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Review

Mechanisms of body fat modulation by conjugated linoleic acid (CLA)

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Abstract

Since its discovery as an anticancer principal from ground beef extract in the 1980s, conjugated linoleic acid (CLA) has drawn much attention due to its variety of biological activities. One of the most interesting aspects of CLA is its ability to reduce body fat while enhancing lean body mass. One explanation for the variety of biological activities of CLA is that CLA is a mixture of geometric and positional isomers, although the primary research focus is on the two main isomers, *cis*-9,*trans*-11 and *trans*-10,*cis*-12. The involvement of eicosanoid metabolism has been suggested as one mechanism for CLAs wide range of biological activities. Incorporation of CLA in the sn-2 position of phospholipid fractions, and the negative correlation between tissue levels of CLA and arachidonic acid support this. Other possible mechanisms of CLA with regard to body fat reduction will be discussed, as well as differences between human and animal studies. Safety concerns regarding the use of CLA in humans are not conclusive and need further investigation.

Keywords: Conjugated linoleic acid; CLA; *cis*-9,*trans*-11 CLA; *trans*-10,*cis*-12 CLA; Body fat

Contents

1. Introduction

Lifestyle factors, including diet, influence the development of many types of diseases. In the 1970s, scientists started to search for mutagens/carcinogens from food in hopes of eventually using this knowledge to help prevent

Abbreviations: CLA, conjugated linoleic acid; TNF-α, tumor necrosis factor-α; peroxisome proliferator-activated receptor, PPAR; nuclear factor-κB, NFκB; IL, interleukin.

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cancer. Based on reports that overcooked meat contained mutagens, Dr. Pariza's group at the University of Wisconsin-Madison investigated the correlation between mutagen formation and cooking temperature and time using ground beef. To their surprise, in addition to mutagens in beef extract they also found a compound that had anti-mutagenic activity (Pariza & Hargraves, 1985). This compound was identified and named conjugated linoleic acid (CLA) based on the structural similarity to linoleic acid (Ha, Grimm, & Pariza, 1987; Pariza, Ashoor, Chu, & Lund, 1979). Further investigation established that CLA inhibited carcinogenesis in several animal models (Banni, Heys, & Wahle, 2003; Pariza, Park, & Cook, 2001). In addition to its originally identified anticancer activity, CLA has been shown a wide range of biologically beneficial activities; decreased severity of atherosclerosis (Lee, Kritchevsky, & Pariza, 1994; Nicolosi, Rogers, Kritchevsky, Scimeca, & Huth, 1997), reduction of adverse effects of immune stimulation (Cook, Miller, Park, & Pariza, 1993; Miller, Park, Pariza, & Cook, 1994), growth promotion in young rats (Chin, Storkson, Liu, Albright, & Pariza, 1994), and a reduction of body fat and an increase in lean body mass in several animal species (Cook et al., 1998; Houseknecht et al., 1998; Park, 1996; Park et al., 1997; Park, Albright, et al., 1999; Park, Storkson, Albright, Liu, & Pariza, 1999).

2. Isomers of CLA

One explanation for the variety of biological activities of CLA is that CLA is a mixture of geometric and positional isomers, with double bonds at [9, 11], [10, 12], [8, 10], [7, 9], and [11, 13]. Although a number of CLA isomers are found in food (Kramer et al., 1998), the primary research focus is on the two main isomers, *cis*-9,*trans*-11 and *trans*-10,*cis*-12. It needs to be pointed out that naturally occurring CLA primarily consists of the *cis*-9,*trans*-11 isomer (>80%) present in food, such as beef, milk, and dairy products, with other minor isomers listed above (Chin et al., 1994). This isomer originates from biohydrogenation of linoleic acid to stearic acid by rumen bacteria (Kepler, Hirons, McNeill, & Tove, 1966). An alternative source of *cis*-9,*trans*-11 CLA isomer is by delta-9 desaturation of *trans*-11 vaccenic acid in mammalian tissues (Corl, Barbano, Bauman, & Ip, 2003; Kay, Mackle, Auldist, Thomson, & Bauman, 2004). The other main isomer of synthetic CLA, *trans*-10,*cis*-12, is present in food in negligible amounts. Thus this isomer is considered to be 'man-made' and may not have much relevance if CLA is obtained from natural sources (Chin et al., 1994; Dhiman et al., 2005; Park & Pariza, 1998). Currently, most CLA research reports on the activities of these two isomers. This is not due to the other isomers having no effects, but rather that their effects are currently unknown. The known biological effect of the minor isomers is that the *trans*-9,*trans*-11 CLA isomer inhibits platelet aggregation (Al-Madaney, Kramer, Deng, & Vanderhoek, 2003; Li, Barnes, Butz, Bjorling, & Cook, 2005) and has antiproliferative effect (Lai, Yin, Li, Zhao, & Chen, 2005). This review will primarily focus on *cis*-9,*trans*-11 and *trans*-10,*cis*-12, the two main isomers.

