Effects of Two Conjugated Linoleic Acid Isomers on Body Fat Mass in Overweight Humans

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Abstract

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Objective: To examine the effects of two different conjugated linoleic acid (CLA) isomers at two different intakes on body composition in overweight humans.

Research Methods and Procedures: Eighty-one middle-aged, overweight, healthy men and women participated in this bicentric, placebo-controlled, double-blind, randomized study. For 6 weeks (run-in period), all subjects consumed daily a drinkable dairy product containing 3 g of high oleic acid sunflower oil. Volunteers were then randomized over five groups receiving daily either 3 g of high oleic acid sunflower oil, 1.5 g of cis-9,trans-11 (c9t11) CLA, 3 g of c9t11 CLA, 1.5 g of trans-10,cis-12 (t10c12) CLA, or 3 g of t10c12 CLA administrated as triacylglycerol in a drinkable dairy product for 18 weeks. Percentage body fat mass and fat and lean body mass were assessed at the end of the run-in and experimental periods by DXA. Dietary intake was also recorded.

Results: Body fat mass changes averaged 0.1 ± 0.9 kg (mean ± SD) in the placebo group and −0.3 ± 1.4, −0.8 ± 2.1, 0.0 ± 2.3, and −0.9 ± 1.7 kg in the 1.5-g c9t11, 3-g c9t11, 1.5-g t10c12, and 3-g t10c12 groups, respectively. Changes among the groups were not significantly different ($p = 0.444$). Also, lean body mass and dietary intake were not significantly different among the treatments.

Discussion: A daily consumption of a drinkable dairy product containing up to 3 g of CLA isomers for 18 weeks had no statistically significant effect on body composition in overweight, middle-aged men and women.

Key words: conjugated linoleic acid isomers, human, body composition, fat mass, lean body mass

Introduction

Conjugated linoleic acid (CLA)[1], a common name for a group of positional and geometrical conjugated isomers of linoleic acid (cis-9,cis-12 octadecadienoic acid), is found mainly in ruminant meat and dairy products (1). Estimated daily intake of CLA, which is constituted for ~80% of cis-9,trans-11 (c9t11) configuration, is low and averages 151 mg in women and 212 mg in men (2). Dietary supplements with CLA, synthesized from linoleic acid (1), are now widely available because of their postulated health effects. In particular, it has been shown that synthetic CLA, containing ~40% c9t11-CLA and 44% trans-10,cis-12

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[1] Nonstandard abbreviations: CLA, conjugated linoleic acid; c9t11, cis-9,trans-11; t10c12, trans-10,cis-12; BIA, bioelectrical impedance analysis; ITT, intention-to-treat; PP, per protocol.

(t10c12)-CLA plus some others isomers, reduces body fat mass in rodents (3–5) and pigs (6). However, it is noteworthy that the various CLA isomers might have different effects on body composition. Indeed, the c9t11-CLA isomer increases body weight gain in growing mice but has no effect on the amount of body fat (3). In contrast, t10c12-CLA does not promote growth but reduces body fat mass (3). Until now, in most human studies, mixtures of CLA isomers have been tested (7–16). However, results are conflicting and less convincing than the animal data, perhaps due to differences in composition of the mixtures used or in daily intakes. The notion, however, of isomer-specific effects is based on animal data (17), not on human data. Therefore, we decided to test the effects on body fat mass of the two major CLA isomers at two different intakes in overweight middle-aged subjects.

