

Conjugated linoleic acids: why the discrepancy between animal and human studies?

Mélanie Plourde, Stephanie Jew, Stephen C Cunnane, and Peter JH Jones

Conjugated linoleic acids (CLA) are positional and geometric isomers of linoleic acid. In animals, CLA consumption reduces body fat but results in humans are less conclusive. This review of the literature on CLA and loss of body fat or body weight in humans was conducted to explore the reasons for the discrepancy between animal and clinical trials. It indicates that the incongruity between human and animal data is largely related to methodological differences in the experimental design, including age and gender and, to a lesser extent, to CLA dose and isomers. The relatively unknown metabolic fate of CLA in humans may also be a contributing factor that helps explain the lack of consistency for CLA efficacy across studies.

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INTRODUCTION

Conjugated linoleic acids (CLA) are a group of polyunsaturated fatty acids with a single pair of conjugated double bonds.¹ CLA are found in ruminant meat and milk products (about 0.4% and 1% of total lipids, respectively). The major natural CLA isomer is 18:2 *cis*-9,*trans*-11, or ruminic acid (RA; Figure 1A). Chemically synthesized CLA mixtures are also available and are usually composed of a 50:50 mixture of RA and 18:2 *trans*-10,*cis*-12 (Figure 1B). Over the past 20 years, numerous studies conducted with rodents, pigs, and chickens have shown that CLA, at doses ranging from 0.5 to 1.0% of total dietary fat, reduces body fat in comparison to vegetable oils free of CLA.²⁻⁶

Over at least the past 7 years, attempts to replicate these results in humans have produced inconsistent results. Recently, a meta-analysis of human trials concluded that at an average dose of 3.2 g/d CLA reduces body fat mass (FM) by 3–4 g/d for each gram of CLA consumed.⁶ However, 12 papers were not included in this meta-analysis, either because they were published after

the meta-analysis or because they did not meet the inclusion criteria chosen for the meta-analysis.⁶ Among the 12 papers not reviewed in the meta-analysis, three reported a loss of 2–8% of FM,⁷⁻¹⁰ but none of these papers reported significant losses of body weight (BW) with CLA supplementation. Hence, the purpose of the present review was to re-evaluate the available data concerning loss of FM and/or BW following CLA supplementation in humans in order to provide a possible explanation for the inconsistent data reported for humans and animals.

CLA AND LOSS OF BODY WEIGHT OR FAT MASS

Normal-weight humans

Studies reporting BW and fat loss (FL) in normal-weight humans given CLA are shown in Table 1. In all of these studies, normal weight was defined as a body mass index of 20–25 kg/m². To date, we are not aware of studies reporting BW loss in normal-weight humans given 0.6–6 g/d CLA for periods of 4–14 weeks, regardless of

Affiliations: *M Plourde* and *SC Cunnane* are with the Research Center on Aging and Departments of Medicine, and Physiology and Biophysics, Université de Sherbrooke, Sherbrooke, Québec, Canada. *S Jew* is with the School of Dietetics and Human Nutrition, McGill University, Ste-Anne-de-Bellevue, Québec, Canada. *PJH Jones* is with the Richardson Centre for Functional Foods and Nutraceuticals, University of Manitoba, Winnipeg, Manitoba, Canada.

Correspondence: *M Plourde*, Research Center on Aging, 1036 Belvédère South, Sherbrooke, Québec J1H 4C4, Canada. E-mail: melanie.plourde2@usherbrooke.ca, Phone: +1-819-821-1170 #45383, Fax: +1-819-829-7141.

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Abbreviations: ARA, arachidonic acid (20:4n-6); BW, body weight; CLA, conjugated linoleic acid; FL, fat loss; FM, fat mass; LA, linoleic acid (18:2n-6); RA, ruminic acid (18:2 *cis*-9, *trans*-11)

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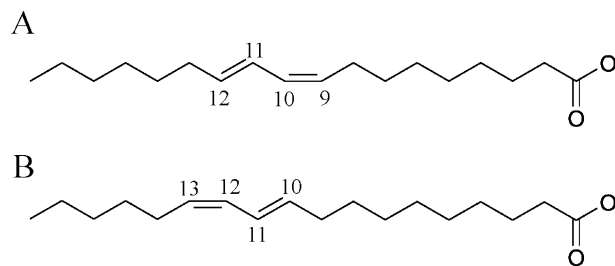


Figure 1 Chemical structures of A) ruminic acid, 18:2 *cis*-9,*trans*-11 and B) 18:2 *trans*-10,*cis*-12.