Studies have found that the wide range of CLAs activities results from interaction between the two major CLA isomers. These major isomers have shown additive, independent, or antagonistic effects. It has been shown that both *cis*-9,*trans*-11 and *trans*-10,*cis*-12 isomers of CLA have anti-cancer effects, which can be additive (Ip et al., 2002; Masso-Welch et al., 2002; Masso-Welch et al., 2004). Others reported independent effects of these two isomers. The *trans*-10,*cis*-12 CLA isomer is known to be responsible for body fat reduction, inhibition of stearoyl-CoA desaturase, and reduction of hepatic apolipoprotein B secretion, while the *cis*-9,*trans*-11 isomer improves growth performance in rodents (Chin et al., 1994; Cook, Drake, Jerome, & Pariza, 1999; Cook et al., 1993; Park, Storkson, et al., 1999; Pariza, Park, & Cook, 2000; Storkson, Park, Cook, & Pariza, 2005; Valeille et al., 2004). Lastly, these two isomers can work against each other (antagonistic effects) such that *trans*-10,*cis*-12 isomer increased insulin resistance but *cis*-9,*trans*-11 isomer corrected this (Song et al., 2004). Hence, the many physiological effects that are reported for CLA appear to be the result of multiple interactions of the biologically-active CLA isomers with numerous metabolic signaling pathways (Pariza, Park, Xu, Ntambi, & Kang, 2003).

3. Incorporation and metabolism of CLA

CLA has been reported to be incorporated and to have a fate similar to other fatty acids in biological systems; supplementation increased tissue CLA levels incorporated into both triacylglyceride and phospholipid fractions, while withdrawal decreased CLA levels (Devery, Miller, & Stanton, 2001; Park, 1996; Park, Albright, et al., 1999; Petrik, McEntee, Johnson, Obukowicz, & Whelan, 2000; Sisk, Hausman, Martin, & Azain, 2001). In addition, CLA was detected in all phospholipid classes analyzed, including phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, and cardiolipin (Banni et al., 2001; Kramer et al., 1998; Sugano et al., 1997).

CLA has also been shown to be metabolized like other fatty acids. The elongated and/or desaturated metabolites of CLA have been detected in animal tissues fed CLA (Banni et al., 2001; Juaneda & Sebedio, 1999; Park, Storkson, Albright, Liu, & Pariza, 2005; Sebedio et al., 1999; Sebedio et al., 2001). In addition, conjugated 16, 14 and 12 carbon fatty acids, possible products from fatty acid β-oxidation of CLA, have also been reported (Park, Storkson, et al., 2005; Ringseis et al., 2006; Sebedio et al., 2001). It is noted here that the effects of CLA on body fat reduction is most likely due to CLA itself rather than its metabolites, based on the observation that conjugated eicosadienoic acid (CEA, conj. 20:2$\Delta^{c11,t13/t12,c14}$) and conjugated eicosatrienoic acid, (CETA, conj. 20:3$\Delta^{c8,t12,c14}$) have similar or less effective effects on body fat reduction than CLA, while CLA was observed in animals fed CEA

(Table 1) (Park, Storkson, et al., 2005). However, biological effects of shorter chain conjugated fatty acids are not known at this point.

Although it has been shown that CLA does incorporate into phospholipid fractions, the exact location of incorporation is not known. Thus, we tested hepatic phospholipids fractions from CLA-fed mice to determine the position of CLA in the phospholipid (Table 2). The phospholipid fractions were incubated with phospholipase A_2, which specifically hydrolyzes fatty acids from the sn-2 position. Then fatty acid composition of free fatty acids and lysophospholipid fraction were analyzed. As shown in Table 2, lysophospholipid fractions had a very small amount (none in one case) of CLA after hydrolysis with phospholipase A_2 suggesting that CLA was primarily incorporated into the sn-2 position. There was no selective incorporation of *cis*-9,*trans*-11 and *trans*-10,*cis*-12 CLA isomers into this position based on the fact that both isomers were detected. Since the sn-2 position of phospholipids is the primary location for arachidonic acid incorporation as well, it was not surprising to observe CLA feeding reduced tissue arachidonic acid levels. However, this effect has not been consistent, possibly due to the variable conditions as well as differing tissue types (Belury & Kempa-Steczko, 1997; Cook et al., 1993; Javadi et al., 2004; Park, 1996; Sugano, Yamasaki, Yamada, & Huang, 1999; Urquhart, Parkin, Rogers, Bosley, & Nicolaou, 2002; Watkins et al., 1997). By performing fatty acid analysis from tissues collected during various feeding periods, we were able to find a consistent negative correlation between levels of CLA and arachidonic acid in muscle but not in liver in rats and chickens (Fig. 1 and unpublished data), particularly muscle phospholipid fractions in mice (Park, 1996). The negative correlation between arachidonic acid and CLA was observed with both *cis*-9,*trans*-11 and *trans*-10,*cis*-12 isomers. Along with the fact that both of the two main CLA isomers are incorporated into the sn-2 position of phospholipids, this suggests that CLA competes with arachidonic acid in phospholipid fractions, resulting in possible involvement of CLA in eicosanoid production (Eder, Schleser, Becker, & Korting, 2003; Liu & Belury, 1998; Ogborn et al., 2003; Watkins et al., 1997; Whigham et al., 2001; Whigham et al., 2002). However, we cannot ignore the fact that simple replacement of arachidonic acid by CLA does not explain the whole extent of the reduction of arachidonic acid due to the relatively small quantities of

Table 1
Comparison of CLA and conjugated eicosadienoic acid on body composition in mice (Park, Storkson, et al., 2005)[A]