Research Methods and Procedures

Subjects

Ninety middle-aged (35 to 65 years) healthy men ($N = 45$) and women ($N = 45$) were included in the study, which was performed simultaneously at two centers (Clermont-Ferrand, France and Maastricht, The Netherlands). Subjects were recruited through announcements in local newspapers. Eligible subjects completed a medical and physical examination, a standard blood test, and a questionnaire to assess energy and nutrient intakes. All subjects were moderately overweight (BMI 25 to 30 kg/m^2) and normolipemic (mean total cholesterol < 6.0 mM and mean triacylglycerol < 1.5 mM, as measured on two separate occasions after an overnight fast). Exclusion criteria were: irregular dietary habits, diastolic blood pressure > 85 mm Hg or systolic blood pressure > 150 mm Hg, unstable body weight or attempts to lower weight during the past 3 months, presence of proteinuria or glucosuria, use of medication, a diet or a clinical condition known to affect lipid or glucose metabolism, drug or alcohol abuse, history of coronary heart disease or malignancy < 5 years ago, or a positive serology for human immunodeficiency virus or hepatitis. The subjects were requested not to change their usual diets, level of physical exercise, smoking habits, or use of alcohol during the study. The Ethical Committees from both centers approved the study protocol. All subjects signed informed consent forms before entering the study.

Study Design

The study was designed as a placebo-controlled, double-blind, randomized trial carried out at the same time at two different centers. During the first 6 weeks of the study (run-in period), all subjects consumed daily a drinkable dairy product providing 3 g of high oleic sunflower oil (placebo). Thereafter, the volunteers were randomly allocated to one of the five groups, stratified for center, gender, and BMI. For the next 18 weeks of the study (intervention period), the first group continued to consume the drinkable dairy product with 3 g of high oleic sunflower oil. The second group received the product daily with 1.5 g of purified c9t11-CLA plus 1.5 g of high oleic sunflower oil, and the third group was given 3 g of purified c9t11-CLA. These two groups are further referred to as the low-c9t11 and high-c9t11 groups, respectively. The fourth and fifth groups received daily a drinkable dairy product containing 1.5 g of purified t10c12-CLA plus 1.5 g of high oleic sunflower oil and 3 g of purified t10c12-CLA isomer, respectively. They are referred to as the low-t10c12 and high-t10c12 groups. During the study, subjects recorded in diaries any signs of illness or any experienced side effects (headache, stomach complaints, nausea, bloated feeling, flatulence, diarrhea, constipation, itching, eruptions, and fatigue).

The two CLA isomers were given as a triacylglycerol and were produced by Natural Lipids LTD (Hovdebygda, Norway). In brief, ethyl-linoleate (94% purity) from safflower oil was treated with a solution of KOH in ethanol. This resulted in the formation of CLA, almost exclusively c9t11-CLA and t10c12-CLA in almost equal amounts. Other isomers ($<1\%$ each) were c9c11, c10c12, t9t11, and t10t12-CLA. The mixture of isomers was dissolved in acetone and cooled to -60 °C. At this temperature, most of the t10c12 ethyl esters precipitated, and the precipitates were collected by filtration. By repeated crystallizations, two concentrates of CLA were obtained, one with more than 80% c9t11-CLA and one with more than 80% t10c12-CLA. After removal of acetone, the concentrates were saponified and converted into triacylglycerols by addition of glycerol and Novozyme 435 (*Candida antartica* lipase, Novo Nordisk A/S, Bagsvaerd, Denmark). Finally, the oil was redistilled to remove free fatty acids and all traces of solvents. This resulted in an oil with $>80\%$ pure CLA-triacylglycerol. The triacylglycerol of each CLA isomer was stored under nitrogen atmosphere in air-tight steel containers.

The CLA was incorporated into an acidified drinkable dairy product by Danone (Palaiseau, France), which contained 67.0% (w/w) water, 20.0% milk [3.2% proteins, 5% lactose, 0.7% minerals (1250 ppm Ca), and 0.05% fat], 4.1% oils, 8% saccharose, 0.4% pectin, 0.35% citric acid, and 0.12% aromas. The product was stored in 100-mL bottles, sealed, and coded by subject number according to a randomization list made by Danone. Bottles were delivered to the investigators in boxes with the shelf life date; each box contained a 2-week supply (14 bottles) for each subject. Subjects had to consume one bottle per day and had to return unused bottles to verify compliance.

