part, through inhibition of VEGF and its receptor Flk-1. Basic fibroblast growth factor (bFGF) is a potent angiogenic factor expressed in many tumors. In a separate study, CLA inhibited bFGF-induced angiogenesis in vivo and decreased bFGF-induced endothelial cell proliferation and DNA synthesis in vitro [158]. A subsequent study by Masso-Welch et al. showed that both c9t11 and t10c12 CLA present at 0.5% and 1.0% of the diet can inhibit angiogenesis in vivo and decrease VEGF in CD2/F(1) mice. However, leptin, the proangiogenic hormone, was decreased only with t10c12 CLA diet [159]. Collectively, the results suggest that CLA and its isomers may inhibit breast cancer, in part, through their antiangiogenic activity. More information is warranted on the antiangiogenic activity of CLA for its clinical application in cancer. To the best of our knowledge, there have been no published reports evaluating the effects of CLA on angiogenesis in humans.

The effect of CLA and purified isomers on cancer cell lines and animal models is summarized in brief in Table 3.

5. Conjugated LA and IR

5.1. Animal studies

In a ZDF rat model for obesity and diabetes, intake of 50:50 CLA mix at 1.5% of the diet normalized impaired glucose tolerance and attenuated hyperinsulinemia [21]. In another study in the same model, 50:50 CLA isomer mix improved glucose tolerance and insulin sensitivity, and decreased fasting glucose and insulin levels [20]. In the same study, 91% enriched c9t11 isomer did not have any effect on these parameters. Results from the two studies seem to suggest that t10c12 may be the isomer involved in having beneficial effects as far as IR goes. However, both studies were of very short duration (14 days). In another interesting study in ZDF rats, intake of 1.5% of both 50:50 CLA mix and t10c12 (90% enriched) decreased glucose and insulin response during an oral glucose tolerance test [160]. On the other hand, 76%-enriched c9t11 was found to be metabolically neutral in this strain of rats. Nagao et al. [104] showed that CLA attenuated plasma glucose and insulin and prevented hyperinsulinenia by enhancing plasma adiponecin levels and mRNA expression in white adipose tissue from ZDF rats.

In contrast, intake of 1% CLA in C57BL6 female mice fed with semipurified diet containing 4 g fat/100 g diet significantly decreased body fat mass with symptoms of lipoatrophic diabetes like IR and hepatomegaly [32]. In a subsequent study, intake of 1% CLA in mice fed with 34 g fat/100 g diet demonstrated normal plasma insulin levels and 45% increase in liver weight, whereas 0.1% CLA did not induce IR and demonstrated hepatomegaly, suggesting that CLA intake in the diet and amount of fat in the diet may be a determinant of lipodystrophy in mice [161]. In another study, intake of t10c12 in ob/ob mice elevated serum glucose and insulin levels and induced IR [97]. Conjugated LA at 1% of the diet did not improve insulin tolerance in high metabolic rate and low metabolic rate mice in another study [162]. Ohashi et al. [163] compared the effects of 0.5% CLA in normal C57BL/6, mildly obese/diabetic KK and morbidly obese/diabetic KKAy mice. The mice showed increased liver weight together with IR associated with hyperglycemia and hyperinsulinenia. Interestingly, in a