whether the CLA was provided as a mixture of two or four CLA isomers, or as a single isomer^{9,11–23} (Table 1). A loss of body FM in normal-weight subjects was reported in five of nine trials after CLA intake.^{9,13,15,16,22} Three of these studies resulted in a FM loss of 4%,^{9,13,15} one showed a FL of 8%²² and the fifth reported a FM loss of 20%.¹⁶ However, two of these studies had potential confounders because the subjects in both the CLA and the control groups were undergoing physical training three times/week.^{9,16} Colakoglu et al.²² also reported that subjects who were both taking CLA and doing physical training experienced a cumulative effect on BW and FM loss.

Overweight and obese humans

BW and FM loss in overweight and obese humans given CLA are summarized in Table 2. Overweight was defined in all studies as a body mass index of 25–30 kg/m², and obesity was defined as a body mass index >30 kg/m². In overweight and obese subjects, 4 of 16 studies^{10,24–26}

reported statistically significant BW loss ($\leq 3\%$ in all cases) during CLA supplementation, but the remaining 11 studies showed no significant effect. Seven of the 16 studies reported a loss of 2–6% of body FM during CLA supplementation (Table 2), amongst which four demonstrated loss of both BW and body FM following CLA intake.^{10,24–26} Thus, there is minimal evidence supporting weight loss in normal-weight, overweight, and obese subjects following CLA supplementation (Tables 1 and 2), yet approximately 50% of the studies claim losses of FM after CLA intake in normal, overweight, and obese subjects. Thus, it may be possible that CLA intake leads to a loss of body fat but, unless new, stronger evidence emerges, CLA supplementation should not yet be considered either as a BW and/or FL agent in humans.

INCONSISTENCIES IN EXPERIMENTAL DESIGN

Variations in experimental design may explain observed differences in BW and FM loss across animal versus

Table 1 Weight and fat mass loss in normal-weight human subjects.

Reference	Subjects / groups	Gender (M/F)	Dose (g/d)	CLA supplement	Duration (weeks)	WL (%)	FL (%)
Zambell et al. (2000) ¹⁹	17	0\17	3.0	Four isomers*	9	NS	NS
Benito et al. (2001) ¹²	10	0\10	3.9	Four isomers*	13	NS	ND
Mougios et al. (2001) ¹³	12	7\5	0.7	CLA mixture [†]	4	NS	NS
Mougios et al. (2001) ¹³	12	7\5	0.7–1.4	CLA mixture [†]	4 + 4	NS	–4%
Thom et al. (2001) ¹⁶	20	10\10	1.8	CLA mixture [†]	12	NS	–20%
Smedman et al. (2001) ¹⁵	26	15\11	4.2	CLA mixture [†]	12	NS	–4%
Noone et al. (2002) ²⁰	16	6\10	3.0	CLA mixture [†]	8	NS	ND
Belury et al. (2003) ¹¹	21	NM	6.0	CLA mixture [†]	8	NS	ND
Petridou et al. (2003) ²¹	16	0\16	2.1	CLA mixture [†]	6	NS	NS
Tricon et al. (2004) ¹⁸	39–49	NM	0.6–2.4	RA	8	NS	NS
Tricon et al. (2004) ¹⁸		NM	0.6–2.5	18:2 <i>trans</i> -10, <i>cis</i> -12	8	NS	NS
Colakoglu et al. (2006) ²²	44	0\44	3.6	CLA mixture [†]	6	NS	–8%
Tricon et al. (2006) ¹⁷	32	32\0	1.4	RA	6	NS	ND
Pinkoski et al. (2006) ⁹	38	19\19	5.0	CLA mixture [†]	7	NS	–4%
Lambert et al. (2007) ²³	64	26\38	3.9	CLA mixture [†]	12	NS	NS
Nazare et al. (2007) ¹⁴	21	NM	3.8	CLA mixture [†]	14	NS	NS

* Mixture of four CLA isomers: RA, 18:2 *trans*-10,*cis*-12, 18:2 *cis*-11,*trans*-13, 18:2 *trans*-8,*cis*-10.

[†] CLA mixture is composed of ruminic acid (RA) and 18:2 *trans*-10,*cis*-12.

Abbreviations: CLA, conjugated linoleic acid; F, female; FL, fat loss; M, male; RA, ruminic acid; ND, not determined; NM, not mentioned; NS, not significant; WL, weight loss.