	ECW (g)	% Fat	% Water	% Protein	% Ash
Control 1 (Corn oil)	24.5 ± 1.2	13.10[a] ± 1.11	61.86[a] ± 0.84	17.34[a] ± 0.37	3.50[a] ± 0.11
Control 2 (EA)	24.4 ± 1.1	13.26[a] ± 1.67	61.82[a] ± 1.23	18.26[ab] ± 0.54	3.78[ab] ± 0.16
CLA	22.4 ± 1.0	6.33[b] ± 0.55	66.59[b] ± 0.48	19.34[b] ± 0.31	3.98[b] ± 0.07
CEA	22.3 ± 1.0	6.33[b] ± 0.56	66.79[b] ± 0.54	19.06[b] ± 0.36	4.00[b] ± 0.11

[A] Female mice were fed treatment diets for 4 weeks. Diet contained 5% corn oil plus 0.5% corn oil (control), CLA, eicosadienoic acid (EA, 20:2$\Delta^{c11,c14}$), or conjugated eicosadienoic acid (CEA, conj. 20:2$\Delta^{c11,t13/t12,c14}$). Numbers are mean ± SE $n = 6$. Means with different letters are significantly different ($p < 0.05$). ECW, empty carcass weight.

Table 2
Fatty acid composition (area %) of free fatty acid and lysophospholipid fractions of mouse liver[a]

Fraction	Fatty acid (Area %)								
	16:0	16:1	18:0	18:1	18:2	CLA[b]	20:3[c]	20:4	22:6
Free fatty acid	7.52	1.67	2.49	13.73	21.87	0.55	2.73	28.39	2.12
Lysophospholipid	33.9	0.45	35.4	3.53	5.47	0.09	0.30	1.78	n.d.[d]

[a] Mice were fed diet containing 0.5% CLA (w/w) for 6–7 weeks. Liver phospholipid fractions were extracted from pooled mouse liver with chloroform: methanol as previously reported (Folch et al., 1957). Phospholipid fraction was separated from non-phospholipid fraction using Sep-Pak® as described before (Juaneda & Rocquelin, 1985). Three to five mg of liver phospholipid was dried under nitrogen, suspended in sodium deoxycholate (0.5% final concentration), then glycylglycine buffer (pH 8.0) and 1 mM $CaCl_2$ were added. The reaction was started by adding 60 Units of phospholipase A_2 (from Bee Venom, Sigma Chemical Co., St. Louis, MO) and incubated in shaking water bath at 37 °C for 1.5–2 h (Jimeno-Abendano & Zahler, 1979). Total fat was extracted from the reaction mixture and free fatty acids were separated from lysophospholipid and unreacted phospholipid by preparative thin layer chromatography (silica gel plates 60 Å, 1000 μm thickness, Merck, Darmstad, Germany). The developing solvent was hexane: diethyl ether: methanol: acetic acid (90:20:5:2, v/v/v/v), and detected by I_2 vapor (Laustiola et al., 1986). Corresponding bands were scraped from plate and extracted with chloroform: methanol. After methylation, samples were subjected to Gas Chromatography to determine fatty acid compositions. Gas chromatography was conducted using a Hewlett Packard 5890 Series II fitted with a flame ionization detector and a 3396A integrator. A Supelcowax-10 fused silica capillary column (60 mm × 0.32 mm i.d., 0.25 μm film thickness, Supelco Inc., Bellefonte, PA) was used with split mode and helium as the carrier gas. Injector and detector temperatures were 250 °C. Oven temperature was programmed from 50 °C to 200 °C, increased 20 °C per min, held there for 50 min, increased 10 °C per min to 230 °C, and held for 20 min. Numbers are means of four analysis, collected from two independent experiments.

[b] CLA, conjugated linoleic acid, both *cis*-9,*trans*-11 and *trans*-10,*cis*-12 isomers were detected. The ratio of *cis*-9,*trans*-11 and *trans*-10,*cis*-12 isomers in free fatty acid fraction was 2.2:1.

[c] 20:3, *cis*-8,*cis*-11,*cis*-14 eicosatrienoic acid, dihomo-γ-linolenic acid.

[d] n.d., not detected.

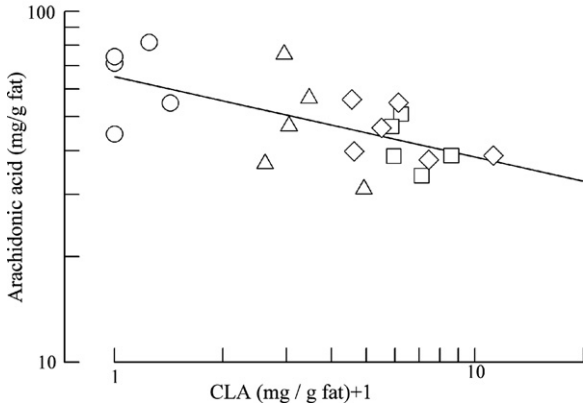

Fig. 1. Correlation between the concentration of CLA and arachidonic acid in rat muscle. Weanling Sprague–Dawley rats were purchased from Harlan Sprague–Dawley (Madison, WI), and housed in a 12 h light-dark cycled room. Semi-purified diet (TD94060, Harlan-Teklad, Madison, WI) was provided upon arrival. After adaptation, animals were divided into two groups, and fed either control diet (5.5% corn oil) or CLA-containing diet (5.0% corn oil plus 0.5% CLA) for 3–28 days (circles, control; triangles, 3 days; squares, 14 days; diamonds, 28 days). After feeding periods, animals were sacrificed by CO_2 suffocation. Muscle of individual animals were collected and subjected to fatty acid analysis as described in Table 2 legend. The correlation coefficient was 0.3735 ($p = 0.0021$).