<table>
<thead>
<tr>
<th>Function</th>
<th>Significant findings</th>
<th>References</th>
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<tr>
<td>Angiogenesis</td>
<td>In vivo ( \downarrow ) angiogenesis in mice</td>
<td>[156–158]</td>
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<tr>
<td>In vitro ( \downarrow ) angiogenesis by c9t11 and t10c12</td>
<td>[156,157]</td>
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<tr>
<td>In vivo ( \downarrow ) angiogenesis by c9t11 and t10c12 in mice</td>
<td>[159]</td>
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<tr>
<td>Prostate cancer</td>
<td>In vivo ( \downarrow ) tumor load and lung metastases in SCID mice</td>
<td>[154]</td>
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<tr>
<td>In vivo( ++ ) growth and development of prostate tumor cells</td>
<td>[155]</td>
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<tr>
<td>Breast cancer</td>
<td>In vivo ( \downarrow ) chemically induced carcinogenesis in rats</td>
<td>[10,143]</td>
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<tr>
<td>In vivo ( \downarrow ) transplatable breast cancer tumor cells and metastasis</td>
<td>[144,145]</td>
<td></td>
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<tr>
<td>In vitro ( \downarrow ) cell cycle in MCF-7 breast cancer cells</td>
<td>[130]</td>
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<tr>
<td>In vitro ( \downarrow ) growth-suppression of MCF-7 breast cancer cells by CLA or isomers</td>
<td>[133–136]</td>
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<td>In vitro ( \downarrow ) VEGF-A in MCF-7 cells cultured with human breast stromal cells by CLA isomers</td>
<td>[142]</td>
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<tr>
<td>CLA isomers ( \downarrow ) proliferation in MCF-7 cells through different mechanisms</td>
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<tr>
<td>CLA isomers have antiestrogenic properties in MCF-7 cells</td>
<td>[139]</td>
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<tr>
<td>In humans, CLA content of diet or adipose tissue not associated with lower risk of breast cancer</td>
<td>[146,147]</td>
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<tr>
<td>No association between dietary or serum CLA and breast cancer risk</td>
<td>[149,150]</td>
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<tr>
<td>( \downarrow ) BAP-induced forestomach tumorigenesis by CLA and isomers (98% purity)</td>
<td>[8,120]</td>
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<tr>
<td>( \downarrow ) chemically induced colon carcinogenesis</td>
<td>[11,112,123,125,127]</td>
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<tr>
<td>( ++ ) azoxymethane induced carcinogenesis</td>
<td>[122,124]</td>
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<tr>
<td>t10c12 carcinogenesis in Min mouse model</td>
<td>[124]</td>
<td></td>
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<tr>
<td>( \downarrow ) metastasis of human gastric and colon cancer cells</td>
<td>[126]</td>
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<tr>
<td>( \downarrow ) HT-29 colon cancer cell growth</td>
<td>[113,114]</td>
<td></td>
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<tr>
<td>t10c12 Caco2 and HT-29 colon cancer cells but c9t11 does not</td>
<td>[115–117]</td>
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recent study in genetically obese C57BL/6 ldpob/lepob mice, intake of diet supplemented with 1.5% CLA mix or CLA enriched in t10c12 for 2 weeks elevated fasting glucose and insulin levels, and failed the glucose tolerance test [44]. However, when diet was continued for 10 weeks, CLA had beneficial effects on both glucose and insulin levels. The study suggests that although initially CLA may have negative effects of IR, long-term treatment with CLA could improve insulin sensitivity and glucose tolerance.

Studies suggest that the effects of CLA may be dependent on fat content of the diet. In an interesting study in SD rats, CLA intake (1.0%) as a mixture of isomers, c9t11 alone or t10c12 alone enhanced glucose tolerance and decreased IR index compared to control high-fat diet-fed mice suggesting that both CLA isomers have beneficial effects on IR [61]. We also found improved insulin sensitivity with decreased serum glucose and insulin with intake of 0.5% CLA in BALB/C mice fed with high-fat (20%) diet (Bhattacharya A, unpublished observation). Interestingly, in a recent study in insulin-resistant mice, intake of 1% CLA induced IR with decreased plasma adiponectin levels [164]. Glucose tolerance test indicated marked increase in insulin secretion; however, it was insufficient to prevent glucose intolerance. But, when diet was high in fat, CLA did not affect any of the parameters.

5.2. Clinical studies

Two studies by Riserus et al. [73] showed that both t10c12 and c9t11 isomers may decrease insulin sensitivity in humans at risk for cardiovascular diseases. In the first study, abdominally obese men were treated with 3.4 g/day CLA isomer mix and purified t10c12 CLA for 12 weeks. In the second study, subjects received 3 g c9t11 CLA/day for 12 weeks [111]. Compared to placebo, c9t11-fed subjects showed 15% decrease in insulin sensitivity and increased lipid peroxidation. The isomer t10c12 increased IR and glycemia, whereas CLA isomer mix (35.9% t10c12+35.4% c9t11) did not induce any significant changes. Interestingly, in a previous study in abdominally obese men, CLA administered at a dose of 4.2 g/day did not induce hyperinsulinemia or hyperglycemia compared to placebo [70]. However, the study was of much shorter duration (4 weeks). Another study showed an association between increased lipid peroxidation and IR with intake of t10c12 CLA isomer in abdominally obese men [165], but the study did not find any association between significantly elevated C-reactive protein and IR. In yet another study in abdominally obese men, t10c12 increased proinsulin, proinsulin/insulin ratio and C-peptide concentrations in comparison with control oil-fed subjects [166]. Adiponectin, however, did not change significantly. The change in proinsulin, but not the proinsulin/insulin ratio, was related to impaired insulin sensitivity. The study concluded that t10c12 isomer induces hyperproinsulinemia in obese individuals, which may be related to impaired insulin sensitivity, independently of changes in insulin concentrations.