Methods

Subjects attended the study center at least every 2nd week to collect the experimental products and/or for measure-

ments. At each visit, subjects were weighed after an overnight fast of at least 12 h, wearing light indoor clothing and no shoes. At weeks 0, 4, 9, 14, 19, and 24, waist circumference between the lowest rib and the iliac crest and hip circumference at the widest part of the hip were measured, each time by the same investigator. DXA (Hologic QDR 4500A; Hologic, Bedford, MA) was used to measure body composition, body fat mass, and lean body mass at week 4 (run-in period) and at weeks 14 and 24 (intervention period). The same type of apparatus, which was cross-calibrated before the study using appropriate phantoms, was used at the two centers. At weeks 0, 4, 9, 14, 19, and 24, whole-body bioelectrical impedance analysis (BIA) was used to estimate body composition with a multifrequency bioelectrical impedance apparatus (Analycor, Eugédia, Paris, France in Clermont-Ferrand; Xitron Hydra ECF-ICF Model 4200, Xitron, San Diego, CA in Maastricht).

The subjects recorded their dietary intake three times during the study period: during week 4 of the run-in period and in weeks 14 and 24 of the intervention period. Food intake was recorded for 5 consecutive days, including 3 week days and 2 weekend days. The data were coded and analyzed by a dietitian using computerized nutrient databases, which were specific for each country (GENI Micro6.0, Villers. Les. Nancy, France).

Fasting blood was sampled twice at the end of the run-in period and in weeks 9, 14, 19, 23, and 24 during the intervention period for analyses to be reported in detail elsewhere. Various plasma and blood parameters were analyzed, including insulin and glucose concentrations and standard hematologic parameters and clinical chemistry: white cells, red blood cells, platelets, inflammation (C-reactive protein), liver function (alanine aminotransferase, aspartate aminotransferase, bilirubin, γ-glutamyltransferase, and alkaline phosphatase), and kidney function (creatinin). In addition, liver size (Clermont-Ferrand only) was determined by ultrasound at weeks 9 and 14. The fatty acid composition of plasma phospholipids (18,19) was monitored in the samples obtained at the end of each period to check the dietary compliance.

Statistical Analyses

Before the start of the study, it was calculated that 16 subjects in each group were needed to reach a power of 80% to detect a difference in fat mass of 2 kg (or 9%) between two treatments with an alpha of 5%. A 9% fat reduction is clinically relevant according to the 1997 WHO "Consultation on Obesity," which advises weight losses in the range of 5% to 15% (20).

The responses to treatment were calculated for each subject as the change between values obtained at the end of the run-in period and values at the end of the intervention period. Differences among the five groups were tested by ANOVA, including center and gender as factors. Moreover, repeated-measures ANOVA, including week 14 values, were used to look for possible treatment × time interactions, but results were comparable and, therefore, are not presented. If the p value was ≤ 0.05, changes in the treatment groups were compared with those in the placebo group using a Dunnett's test to control for multiple comparisons. Statistical calculations were performed using the StatView software (SAS Institute Inc., Cary, NC). Values are expressed as means ± SD. Results have been analyzed according to intention-to-treat (ITT) and per protocol (PP) analyses. Results of 84 subjects could be used for the ITT analysis because six subjects had already withdrawn during the run-in period before the first measurements. The PP analysis was performed on 81 subjects because results of an additional three subjects could not be used. When necessary, missing data were estimated with the last observation carried forward method. Because the results of ITT and PP analyses were comparable, only the results of the PP analysis are shown.