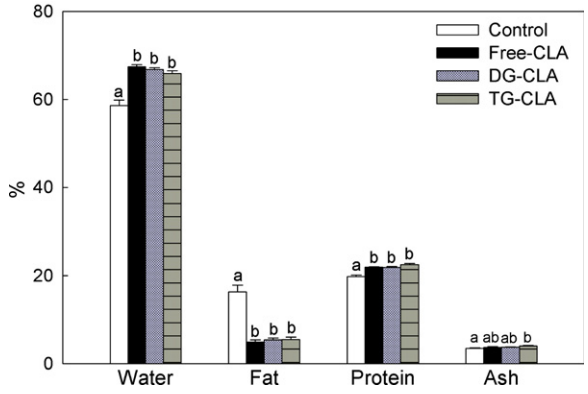

Fig. 2. Effects of CLA free fatty acid (Free-CLA), diacylglyceride containing CLA (DG-CLA), and triacylglyceride containing CLA (TG-CLA) on body composition. Four-week old ICR male mice were fed semi-purified diet containing 0.7% of CLA (w/w, equivalent to 0.5% CLA at final) with different preparations for 4 weeks. All CLA preparations contained about 73% CLA (*cis*-9,*trans*-11, 33.4–33.9; *trans*-10,*cis*-12, 34.4–35.5; *cis*, *cis*, 2.3-2.4; *trans*, *trans*, 1.6–2.0), provided by Rinoru Oil Mills Company Ltd. (Nagaya, Japan). For diet composition (TD 94090, Halran Teklad, Madison, WI) and method for body compositional analysis, please see (Park et al., 1997). Numbers are means ± SE of 6–7 animals. In each variable, means with different letters are significantly different ($p < 0.0001$ for fat, water, and protein and $p < 0.05$ for ash).

CLA compared to the changes in arachidonic acid level in the same tissue (Table 2 and Fig. 1). In fact, it has been reported that CLA can inhibit cyclooxygenase activities, which is the rate limiting enzyme for prostaglandin formation (Li et al., 2005; Zhang et al., 2005).

4. Body fat reduction by CLA

One aspect of CLA that has drawn much attention is its ability to reduce body fat in animals, first reported in 1995 (Park, Albright, Liu, Cook, & Pariza, 1995). It has since been confirmed that the *trans*-10,*cis*-12 isomer is the isomer responsible for this activity (Park, Storkson, et al., 1999). Most studies used the free fatty acid form of CLA. There are few reports comparing the effectiveness of CLA on body fat reduction, as well as its anti-cancer effects, using the triacylglyceride form (Ip et al., 2002; Rahman et al., 2001; Terpstra et al., 2003). We also tested the diacylglyceride and triacylglyceride forms of CLA, which showed the same effect on body composition as the free form of CLA (Fig. 2). When derivatives other than the acid form of CLA were tested, there was no effect on fat metabolism, suggesting the carboxyl end is necessary for CLAs activities (Cook, Drake, & Pariza, 2000; Park et al., 2004).

Earlier studies used relatively young rapidly growing animals, and thus were not able to distinguish whether CLAs reduction of body fat was due to reduced body fat accretion or reduction of existing fat. Older mice (retired breeder, older than 6 months) were used to test CLAs effect on existing fat in comparison to body fat accretion. From these studies, it was shown that CLA indeed reduced existing body fat in older mice (Mirand et al., 2004; Pariza et al., 2001; Park, Albright, Storkson, Liu, & Pariza, 2005, & unpublished data).

5. Mechanism of body fat reduction by CLA

Multiple mechanisms for CLAs effect on body fat reduction have been suggested; i.e. increasing energy expenditure, modulating adipocyte metabolism, modulating adipokines and cytokines and increasing fatty acid β-oxidation.

First, CLA may reduce body fat by increasing energy expenditure. Numerous studies have suggested that CLA increases energy expenditure as shown by increased oxygen consumption (Choi et al., 2004; Nagao et al. 2003; Ohnuki, Haramizu, Ishihara, et al., 2001; Ohnuki, Haramizu, Oki, et al., 2001; Terpstra et al., 2002; Terpstra et al., 2003; West et al., 2000), and by increased expression of uncoupling proteins by CLA, both of which are indicators for energy expenditure (Choi, Jung, Park, & Song, 2004; Ealey, El-Sohemy, & Archer, 2002; Nagao, Inoue, et al., 2003; Peters, Park, Gonzalez, & Pariza, 2001; Roche et al., 2002; Ryder et al., 2001; Tsuboyama-Kasaoka et al., 2000; Tsuboyama-Kasaoka, Miyazaki, Kasaoka, & Ezaki, 2003).