In contrast to previous reports, CLA was found to improve insulin sensitivity in young sedentary humans [167]. Subjects were fed with CLA isomer mix or SFO placebo for 8 weeks, and oral glucose tolerance test was performed at 4 and 8 weeks. Conjugated LA increased insulin sensitivity index after 8 weeks, which correlated with decreased fasting insulin levels. However, there was considerable variation within the CLA group. Moreover, sample size was considerably small (CLA, 10 subjects; placebo, 6 subjects).

In a recent study in moderately overweight subjects with LDL phenotype B, intake of 3 g/day c9t11 or 3 g/day t10c12 for 13 weeks did not induce any changes in plasma levels of glucose or insulin [112]. In long-term studies by Gauillier et al. for 1 year and thereafter for 2 years, CLA did not increase fasting blood glucose and insulin levels in both the studies, suggesting the absence of adverse effects with long-term administration of CLA unlike those reported in short-term studies [77,78].

In summary, studies evaluating the effects of CLA in IR have differed in terms of duration of study (14 days vs. 5 months), metabolic state of the animal (normal vs. diabetic) and strain (mice vs. rats) used. While CLA seems to be beneficial in rat models, it seems to induce IR in mice models, which could be associated with rapid loss of fat mass together with hepatomegaly. Interestingly, rat studies have not shown enlarged livers. In our study, CLA did not induce lipodystrophy in high-fat diet-fed BALB/C mice (Bhattacharya A, unpublished observation). Moreover, t10c12 and CLA isomer mix (50:50), but not c9t11, improves glucose tolerance and IR in rat models indicating that t10c12 could be the biologically active isomer. More studies are required to fully understand the mechanisms involved in the beneficial or deleterious effects of CLA and the purified isomers in IR. The effects of CLA or individual isomers on IR are summarized in Table 4. More clinical studies of longer duration with large sample size are required to analyze the effects of purified isomers on IR and hyperinsulinemia before conclusions can be drawn regarding the influence of CLA in developing IR.

6. Conjugated LA and mediators of inflammatory response

Proinflammatory cytokines (TNF-α, IL-6, IL-1, etc.), anti-inflammatory cytokines [IL-10, interferon-γ (IFN-γ), etc.], eicosanoids (prostaglandins, leukotrienes) and nitric oxide (NO) are key inflammatory mediators that are regulated by dietary intake of PUFA including ω-6 and ω-3 fatty acids.

6.1. In vitro studies

In Jurkat T cells, CLA significantly inhibited cell proliferation and increased the expression of IL-2 and IFN-γ [168]. When RAW 264.7 macrophage cells were stimulated with IFN-γ, CLA decreased production of inflammatory mediators like prostaglandin PGE2, TNF-α,
Table 4

<table>
<thead>
<tr>
<th>Model</th>
<th>Significant findings</th>
<th>References</th>
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<tr>
<td>ZDF fat model of obesity and diabetes</td>
<td>CLA ↓ hyperinsulinemia, fasting glucose and insulin ↑ adiponectin; glucose tolerance, insulin sensitivity ↑ 76% and 91% enriched c9t11, 90% enriched t10c12 ↓ glucose tolerance</td>
<td>[20,21,104,160]</td>
</tr>
<tr>
<td>SD rats + high-fat diet</td>
<td>CLA, ↑ c9t11 and t10c12 ↓ IR index glucose tolerance</td>
<td>[61]</td>
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<tr>
<td>BALB/C mice + high-fat diet</td>
<td>CLA ↓ glucose, insulin, IR index</td>
<td>[50], unpublished data</td>
</tr>
<tr>
<td>C57BL/6 mice</td>
<td>↑ IR, hepatomegaly with low-fat diet ↔ IR ↓ liver lipodystrophy with high-fat diet ↑ liver lipodystrophy, IR, hyperinsulinemia, hyperglycemia t10c12 ↓ glucose, insulin after 2 weeks ↓ glucose, insulin after 10 weeks</td>
<td>[32,161], [163], [44]</td>
</tr>
<tr>
<td>Abdominal obese men</td>
<td>IR, glycemia, proinsulin, proinsulin/insulin ratio, lipid peroxidation c9t11 ↓ insulin sensitivity ↑ lipid peroxidation CLA++ hyperinsulinemia, hyperglycemia, insulin sensitivity t10c12 ↓ glucose, insulin, IR t10c12 ↓ insulin sensitivity ↑</td>
<td>[70,73,111]</td>
</tr>
<tr>
<td>Young sedentary humans</td>
<td>↑ Insulin sensitivity index ↓ fasting insulin</td>
<td>[167]</td>
</tr>
<tr>
<td>Overweight with LDL phenotype B</td>
<td>↔ 13 weeks c9t11 and t10c12 — plasma levels of glucose and insulin</td>
<td>[112]</td>
</tr>
<tr>
<td>Healthy overweight humans</td>
<td>12 and 24 months ↔ blood levels of glucose and insulin</td>
<td>[77,78]</td>
</tr>
</tbody>
</table>