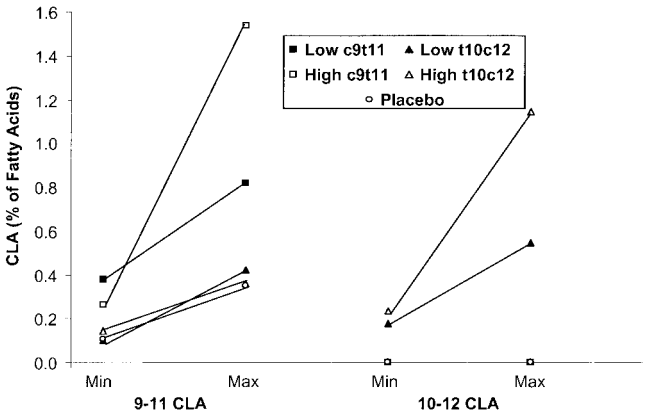

Figure 1: Range (minimum to maximum) of the percentage of CLA detected in plasma phospholipids at the end of the intervention period for each group.

Results

Eighty-two of the 90 subjects completed the study. Six subjects withdrew during the run-in-period, and one woman from the low-t10c12 group and one woman from the high-t10c12 group dropped out at week 13. Moreover, a woman from the low-t10c12 group was excluded because she had undetectable levels of t10c12 CLA during the intervention period, indicating noncompliance. The range of the percentage of CLA detected in the plasma phospholipids at the end of the supplementation for each group is presented in Figure 1. The t10c12 CLA isomer was detected in the plasma from only the volunteers who belonged to either the low-t10c12 or high-t10c12 groups. The five treatment groups were not significantly different with respect to sex ratio (not shown) and age (placebo,

Table 1. Body weight and BMI of the subjects at the end of the run-in and intervention periods

	n	Run-in period	Intervention period	Change	95% CI
Weight (kg)					
Placebo	15	78.7 ± 8.0	78.5 ± 8.2	−0.1 ± 1.1	
Low c9t11	18	79.8 ± 8.9	79.4 ± 8.7	−0.4 ± 2.0	−2.0; 1.4
High c9t11	18	81.0 ± 10.2	79.1 ± 10.1	−1.9 ± 3.2	−3.4; 0.0
Low t10c12	15	84.5 ± 12.4	83.9 ± 13.6	−0.5 ± 2.9	−2.3; 1.3
High t10c12	15	78.4 ± 6.8	77.1 ± 6.3	−1.2 ± 2.3	−2.9; 0.7
BMI (kg/m^2)					
Placebo	15	27.7 ± 1.6	27.6 ± 1.7	0.0 ± 0.4	
Low c9t11	18	27.9 ± 1.7	27.7 ± 1.6	−0.1 ± 0.7	−0.7; 0.5
High c9t11	18	27.7 ± 1.2	27.1 ± 1.6	−0.6 ± 1.0	−1.2; 0.0
Low t10c12	15	28.4 ± 2.1	28.3 ± 2.7	−0.2 ± 0.9	−0.7; 0.4
High t10c12	15	27.1 ± 1.3	26.7 ± 1.0	−0.4 ± 0.8	−1.0; 0.2

Results are expressed as mean ± SD. 95% Confidence intervals (CIs) have been calculated relative to the change in the placebo group.

48.3 ± 9.7; low-c9t11 group, 47.5 ± 7.7; high-c9t11 group, 49.9 ± 8.1; low-t10c12 group, 48.1 ± 6.8; high-t10c12 group, 48.2 ± 8.6 years). At the end of the run-in period, weight and BMI (Table 1), fat and lean body mass (Table 2), and daily energy intake (Table 3) were not significantly different among the treatment groups. No center or gender effect was detected for any of the parameters.