Secondly, CLA reduces body fat by reducing adipose cell mass and/or cell numbers. This can be achieved in part by (1) inhibiting lipoprotein lipase at adipose cells; (2) inhibiting stearoyl-CoA desaturase activities; (3) enhancing apoptosis of preadipocytes and adipocytes; and (4) modulating lipolysis. Since lipoprotein lipase is the key enzyme for fat uptake, inhibition of adipose lipoprotein lipase results in reduced fat uptake (Lin, Kreeft, Schuurbiers, &

Draijer, 2001; Park et al., 1997; Park, Storkson, et al., 1999; Park et al., 2004). This has been shown consistently with the *trans*-10,*cis*-12 isomer but not with *cis*-9,*trans*-11 (Lin et al., 2001; Park, Storkson, et al., 1999; Park et al., 2004). Stearoly-CoA desaturase is the rate-limiting enzyme for converting saturated fatty acids to monounsaturated fatty acids, the main substrate for fat deposit in adipose tissue (Cohen et al., 2002; Ntambi et al., 2002). Thus inhibition of stearoly-CoA desaturase by CLA may in part contribute to reduced fat mass (Choi, Park, Storkson, Pariza, & Ntambi, 2002; de Veth, Griinari, Pfeiffer, & Bauman, 2004; Lin, Loor, & Herbein, 2004; Park et al., 2000). CLA is also reported to reduce adipose tissue mass and cell numbers by increased apoptosis of preadipocytes and adipocytes (Brodie, Manning, Ferguson, Jewell, & Hu, 1999; Brown, Halvorsen, Lea-Currie, Geigerman, & McIntosh, 2001; Brown et al., 2003; Cohen et al., 2002; Evans et al., 2000; Evans et al., 2001; Granlund, Juvet, Pedersen, & Nebb, 2003; Hargrave et al., 2002; Hargrave et al., 2004; Kang, Liu, Albright, Park, & Pariza, 2003; McNeel & Mersmann, 2003; McNeel, Smith, & Mersmann, 2003; Miner, Cederberg, Nielsen, Chen, & Baile, 2001; Satory & Smith, 1999; Tsuboyama-Kasaoka et al., 2000; Warren et al., 2003). An effect of CLA on lipolysis has been suggested, but it has not been consistently shown (Chung, Brown, Sandberg, & McIntosh, 2005; Park et al., 1997; Park, Storkson, et al., 1999; Simon, Macarulla, Fernandez-Quintela, Rodriguez, & Portillo, 2005; Xu et al., 2003).

It has been suggested that CLAs effect on adipocytes may be linked to interaction between CLA and peroxisome proliferator-activated receptor-γ (PPAR-γ). PPAR-γ is the nuclear receptor super family, controls lipid metabolism in adipose tissue, and regulates adipocyte differentiation, proliferation, and lipogenesis (i.e. LXRα, aP2, or CD36) (Gregoire, Smas, & Sul, 1998; Ntambi, Choi, & Kim, 1999; Tontonoz, Hu, Graves, Budavari, & Spiegelman, 1994). Thus reduced PPAR-γ can result in effects seen by CLA. In fact CLA has been shown to reduce PPAR-γ expression in a number of publications, while others found that CLA increased PPAR-γ expression (Brown et al., 2001; Brown et al., 2003; Choi et al., 2000; Clement et al., 2002; Corino, Mourot, Magni, Pastorelli, & Rosi, 2002; Granlund, Pedersen, & Nebb, 2005; Lin et al., 2004; McNeel & Mersmann, 2003; McNeel et al., 2003; Zabala et al., 2004). It has also been reported that CLA acts as a strong agonist for PPAR-α (predominant form in liver) (Moya-Camarena, Van den Heuvel, & Belury, 1999; Moya-Camarena, Vanden Heuvel, Blanchard, Leesnitzer, & Belury, 1999).

Further studies suggested that CLA may affect PPAR-γ through nuclear factor-κB (NFκB) which controls important cell signaling, including mitogen-activated protein kinase/extracellular signal-regulated kinase, and tumor necrosis factor-α (TNF-α) (Brown et al., 2004). Activation of NFκB by CLA has been reported but results are not consistent; CLA decrease NFκB activation when stimulated by lipopolysaccharide, TNF-α, or interferon-γ, but others reports showed either no effect or increased NFκB activation by CLA, particularly by the *trans*-10,*cis*-12 isomer (Bassaganya-Riera et al., 2004; Cheng, Lii, Chen, Lin, & Liu, 2004; Chung, Brown, Provo, Hopkins, & McIntosh, 2005; Li et al., 2005; Loscher et al., 2005; Rajakangas, Basu, Salminen, & Mutanen, 2003; Ringseis, Saal, Muller, Steinhart, & Eder, 2004; Schleser, Ringseis, & Eder, 2005; Sheu et al., 2006). Chung, Brown, Provo, et al. (2005) recently demonstrated that *trans*-10,*cis*-12 CLA activates NFκB both in stromal vascular cells and adipocytes but with different roles. CLA activates NFκB in storomal vascular cells, resulting in secretion of interleukin-6, interleukin-8, and TNF-α. Subsequently, in adipocytes, these cytokins activate NFκB, and also activate extracellular signal-related kinase (ERK), which phosphorylates NFκB and other transcription factors including PPAR-γ, resulting in reduced adipogenic gene expression, although the mechanisms of interaction between ERK and other transcription factors are not fully understood (Chung, Brown, Provo, et al., 2005).