IL-1β, IL-6 and NO. Moreover, reporter assays showed that CLA isomers activate PPAR-γ in RAW 264.7 cells and decrease mRNA expression of cyclooxygenase (COX) 2, inducible NOS and TNF-α [23]. In another study, mixed isomers of CLA and c9t11 isomer alone inhibited TNF-α production, but there was no effect of t10c12 isomer [22]. We found that CLA significantly inhibited receptor activator of NF-κB ligand (RANKL)-stimulated expression of TNF-α in RAW 264.7 cells compared to LA control (Rahman MM, personal communication). Conjugated LA diminished LPS-induced mRNA and protein expression of inducible NO synthase (iNOS) and COX-2 as well as production of NO and PGE₂ in RAW 264.7 macrophage cells [24]. Conjugated LA significantly diminished LPS-induced protein expression of the cytoplasmic phosphorylated IκBα and nuclear p65 as well as NF-κB nuclear protein–DNA binding affinity. The data from the interesting study suggested that CLA may inhibit LPS-induced inflammatory events in RAW 264.7 macrophages through modulation of NF-κB activation. We recently noted significant inhibition of NF-κB DNA binding activity in CLA-treated RAW 264.7 macrophages compared to LA-treated cells, when they were stimulated with RANKL to measure osteoclastogenesis (Rahman MM, unpublished observation).

When human aortic endothelial cells were treated with pure CLA isomers, there was less production of NO, PGE₂, 6-keto F₁₀₂ and TXB₂ compared to untreated cells [169]. Moreover, there was lower activity and mRNA expression of phospholipase A₂ in CLA-treated cells, indicating that eicosanoid formation was impaired by decreased availability of arachidonic acid for the COX pathway. In co-cultured human bronchial epithelial cells and eosinophils, c9t11 isomer was more potent in inhibiting eosinophil cationic protein formation compared to t10c12 isomer or LA [170]. C9t11 isomer also inhibited cell growth and reduced IL-8 mRNA and protein levels in the same study. In peripheral blood mononuclear cells (PBMCs) isolated from weaned pigs, cultured and treated with c9t11 and t10c12 isomers, both isomers suppressed production and expression of IL-1α, TNF-α and IL-6 and enhanced PPAR-γ activation. However, the study concluded that inhibitory activity of CLA on proinflammatory cytokines is attributable to the t10c12 isomer [171]. Various studies in cancer cells have shown either no effect or decrease in prostaglandin production and other inflammatory mediators [127,134,135,137,172].

6.2. Animal and ex vivo studies

Conjugated LA has been shown to inhibit proinflammatory cytokines in some animal studies. We recently examined the effects of CLA and moderate exercise in high-fat diet-fed BALB/C mice and found that CLA lowered TNF-α and IL-6 in serum of sedentary mice. Moreover, CLA prevented exercise-mediated increase in TNF-α production compared to control diet-fed mice [50]. Dietary intake of 1% CLA in male ICR mice for 8 weeks decreased serum TNF-α and leptin levels in another study when compared to diet enriched in LA [173]. The results correlated with decrease of fat mass in these mice [173]. In contrast, another study by the same group in male rats fed with different diets and 1% CLA for 3–4 weeks found no effect on serum levels of leptin and TNF-α, although epididymal and perirenal fat decreased significantly [174]. Another study in SD rats showed that 1.5% CLA in the diet can decrease serum TNF-α irrespective of fat content of the diet [175]. We recently found lower levels of TNF-α and IL-6 in serum of 12-month-old C57BL/6 female mice fed with 0.5% CLA isomer mix (50:50) for 10 weeks (Bhattacharya A, unpublished data). However, in another study, TNF-α mRNA expression was increased by 12-fold in adipocytes isolated from C57BL/6 female mice fed with 1% CLA [32].