Table 2 Weight and fat mass loss in trials with over weight and obese human subjects.

Reference	Subjects / groups	Gender (M/F)	Dose (g/d)	CLA supplement	Duration (weeks)	WL (%)	FL (%)
Berven et al. (2000) ²⁹	47		3.4	CLA mixture [†]	12	NS	ND
Blankson et al. (2000) ³⁰	12	4\8	1.7	CLA mixture [†]	12	NS	-3%
Blankson et al. (2000) ³⁰	8	3\5	3.4	CLA mixture [†]	12	NS	-6%
Blankson et al. (2000) ³⁰	11	4\7	5.1	CLA mixture [†]	12	NS	NS
Blankson et al. (2000) ³⁰	11	4\7	6.8	CLA mixture [†]	12	NS	-4%
Riserus et al. (2001) ⁶⁰	14	14\0	4.2	CLA mixture [†]	4	NS	ND
Riserus et al. (2002) ²⁶	19	19\0	3.4	CLA mixture [†]	12	NS	-3%
Riserus et al. (2002) ²⁶	19	19\0	3.4	18:2 <i>trans</i> -10, <i>cis</i> -12	12	-1%	-3%
Gaullier et al. (2004) ²⁴	61	10\51	3.6	CLA mixture [†]	52	-1%	-5%
Riserus et al. (2004) ²⁷	13	13\0	3.0	RA	12	+2%	NS
Malpuech-Brugere et al. (2004) ³⁹	18	NM	1.5-3.0	RA	6	NS	NS
Malpuech-Brugere et al. (2004) ³⁹	15	NM	1.5-3.0	18:2 <i>trans</i> -10, <i>cis</i> -12	6	NS	NS
Desroches et al. (2005) ⁴¹	16	16\0	2.6	RA	4	NS	ND
Gaullier et al. (2005) ²⁵	46	10\36	3.4	CLA mixture [†]	104	-2%	-6%
Naumann et al. (2006) ⁴⁰	34	NM	3.0	RA	13	NS	ND
Naumann et al. (2006) ⁴⁰	19	NM	3.0	18:2 <i>trans</i> -10, <i>cis</i> -12	13	NS	ND
Taylor et al. (2006) ⁶¹	21	21\0	4.5	CLA mixture [†]	12	NS	NS
Gaullier et al. (2007) ⁸	55	11\44	3.4	CLA mixture [†]	24	NS	-2%
Laso et al. (2007) ⁷	10	6\4	3.0	CLA mixture [†]	12	NS	-2%
Laso et al. (2007) ⁷	10	9\1	3.0	CLA mixture [†]	12	NS	NS
Steck et al. (2007) ⁶²	16		3.2-6.4	CLA mixture [†]	12	NS	NS
Syvrtsen et al. (2007) ³³	27	7\17	4.5	CLA mixture [†]	24	NS	NS
Watras et al. (2007) ¹⁰	22	5\17	3.2	CLA mixture [†]	24	-1%	-4%

[†] CLA mixture is composed of ruminic acid (RA) and 18:2 *trans*-10,*cis*-12.

Abbreviations: CLA, conjugated linoleic acid; F, female; FL, fat loss; M, male; RA, ruminic acid; ND, not determined; NM, not mentioned; NS, not significant; WL, weight loss.

human trials. Inconsistencies in age, gender, and genetic predisposition to fat accumulation in the experimental design also exist between animal and human trials. For example, growing mice accumulate 70% less body fat on a diet containing 0.5 weight % of a 50:50 CLA mixture of RA and 18:2 *trans*-10,*cis*-12, compared to controls given corn oil.²⁸ However, the first human trials attempting to link CLA supplementation to body FL were all conducted in adult men.^{19,29,30} Among these three trials, only one³⁰ achieved significance for FM loss. Thus, in animal trials, mice were in a growing state, i.e., approximately equivalent to the adolescent stage in humans, whereas in the human trials, weight-stable adults were utilized. Such differences in developmental state may confound the results, because there are important differences in metabolic rate, growth rate, and energy requirements between these two physiological states.