Thirdly, CLA may reduce body fat by modulating adipokines and cytokines. CLA has been shown to reduce expression and secretion of leptin (Inoue, Nagao, Hirata, Wang, & Yanagita, 2004; Kang & Pariza, 2001; Nagao, Inoue, et al., 2003; Rahman et al., 2001; Ryder et al., 2001). Reduction of leptin levels by CLA may be explained by the fact that CLA reduced the total amount of adipose tissue, however, the lack of effects by reduced levels of leptin (increasing food intake) is still in question. CLA increased adiponectin and decreased TNF-α (Inoue et al., 2004; Nagao, Inoue, et al., 2003; Nagao, Inoue, Wang, Shirouchi, & Yanagita, 2005; Pariza et al., 2000; Park et al., 2003; Yang & Cook, 2003), which may help improve insulin sensitivity and appears to be a key mediator in many chronic pathologies including obesity (Hotamisligil & Spiegelman, 1994). Others have reported modification of interleukins by CLA as well (Bassaganya-Riera et al., 2003; Bhattacharya et al., 2005; Brown et al., 2004; Changhua et al., 2005; Chung, Brown, Provo, et al., 2005; Loscher et al., 2005; Luongo, Bergamo, & Rossi, 2003). Control of cytokines and adipokines may be directly and indirectly linked in part to cellular signal transduction pathways as discussed above.

Lastly, CLA increases fatty acid β-oxidation in skeletal muscle (Pariza et al., 2001; Wahle, Heys, & Rotondo, 2004). This was suggested by enhanced activity and/or expression of carnitine palmitoyl transferase I (CPT I, the rate-limiting enzyme for fatty acid β-oxidation) in skeletal muscle (Bouthegourd et al., 2002; Degrace et al., 2004; Nagao et al., 2005; Park et al., 1997; Peters et al., 2001). An interpretation is that in biological systems CLA causes fat to be preferentially used as an energy source compared to control, which in turn helps to reduce body fat deposit. Degrace et al. (2004) reported that CLA may reduce fatty acid oxidation due to an increased level of malonyl-CoA, which is the natural inhibitor for CPT. However, in the same report malonyl-CoA concentrations in liver were

determined but not in muscle, where fatty acid oxidation is directly linked to body fat use as energy.

6. Body fat reduction in human studies

Effects of CLA on body fat in human studies are summarized in Table 3. Compared to the body fat reduction seen in mice, there are limited responses to CLA in other species. For example, CLA was less effective in rats (particularly males), pigs, and especially in humans (Dugan, Aalhus, & Kramer, 2004; Mirand et al., 2004; Park, 1996; Park, Albright, & Pariza, 2005; Terpstra, 2004; Wang & Jones, 2004). Differences in CLAs effect on body fat reduction in humans compared to the mouse model may be due first to the relatively low dose used in human studies compared to that used in mice. Mice were fed diet containing 0.5 w/w% CLA, which is equivalent to about 56 g CLA/day/70 kg (Malpuech-Brugere et al., 2004). CLA doses used in human studies were between 0.7 g and 6.8 g per day, which is lower than doses used in mice. Secondly, different responses to CLA between mice and humans may be due to differences in metabolism. The fact that mice have a higher rate of fat turnover than other species may explain why CLA was most effective in older mice (Terpstra, 2001). Thirdly, most human clinical trials assume CLA will reduce pre-existing body fat in adult humans (Ostrowska et al., 2003). Although CLA reduced accumulated body fat in mice (Pariza et al., 2001), CLAs effectiveness in reducing existing fat in humans is less clear. Lastly, human studies involved different dietary regimes, *ad libitum* in animal models (especially in growth phase, a positive energy balance) compared to calorie restriction in some human trials (negative energy balance). In fact, when mice were given CLA during dietary restriction (negative energy balance), no additional benefits of CLA were observed on body weight and body fat. However, if given during positive energy balance CLA reduced body fat gain (Park, Albright, Storkson, et al., 2005). Similarly, it has been reported in human studies that CLA may be effective in reducing fat mass gain during weight gain period (a positive energy bal-

Table 3
Summary of conjugated linoleic acid's effects on body mass in human studies