In a recent study, when weaned pigs were fed with 2% CLA for 14 days and challenged with LPS, CLA alleviated growth depression, prevented production and mRNA expression of IL-6 and TNF-α, and enhanced the expression
of PPAR-γ and IL-10 in spleen and thymus [171]. Yang and Cook [22] used BALB/c mice to determine the effects of dietary CLA on body weight wasting and feed intake after LPS injection and found that CLA was protective against LPS-induced body weight wasting and anorexia. Plasma TNF-α level was lower in the CLA-fed mice compared with the CO-fed mice. In the same study, peritoneal resident macrophages were obtained for measuring TNF-α and NO production after in vitro exposure to IFN-γ and/or LPS. TNF-α production was not found to be different in peritoneal macrophages from mice fed with the dietary treatments, but less NO was produced in macrophages from CLA-fed mice upon stimulation when compared to control-fed mice. Interleukin 4 was decreased in CLA-fed mice when splenocytes were stimulated with concanavalin A (conA) for 44 h; however, IL-2 and the IL-2/IL-4 ratio was elevated.

Whigham et al. tested the effectiveness of CLA in reducing ex vivo antigen-induced release of eicosanoids in a type I hypersensitivity model. Sensitizing with ovalbumin (OVA) significantly decreased production of 6-keto PGF\(_{1\alpha}\), PGF\(_{2\alpha}\), PGD\(_2\) and PGE\(_2\) in lungs, trachea and bladders of CLA mice compared to SFO-treated mice. Moreover, leukotrienes C\(_4\), D\(_4\) and E\(_4\) also decreased in CLA-treated mice, suggesting that CLA decreases lipid-derived inflammatory mediators in this model [176]. In yet another hypersensitivity model, the same group showed that CLA significantly inhibits antigen-induced histamine and PGE\(_2\) levels [177]. Moreover, dietary intake of 2% CLA for 28 days showed improved lymphocyte proliferation, increased CD8\(^+\) lymphocyte population and reduced PGE\(_2\) and IL-1β in weaned pigs injected with OVA [178].

In a previous study, young C57BL/6NCr1BR mice (4 months) fed with 1% CLA for 8 weeks had greater splenocyte proliferation in response to conA and phytohemagglutinin (PHA) than in control mice. In old mice (22 months), splenocyte proliferation in response to conA was much higher than control mice. Young mice fed with CLA had higher splenocyte IL-2, while, in contrast, CLA-fed old mice had lower IL-2 compared to control mice [179]. Yamasaki et al. [180] isolated splenocytes from C57BL/6 mice fed with control, CLA mix, c9t11 and t10c12 and stimulated them in vitro with conA. The c9t11 isomer significantly increased TNF-α production compared to control- and t10c12-fed mice. Kelley et al. showed that intake of 0.5% c9t11 and 0.5% t10c12 CLA isomers for 56 days similarly affect immune cell function in C57BL/6 female mice. Although there was no effect of isomers on lymphocyte proliferation, number of immune cells or prostaglandin secretion in vitro, both the isomers did not positively influence the cytokine response with increased TNF-α and IL-6 and decreased IL-4 production [181]. This study indicated that there was no difference in activity between c9t11 and t10c12 isomers as far as effect on immune function is concerned. In a recent study in broiler chicks, CLA was fed in different doses (0, 2.5, 5.0 and 10.0 g/kg diet) for 6 weeks [182]. Conjugated LA enhanced PBMC proliferation in response to conA and elevated antibody production in response to sheep red blood cells. Moreover, systemic and peripheral blood lymphocytic PGE\(_2\) synthesis was decreased with 1% CLA in the diet. The study concluded that CLA enhances immune function in broiler chicks without altering growth performance.

Two studies by Yang et al. [183] evaluated the effect of CLA in autoimmune-prone NZB/W F1 mice. The first study showed that when CLA was administered after weaning, CLA prevented loss of body weight associated with kidney disease and prolonged survival compared to control diet-fed mice, although proteinuria developed first in CLA-fed mice. In the second study, CLA or CO as control diet was administered only after the onset of kidney disease in mice maintained on laboratory chow diet since weaning [184]. Here too mice survived longer in CLA-treated mice with much lower decrease in body weight. Both the studies confirmed that CLA has some protective effect against down-regulating autoimmunity.

6.3. Clinical studies

There have been very few studies that have evaluated the effects of CLA on immune function in humans. Initial study by Kelley et al. in 20- to 41-year-old young women showed no effect of 3.9 g/day CLA supplementation for 9 weeks on indices of immune status such as number of circulating white blood cells, lymphocytes and their subsets, monocytes, T-cell and B-cell lymphocyte proliferation in response to PHA, and serum antibody titers after immunization with influenza vaccine. When PBMCs, isolated from the subjects, were stimulated in vitro, CLA did not alter prostaglandin PGE\(_2\), LTB\(_4\), IL-1β, TNF-α and IL-2. It also did not change the percentage of T cells producing IL-2 and IFN-γ, and percentage of monocytes producing TNF-α [185,186]. A subsequent study in men showed similar findings when CLA isomer mix (50% c9t11+50% t10c12) or c9t11-enriched CLA (80% c9t11+20% t10c12) failed to alter immune response like TNF-α, IL-6, IFN-γ, IL-2, IL-4, PGE\(_2\) and lymphocyte proliferation in stimulated PBMCs [187]. Nugent et al. [188] showed that supplementation with similar ratio of CLA isomers did not have any immunological benefits compared to control LA.