Also, CLA-induced loss of body fat in growing mice may depend on the proportion of brown adipose tissue in young mice,³¹ a depot which is lost in older animals and does not occur beyond infancy in humans.³² Brown adipose tissue is used mainly for energy and heat production in young animals and human infants.³²

Gender may also influence the efficacy for weight loss in humans. In animals, Park et al.²⁸ assumed that male and female mice were equally sensitive to CLA-induced

body fat gain. However, in humans, five of the clinical trials in which CLA induced loss of body fat in overweight and obese subjects, had 2-5 times more women than men,^{8,10,24,25,30} suggesting that gender affects body FL. On the other hand, one CLA study with a female : male ratio of 2 reported no body FL.³³ Three of these studies were among the largest published human CLA feeding trials (Table 2).^{8,24,25} However, no one has directly evaluated whether gender impacts the efficiency of CLA for FM loss in humans.

Another factor that may influence body FL is genetic predisposition to fat accumulation. In rats given CLA at 0.5 weight % of the diet, those with a genetic predisposition to fat accumulation such as obese Zucker rats, had retroperitoneal fat pads that were 15% heavier than those of obese Zucker rats on control diets. Yet in wild-type (lean) Zucker rats given CLA, retroperitoneal fat pads were 24% smaller compared to controls.³⁴ Hence, it was suggested that a genetic predisposition to fat accumulation could play an important role in the effectiveness of CLA in rats. To our knowledge, no human trials using CLA supplementation have tried to match genetic predisposition to fat accumulation with efficacy of CLA on body FL or risk factors for other diseases such as atherosclerosis.

It would be of interest to see if CLA given to humans regulates genes of lipid metabolism such as fatty acid-

binding protein, peroxisomal proliferator-activator receptor, or fatty acid synthase, which could help our understanding of the precise roles for CLA in biological and physiological processes and assist in explaining the inter-individual responsiveness. Another factor that may influence the outcome of the clinical trials is the caloric intake of the subjects. In animal studies, the caloric intake is usually well controlled and/or monitored. In some animal studies, food is provided *ad libitum*, i.e., intakes are not controlled but at least the amount of food consumed is monitored and the daily nutritional value is known because the animal has eaten only one type of food. In contrast, human trials performed to date have failed to control and/or monitor the dietary intake of the subjects for the whole experimental period.

Thus, the lack of reproducibility between animal and clinical trials using CLA to lower BW and FM may be largely influenced by age, gender, and genetic predisposition to fat accumulation, but results may also be influenced by other confounders such as dietary intake. Differences between experimental designs used in animal and human trials may contribute to low reproducibility of BW and FL results obtained in animal studies compared to clinical trials. The poor reproducibility between human trials is also possibly linked to differences in experimental design. For instance, most of the clinical studies that report a loss of either weight or FM were conducted in Norway or Sweden,^{8,15,16,24–26,30} so the environmental background of the subjects, such as their country and culture, could have been confounders in an unknown way.

WHICH CLA DOSE AND ISOMER(S) SHOULD BE USED?

In the human trials demonstrating statistically significant weight^{10,24–26} and body fat^{7–11,15,16,22,24–26,30} losses after CLA intake, the average dose was 3.2 g/d,⁶ which is more than 10 times the habitual normal (0.1–0.3 g/d) intake of CLA in humans.¹ There is no indication to date that the CLA dose given to humans was too low (generally between 0.5 and 1% of dietary lipids) to be able to reproduce in humans the loss of weight and fat observed in animals.

Apart from the CLA dose, different mixtures of CLA isomers may contribute to different outcomes of clinical trials, since some single CLA isomers or mixtures have opposing effects.^{18,35} Considering that most CLA supplements are chemically synthesized and would thus contain impurities such as *trans, trans* CLA, purification of the mixtures is essential because *trans, trans* CLA might be toxic to humans if consumed in large quantities.³⁵ Usually, CLA exists as a mixture of two isomers (50:50 of RA and 18:2 *trans*-10,*cis*-12). However, several animal studies have suggested that the single conjugated isomer 18:2 *trans*-10,*cis*-12^{26–38} is the body fat-lowering agent,

whereas RA is proposed to be implicated more in growth modulation.³⁸

Some human trials have thus been designed to study the question of whether one CLA isomer is more potent to lower body FM than another. Of four human trials using pure 18:2 *trans*-10,*cis*-12,^{18,26,39,40} only one reported a loss of weight and FM.²⁶ Six clinical trials used either pure RA^{18,27,39,40} or dairy products naturally enriched in RA^{17,41} and none reported a loss of weight. Moreover, none of these studies reported a gain of lean body mass following the intake of RA, as was observed in an animal study using pure RA.³⁸

Thus, although a CLA isomer-dependant effect for body FL has been suggested in animals, in humans, clear evidence to support this hypothesis is lacking, suggesting that the incongruity between the results obtained in animals and humans for body FL may be in part related to the CLA isomer used. Thus, differences in experimental design may contribute importantly to the incongruity across data.