References	Dose (g/d)	Subjects (BMI, kg/m^2)	*n*	Duration	Results[a]
Atkinson (1999)	2.7	MF (28–30)	80	6 months	↓ BF, – BW, ↓ BFG
Blankson et al. (2000)	1.7, 3.4, 5.1, 6.8	MF (25–35)	47	12 wk	↓ BF, – BMI, ↑ FFM with 6.8 g
Berven et al. (2000)	3.4 (4.5 g 80%)	MF (27–39)	55	12 wk	↓ BW, ↓ BMI
Zambell et al., 2000	1.95	F	17	8 wk	– FFM, – BF, slight ↓ BW – energy expenditure
Riserus et al. (2001)	4.2	M (32)	24	4 wk	↓ SAD, – BMI, – BW
Smedman and Vessby (2001)	4.2	MF (25)	53	12 wk	↓ BF, – BW, – BMI, – SAD
Mougios et al. (2001)	0.7 and 1.4	MF (<30)	22	4wk each Total 8wk	↓ BF
Thom et al. (2001)	1.08	MF (<25)	10	12 wk	↓ BF, – BW, – BMI
Riserus, Arner, et al. (2002)	3.4, mix or 10,12	M (27–39)	57	12 wk	↓ BF, ↓ SAD, – BMI, – BW (↓ BW, ↓ BMI for *t*10,*c*12 only)
Kreider et al. (2002)	3.9	M [exercise]	23	4 wk	– BM, – FFM, – BF, – bone M, no benefit of exercise
Noone et al. (2002)	3 (50:50, 80:20)	MF (<25)	51	8 wk	– BW [exercise]
Kamphuis et al. (2003a)	1.8 or 3.6	MF (28)	54	13 wk	– BWG, favorable appetite effects
Kamphuis et al. (2003b)	1.8 or 3.6	MF (28)	54	13 wk	– BWG, ↑ FFM gain, ↓ BF ↑ resting metabolic rate
Belury et al. (2003)	6	MF [T2DM][b]	21	8 wk	↓ BW, ↓ BF with *t*10,*c*12
Gaullier et al. (2004)	3.4, TG, FFA	MF (25–30)	180	1 yr	↑ FFM, ↓ BW, ↓ BMI, ↓ BF ↓ Bone Mass
Malpuech-Brugere et al. (2004)	1.5 or 3 g of *c*9,*t*11 or *t*10,*c*i12	MF (overweight)	81	18 wk	Slight ↓ BF (both isomers) – BW, slight ↓ BF
Riserus, Vessby, Arner, et al. (2004)	3.4, mix or 10,12	MF (30)	57	12 wk	Slight ↓ BMI
Riserus, Vessby, Arnlov, et al. (2004)	3 (83% mainly *c*9,*t*11)	MF (27–35)	25	12 wk	Slight ↓ BMI, ↓ BW, – BF
Eyjolfson et al. (2004)	3	MF	16	8 wk	– BW, – BMI, – BF
Whigham et al. (2004)	6	MF (27–35)	47	16 wk+ 24 wk	– BW, – BF
Gaullier et al. (2005)	3.4	MW	134	2 yrs	↓ BF, ↓ BW, ↓ BMI
Larsen et al. (2006)	3.4	MW (>28)	83	1 yr	– BWG, – BFG, – FFM (weight gain period)

[a] ↑ increased; ↓ decreased; –, no change; M, male; F, female; TG, triacylglyceride; FFA, free fatty acid; BW, body weight; BMI, body mass index; BF, body fat; FFM, fat free mass; BWG, body weight gain; FMG, fat mass gain; slight indicates non-significant change.
[b] Type 2 Diabetes mellitus.

ance) following weight loss, although the re-gain of body weight per se was not reduced by CLA (Atkinson, 1999; Kamphuis, Lejeune, Saris, & Westerterp-Plantenga, 2003a; Kamphuis, Lejeune, Saris, & Westerterp-Plantenga, 2003b; Larsen, Toubro, Gudmundsen, & Astrup, 2006). Thus CLA may be a useful tool to control body fat mass gain, particularly in positive energy balance populations.

7. Safety and health considerations

The safety of commercial CLA preparations has also been evaluated in numerous human clinical trials. As pointed out earlier, typically commercial CLA preparations intended for human use consist almost entirely (i.e. >90%) of the two biologically active isomers in approximately equal amounts (i.e. about 45% each), as reviewed previously (Gaullier, Berven, Blankson, & Gudmundsen, 2002). There is no evidence to indicate that such high-quality CLA, when consumed at 3–6 g per day, will induce adverse effects in healthy humans (Gaullier et al., 2005). Currently, results have been reported for five long-term CLA studies, three human (Gaullier et al., 2004, 2005; Larsen et al., 2006; Whigham, O'Shea, Mohede, Walaski, & Atkinson, 2004; two reports by Gaullier et al. on the same subject with 1 and 2 year supplementation) and two rat studies (Park, Albright, & Pariza, 2005; Scimeca, 1998) lasting between 12 and 24 months. None of these studies reported any significant adverse effects of CLA supplementation.

Despite these conclusions some researchers have expressed concerns over the potential safety of CLA for humans (Kelley & Erickson, 2003; Larsen, Toubro, & Astrup, 2003; Riserus, Basu, et al., 2002; Tricon & Yaqoob, 2006). The main concerns over CLA use identified so far are lipodystrophy, fatty liver, glucose intolerance, and oxidative stress (Clement et al., 2002; Pariza, 2004; Poirier, Niot, Clement, Guerre-Millo, & Besnard, 2005; Tricon & Yaqoob, 2006).

Lipodystrophy, seen only in mice, and fatty liver may be the results of CLAs pronounced effects of body fat mobilization (Clement et al., 2002; Tsuboyama-Kasaoka et al., 2000). Yanagita, Wang, Nagao, Ujino, and Inoue (2005) reported that CLA promoted fatty acid synthesis in the liver. Fatty liver, the occurrence of which has been inconsistent, may or may not be a temporary response of biological systems to CLA, and may be reversible (O'Hagan & Menzel, 2003). We have performed patho-physiological evaluations of liver tissue, even following an 18 month feeding regimen, and found no evidence that CLA supplementation results in fatty liver (Park, Albright, & Pariza, 2005, & unpublished observations).