A study in healthy human subjects showed that intake of both 80% c9t11 and t10c12 CLA for 8 weeks may positively influence immune function [189]. Both isomers similarly decreased T-cell lymphocyte activation, which negatively correlated with c9t11 and t10c12 content of PBMC. There was, however, no influence of the isomers on lymphocyte subset population, cytokine production or serum concentrations of C-reactive protein. A recent study investigated the effects of 3 g/day supplementation of CLA-TG form (50:50 c9t11 and t10c12) for 12 weeks on immune response in young healthy volunteers with SFO as control [190]. The study found plasma levels of IgA,
IgM and anti-inflammatory cytokine IL-10 to be increased with concomitant decrease in levels of IgE, and proinflammatory cytokines, TNF-α and IL-1β in CLA-supplemented subjects. Delayed-type hypersensitivity was also decreased with CLA intake. This was the first study that showed positive results of CLA supplementation on immune function.

The anti- or proinflammatory effects of CLA and individual isomers are summarized in Table 5. Both in vitro and in vivo studies in various animal models indicate that CLA has mediatory effects on cytokine and prostaglandin production, which could influence the inflammatory response. Although some studies indicate that t10c12 isomer may be more anti-inflammatory compared to c9t11 isomer, we feel that this aspect still remains to be established in both cell line as well as animal studies. Cell line and animal studies are consistent as far as the established in both cell line as well as animal studies.

It is clearly established that CLA decreases PGE2 and NO production, which could influence the inflammatory response. Although some studies indicate that t10c12 isomer may be more anti-inflammatory compared to c9t11 isomer, we feel that this aspect still remains to be established in both cell line as well as animal studies. Cell line and animal studies are consistent as far as the effect of CLA on PGE2 and NO production is concerned. It is clearly established that CLA decreases PGE2 and NO through inhibition of COX-2 and iNOS protein and mRNA expression.

Clinical studies in either plasma or PBMCs isolated from experimental subjects have not supported the anti-inflammatory effects of CLA seen in some animal and cell line studies. A recent study in young healthy volunteers showed decrease in TNF-α and IL-1β, and increase in proinflammatory IL-10 production [190]. More studies in obese and healthy humans are required before CLA or its isomers can be recommended to improve immune function.

### 7. Conjugated LA and bone health

The interest on the effects of CLA on bone arises from the fact that CLA effectively reduces body fat mass [77], which in turn reduces body weight. Reduction in body weight is associated with reduction of bone mass [191–194]. But there is convincing evidence that CLA supplementation does not reduce bone mass; rather, it actually is found beneficial to bone.

#### 7.1. In vitro studies

The effects of CLA on certain cell lines have been reported as early as in 1999, when human osteoblast-like cell lines MG63 and SaOS2 cells were treated with physiologically equivalent levels of CLA. Conjugated LA did not show any adverse effects since there was no cytotoxicity or apoptosis of cells [195]. Indirect effects of CLA on bone have been demonstrated in a study using human intestinal-like CaCo2 cells. These cells, when treated with CLA, were able to mobilize more calcium into the cells [196]. In our laboratory, we used RAW 264.7 cells to carry out a CLA dose response study. The cells were treated for 5 days and then stained for TRAP. The number of multinucleated TRAP-positive cells was found significantly reduced at the lowest concentration (1 μM) of CLA tested (Rahman et al., unpublished data).