METABOLIC FATE OF CLA

The lack of knowledge about the mechanisms by which single isomers or mixtures of CLA induce body FL in animals and in a few clinical trials may also contribute to this discrepancy as well as to the unknown metabolic fate of CLA after ingestion. In animal studies, the absorption of CLA given either as a free fatty acid or as a triglyceride was around 99%, whether assessed by lymphatic recovery⁴² or fecal analysis after CLA supplementation.⁴³ In animals, CLA are incorporated similarly to oleic acid into different organs, preferentially accumulating in neutral lipids.⁴⁴ The only study reporting CLA absorption in humans showed that incorporation into chylomicrons of CLA given as ethyl esters was almost 50% less than CLA given as a triglyceride.⁴⁵ In humans, CLA are incorporated into plasma and leukocyte lipids in proportion to dietary intake^{46,47} and have also been identified in milk and breast adipose tissue.^{48,49} Aside from these few studies, the metabolic fate of CLA in humans remains largely unknown. Without knowing the exact process of disposal of CLA, potential heterogeneity in such a system between and within subjects cannot be evaluated, which could account for variations in metabolism and, thus, in efficacy of CLA. Indeed, it is unknown if CLA are either β -oxidized, elongated or desaturated, or compete with other fatty acids such as linoleic acid for signalling processes in lipid metabolism. In rats, one study has reported that ~70% of ¹⁴C-CLA was completely β -oxidized within 24 h, a rate similar to that of α -linolenic acid, and 18% more than linoleic acid.⁵⁰ At present, there are no available data on the β -oxidation of CLA in humans, partly due to a lack of availability of suitable tracers for CLA.

CLA that are not β -oxidized could be elongated and desaturated in rats. Two animal studies have shown that conjugated arachidonic acid (ARA) accounted for 5% and 22% in rats given CLA.^{51,52} Higher conjugated ARA was obtained in livers of rats that had been reared on a fat-free diet for 2 weeks before being fed with 180 mg/day CLA for 6 days.⁵³ One study reported data on CLA metabolites in six healthy adult women following consumption of deuterated conjugated CLA mixtures of ethyl esters. No metabolites of deuterated RA were identified but ~5% of 18:2-*d*₄ *trans*-10,*cis*-12 was Δ 6 desaturated to 18:3-*d*₄ *cis*-6,*trans*-10,*cis*-12.⁵⁴ Thus, it appears that conversion of RA and 18:2 *trans*-10,*cis*-12 to conjugated ARA in humans is relatively inefficient or does not occur. Recent data show that the conversion rate of LA to ARA in humans is less than 0.1%.⁵⁵ The significance of possibly producing conjugated ARA to explain discrepancies in the results on BW and FL in animals and humans is partly linked to the role of ARA as a ligand for nuclear receptors, such as peroxisomal proliferator-activator receptors and liver X receptor, both of which are involved in multiple cellular processes such as lipid anabolism and catabolism.⁵⁶ CLA is a ligand to peroxisomal proliferator-activated receptors alpha and gamma in vitro and in vivo.^{57,58} Considering that the conversion of CLA to conjugated ARA seems to be very limited both in animals and humans, this pathway would not seem to explain the discrepancy between animals and humans for losses in weight and fat.

CLA increases β -oxidation of other fatty acids in liver mitochondria and peroxisomes in mice by increasing the expression of specific enzymes such as carnitine palmitoyltransferase-1 and acyl coenzyme A.⁵⁹ However, none of these mechanisms proposed by animal models have yet been evaluated in humans. The unknown fate and poor understanding of the metabolic pathways of CLA in humans is thus probably part of the explanation for the ongoing discrepancy in CLA efficacy between animal and human data.

CONCLUSION

Our assessment of the CLA literature is that body FL is not consistently observed in human subjects given a CLA supplement, and that BW loss is rarely observed after such supplementation. Major reasons for inconsistencies in the results obtained across animal and human trials include the substantially different experimental designs utilized in humans compared to animals, the unknown metabolic fate of CLA, and the absence of confirmed or even plausible mechanisms by which CLA reduce BW and FM. For these reasons, we suggest it is inappropriate to extrapolate to humans results obtained in animals. Until new evidence emerges addressing current limita-

tions, CLA supplementation should not be recommended either as a BW and/or a FL agent in humans.

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