The effects of CLA on glucose metabolism in human studies are still inconclusive and possible effects of CLA on metabolic syndrome remain controversial (Tricon & Yaqoob, 2006). CLA has been reported to improve glucose metabolism as shown by decreasing glucose or insulin concentrations, or glucose tolerance in diabetic models and one study with sedentary young subjects (Belury, 2003; Eyjolfson, Spriet, & Dyck, 2004; Houseknecht et al., 1998; Nagao, Inoue, et al., 2003; Riserus, Vessby, Arner, & Zethelius, 2004; Ryder et al., 2001). Some authors have reported that CLA had no effects on fasting blood glucose and plasma insulin concentrations (Kamphuis et al., 2003b; Larsen et al., 2006; Medina et al., 2000; Noone, Roche, Nugent, & Gibney, 2002; Smedman & Vessby, 2001), while others reported worsening effects by CLA on glucose metabolism (Tricon & Yaqoob, 2006). Although the *trans*-10,*cis*-12 CLA isomer is linked with insulin resistance, it is interesting that the mixed isomer *trans*-10,*cis*-12 and *cis*-9,*trans*-11 CLA preparation did not cause insulin resistance (Moloney, Yeow, Mullen, Nolan, & Roche, 2004; Riserus, Arner, Brismar, & Vessby, 2002; Riserus, Vessby, Arner, et al., 2004; Riserus, Vessby, Arnlov, & Basu, 2004).

The mechanism of insulin resistance by CLA may be due to enhanced fatty acid β-oxidation, the experimental period, and/or be linked with the effects on adipokines and cytokines. Enhanced fatty acid β-oxidation can cause mild glucose tolerance as shown previously (Dumke et al., 2000). It is generally known that reduced body fat can improve insulin response, which is what would be expected with CLA but has not been consistently shown. Based on the observation that CLA increased insulin resistance in normal subjects but improves it in obese model, this effect may be temporary in that CLA feeding may improve insulin resistance after a period of increased insulin resistance (O'Hagan & Menzel, 2003; Wargent et al., 2005). This is supported by our recent observation that 18-month CLA feeding reduced both fasted and fed state serum glucose concentrations (Park, Albright, & Pariza, 2005).

It was also suggested that the effects of CLA on insulin resistance may be linked to TNF-α, which is involved in a variety of biologically important events, such as lipid metabolism, adipocyte differentiation, and insulin sensitivity, as well as inflammatory responses (Ventre et al., 1997). It has been shown that both the *cis*-9,*trans*-11 and the *trans*-10,*cis*-12 isomers reduce TNF-α levels in macrophage cell line and in mice (Akahoshi et al., 2002; Bhattacharya et al., 2005; Pariza et al., 2000; Park et al., 2003; Sugano et al., 2001; Yang & Cook, 2003). Effects of CLA on TNF-α levels in human studies, however, were less consistent (Albers et al., 2003; Nugent et al., 2005; Smedman, Basu, Jovinge, Fredrikson, & Vessby, 2005; Song et al., 2005). Recently, it was reported that *cis*-9,*trans*-11 and *trans*-10,*cis*-12 isomers cause different inflammatory responses and these results suggest a possible link to modified inflammatory responses by CLA and insulin resistance as well (Poirier, Shapiro, Kim, & Lazar, 2006; Ramakers, Plat, Sebedio, & Mensink, 2005).

CLA also reduced the activities and expression of glucose transporter 4 (GLUT4), particularly by the *trans*-10,*cis*-12 isomer, but this was corrected by the *cis*-9,*trans*-11 isomer (Pariza et al., 2003). Chung, Brown, Provo, et al. (2005) suggested that reduced GLUT4 is mediated in part by nuclear factor-κB (NFκB) and mitogen-activated protein kinase/ extracellular signal-related kinase

mechanisms. A recent report by Poirier et al. (2005) suggested that CLA can increase insulin secretion due to pancreatic β-cell hyperplasia, although the significance of this study needs further evaluation. Overall, the exact mechanism of how CLA modulates glucose metabolism is not known. Thus it is necessary to further evaluate CLAs effect on glucose homeostasis.

The other concern over using CLA is increased oxidative stress as observed by increased oxidative products, in particular increased C-reactive protein, and urinary isoprostanes (Basu, Riserus, Turpeinen, & Vessby, 2000; Basu, Smedman, & Vessby, 2000; Riserus, Basu, et al., 2002; Riserus, Vessby, Arner, et al., 2004; Riserus, Vessby, Arnlov, et al., 2004; Smedman et al., 2005; Taylor, Williams, Rhys, James, & Frenneaux, 2006). However, others reported no changes in C-reactive protein (Ramakers et al., 2005). It is again noted that Riserus et al. (Riserus, Vessby, Arner, et al., 2004) suggested a link between the *trans*-10,*cis*-12 CLA isomer but not mixed CLA isomers with regard to this increased oxidative stress.

8. Conclusion

CLA research has drawn much attention in the last two decades, in areas ranging from anticancer to obesity. With so many research papers published nowadays with regard to CLAs additional biological functions, mechanisms, and toxicity, it is clear that we do not know how this relatively simply structured fatty acid can have such a variety of functions. Safety concerns regarding the use of CLA in humans persist and need further investigation. Speculations and hypothesis are only the start and we still need much more research to understand the underlying mechanism(s).

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