<table>
<thead>
<tr>
<th>Table 5 Effect of CLA or isomers on inflammatory mediators</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Model</strong></td>
</tr>
<tr>
<td>Jurkat T cells</td>
</tr>
<tr>
<td>RAW cells stimulated with IFN-γ/LPS</td>
</tr>
<tr>
<td>RAW cells stimulated with RANKL/LPS-stimulated RAW cells</td>
</tr>
<tr>
<td>Human bronchial epithelial cells</td>
</tr>
<tr>
<td>RAW cells stimulated with LPS</td>
</tr>
<tr>
<td>Human aortic endothelial cells</td>
</tr>
<tr>
<td>Cultured PBMCs from pigs</td>
</tr>
<tr>
<td>Bone organ culture</td>
</tr>
<tr>
<td>Murine keratinocytes and mouse epidermis</td>
</tr>
<tr>
<td>Human osteoblast-like cell lines</td>
</tr>
<tr>
<td>Osteoarthritic chondrocytes in culture</td>
</tr>
<tr>
<td>SD rats</td>
</tr>
<tr>
<td>High-fat diet BALB/C mice</td>
</tr>
<tr>
<td>C57BL/6 mice</td>
</tr>
<tr>
<td>ICR mice</td>
</tr>
<tr>
<td>Young and old C57BL/6 mice</td>
</tr>
<tr>
<td>Pigs</td>
</tr>
<tr>
<td>Colon tumor-induced rats</td>
</tr>
<tr>
<td>BALB/C mice/cachexia</td>
</tr>
<tr>
<td>Adipocytes — C57BL/6 mice</td>
</tr>
<tr>
<td>Young women</td>
</tr>
<tr>
<td>Men/healthy volunteers</td>
</tr>
<tr>
<td>Healthy subjects</td>
</tr>
<tr>
<td>Healthy volunteers</td>
</tr>
</tbody>
</table>
7.2. Animal studies

First bone-related studies on the effects of CLA on animals have been studied as early as 1999, when Li et al. reported that CLA regulated bone metabolism by modulating IGF-I and IGFBP in young male SD rats after 42 days of treatment. More recently, a study on young male Wistar rats showed that CLA supplementation for over 8 weeks enhanced calcium absorption but showed no measurable effect on bone mass [197]. In yet another study in 12-month-old ovariectomized Fisher rats, CLA supplementation for 8 weeks showed reduced bone resorption rates [198]. Experiments conducted in our laboratory have shown that young BALB/C male mice had increased bone mass in the lumbar, cancellous and cortical bone mass in the proximal tibial metaphysis and in pure cortical bone mass in the tibia fibula junction (Banu et al., submitted for publication). Another study using female C57BL/6 retired breeders, treated with CLA for 10 weeks, also showed that there was increased bone mass in the fourth lumbar vertebra and the femoral diaphysis (unpublished data).

7.3. Clinical studies

In humans, a few clinical studies have been conducted to elucidate the effects of CLA on bone. The first clinical study, reporting the effects of CLA, was on male athletes undergoing resistance training exercise. It was reported that there were no significant changes in the markers of bone turnover, bone mass and strength [72]. Very recently, another study on healthy men reported that CLA supplementation did not change markers of bone metabolism [199]. Postmenopausal women who consumed more CLA in their regular diet had a positive relationship between intake of CLA and BMD values especially in the Ward’s triangle and total forearms. However, statistically significant differences were observed only in the total forearm BMD in those that consumed more CLA [200].

The in vitro studies have shown that CLA is not toxic to osteoblast cells, which are primarily involved in bone formation. It has also been shown that CLA can increase the rate of absorption of calcium into cells and also that CLA is capable of reducing osteoclastogenesis. There is strong evidence from animal models that CLA increases bone mass in rats and mice. Animal studies also compliment in vitro studies in relation to increased calcium absorption, suggesting that this is one of the mechanisms by which CLA increases bone mass. Data from clinical studies have also been very supportive with respect to CLA, showing that there are no negative effects on bone mass while it successfully reduces body weight. Although we can conclude that CLA is not deleterious to bone, rather it may be beneficial to bone, new clinical studies are urgently required before CLA can be used particularly to treat or prevent postmenopausal bone loss as hormone replacement therapy is found to cause adverse side effects. Effects of CLA on bone biology noted so far are summarized in Table 6.

### Table 6: Effect of CLA on bone health

<table>
<thead>
<tr>
<th>Subjects/models</th>
<th>Findings</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>MG63, SaOS2 cell lines</td>
<td>No toxicity or apoptosis</td>
<td>[195]</td>
</tr>
<tr>
<td>CaCo2 cell lines</td>
<td>↑ calcium absorption</td>
<td>[196]</td>
</tr>
<tr>
<td>RAW 264.7 cell lines</td>
<td>↓ osteoclastogenesis</td>
<td>Rahman et al. (unpublished data)</td>
</tr>
<tr>
<td>SD rats</td>
<td>Modulates IGF-I, IGFBP</td>
<td>[201]</td>
</tr>
<tr>
<td>Wistar rats</td>
<td>↑ calcium absorption</td>
<td>[197,198]</td>
</tr>
<tr>
<td>Ovariectomized Fisher rats</td>
<td>↓ bone resorption rates</td>
<td>[198]</td>
</tr>
<tr>
<td>BALB/C mice</td>
<td>↑ bone mass</td>
<td>Banu et al. (submitted for publication)</td>
</tr>
<tr>
<td>C57Bl/6 mice</td>
<td>↑ bone mass</td>
<td>Unpublished data</td>
</tr>
<tr>
<td>Athletes</td>
<td>↔ markers of bone turnover</td>
<td>[72]</td>
</tr>
<tr>
<td>Healthy adults</td>
<td>↔ markers of bone turnover</td>
<td>[199]</td>
</tr>
<tr>
<td>Postmenopausal women</td>
<td>↑ bone mass</td>
<td>[200]</td>
</tr>
</tbody>
</table>

The mechanism by which CLA acts on bone is still a mystery. Based on the direct and indirect evidences available on the effects of CLA on bone, we can list down the following modes of action: (1) decrease osteoclastogenesis (Rahman et al., unpublished data); (2) down-regulate PGE2, thereby influencing IGFs and IGFBPs and bone formation [201]; (3) alter PGE2-dependent bone resorption in the presence of PUFA [202,203]; (4) regulate leptin and in turn reduce bone resorption and increase bone formation [204–207]; (5) reduce proinflammatory cytokines like TNF-α, IL-1 and IL-6, thereby reducing bone resorption [208–211].

8. Conclusion

Hyperlipidemia, obesity, cancer, osteoporosis and diabetes are serious health problems plaguing the Western society during the aging process, which may be associated with dietary and lifestyle choices. Polyunsaturated ω-3 fatty acids and CLA significantly alter membrane composition and thereby alter cellular function. Recently, a wealth of literature available mainly from cell line and animal studies indicates that CLA and individual isomers (c9t11 and t10c12) may have numerous health benefits. Comparatively, there is very limited literature on the effects of CLA in human health. Moreover, there are considerable variations between studies, and the dramatic beneficial effects seen in some animal models have not been reflected in human studies. This can be attributed to difference in dosage of CLA used in animal and clinical studies and differences in source of CLA [whether CLA was supplemented (e.g., in form of capsules) or obtained from the diet]. For instance, body composition-, cardiovascular health- and IR-related studies have primarily used CLA supplementation, whereas studies related to cancer have correlated disease activity with CLA present in the diet. Conjugated LA obtained from the diet is high in the c9t11 isomer and contains very low
levels of t10c12 isomer. Since some human studies with CLA supplementation have yielded better results compared to studies in which CLA was obtained in the diet, CLA intake (in form of capsules) may be more effective because of the availability of enriched c9t11 and t10c12 isomers in different ratios.

There is very limited literature on human studies with individual isomers (c9t11 and t10c12) and different ratios of c9t11/t10c12, which makes it difficult to clearly establish the protective role of the biologically active CLA isomers in improving human health. While t10c12 lowers fat mass in animal models, there is insufficient data in humans to draw the same conclusion. Some studies in mice models and short-term human studies with t10c12 isomer have also reported hyperinsulinemia and IR. In contrast, adverse events were not reported with CLA 50:50 isomer mixture and the c9t11 isomer. Interestingly, recent studies in animals and humans suggest that CLA may not have adverse effects with long-term intervention and may actually be beneficial in reducing fat mass and atherogenic lipids. The c9t11 isomer-enriched CLA seems to be more effective in improving cardiovascular health. Lack of CLA supplementation studies in cancer patients makes it difficult to make any favorable conclusions regarding CLA, although both c9t11 and t10c12 isomers have proved to be beneficial in cell line studies. Recent and ongoing studies in animal models by our group suggest that CLA may have beneficial effects on bone metabolism. However, very few clinical studies have focused on the effects of CLA on bone health and cancer, and there are also differences in the way CLA was supplemented. Effect of CLA and its isomers on inflammatory mediators has not been the subject of extensive research in humans and need to be pursued urgently.

Since some adverse events are associated with the t10c12 isomer, direction of CLA studies should be shifted from 50:50 isomer mixture toward studies that use different ratios of c9t11 and t10c12 isomers (e.g., 80:20 and 20:80) to establish whether 80:20 isomer ratio will have beneficial effects on bone metabolism. However, without the side effects associated with intake of pure or enriched t10c12 isomer. Moreover, since polyunsaturated fatty acids like CLA are highly susceptible to lipid peroxidation, precautions should be taken to ensure that CLA or isomer preparations used in animal and human studies are adequately supplemented with antioxidants to prevent rancidity and to improve their shelf life. This may considerably improve experimental results and prevent some adverse side effects like lipid peroxidation. More well-controlled studies are urgently needed before CLA or enriched isomers can be recommended to humans with confidence to improve health and quality of life. Till that time, we should proceed with caution in recommending CLA to prevent health-related diseases and disorders in humans.

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