CLA supplementation was well tolerated. The blood parameters analyzed did not reveal any statistically significant differences among the five treatment groups, and the few reported complaints were not related to treatment. Also, no treatment effects were found on changes in concentrations of insulin ($p = 0.534$) and glucose ($p = 0.575$). At the end of the intervention period, plasma insulin and glucose concentrations had changed by −0.33 ± 1.85 mU/L and −0.13 ± 0.47 mM, respectively, in the placebo group, by +0.56 ± 1.87 mU/L and −0.01 ± 0.43 mM in the low-c9t11 group, by −0.43 ± 3.08 mU/L and 0.00 ± 0.35 mM in the high-c9t11 group, by −0.94 ± 2.48 mU/L and 0.06 ± 0.24 mM in the low-t10c12 group, and by −0.58 ± 3.36 mU/L and 0.10 ± 0.50 mM in the high-t10c12 group. Liver echography did not suggest that liver ultrastructure or morphology had changed during the study in any of the five groups. There were also no indications of hepatic lipodystrophy. However, two women were excluded at week 13: one woman from the low-t10c12 group because of increased concentrations of γ-glutamyltransferase and alanine aminotransferase (3 and 2 times the upper limit) and one woman from the high-t10c12 group because of menstrual complaints. Safety parameters will be discussed in detail elsewhere (C.M.-B., R.P.M., J.L.S., et al., manuscript in preparation).

There were no significant changes among the five treatment groups in weight, BMI (Table 1), and the waist-to-hip ratio, which changed by −0.02 ± 0.05 in the placebo group, by −0.01 ± 0.05 in the low-c9t11 group, by −0.03 ± 0.05 in the high-c9t11 group, by 0.00 ± 0.5 in the low-t10c12 group, and by −0.01 ± 0.05 in the high-t10c12 group. Irrespective of the dose, consumption for 18 weeks of either c9t11-CLA or t10c12-CLA did not significantly change the percentage of body fat ($p = 0.564$), body fat mass ($p = 0.444$), or lean body mass ($p = 0.136$), as measured by DXA (Table 2). At the highest intakes (3 g/d), however, slight decreases were observed in body fat mass of 0.8 ± 2.1 and 0.9 ± 1.7 kg in the c9t11 or the t10c12, groups, respectively (not significant). The loss of fat mass was not related to the percentage of CLA detected in plasma phospholipids ($R^2 = 0.0002$ for the high-c9t11 group, $p = 0.965$; $R^2 = 0.01$ for the high-t10c12 group, $p = 0.679$). Eight weeks after the intervention had started (week 14), body fat mass had changed by −0.4 ± 0.7 kg in the placebo group, by −0.1 ± 0.9 in the low-c9t11 group, by −0.8 ± 1.5 in the high-c9t11 group, by −0.1 ± 1.2 in the low-t10c12 group, and by −0.3 ± 0.7 in the high-t10c12 group. Body composition, as measured with bioelectrical impedance, also did not reveal any significant treatments effects (data not shown).

Table 2. Body fat mass (percentage and kilograms) and lean body mass of the subjects at the end of the run-in and intervention periods

	n	Run-in period	Intervention period	Change	95% CI
Fat mass (%)					
Placebo	15	29.0 ± 7.3	29.2 ± 7.1	0.1 ± 1.2	
Low c9t11	18	29.3 ± 7.6	29.0 ± 7.8	−0.2 ± 1.2	−1.4; 0.6
High c9t11	18	29.6 ± 5.4	29.2 ± 5.6	−0.3 ± 1.7	−1.5; 0.6
Low t10c12	15	30.2 ± 6.9	30.2 ± 7.0	0.0 ± 1.8	−1.3; 0.8
High t10c12	15	29.4 ± 6.3	28.7 ± 6.1	−0.7 ± 1.4	−1.9; 0.2
Body fat mass (kg)					
Placebo	15	22.5 ± 4.9	22.6 ± 4.7	0.1 ± 0.9	
Low c9t11	18	23.1 ± 5.5	22.8 ± 5.6	−0.3 ± 1.4	−1.6; 0.8
High c9t11	18	23.6 ± 3.0	22.8 ± 3.2	−0.8 ± 2.1	−2.1; 0.4
Low t10c12	15	25.2 ± 6.2	25.2 ± 7.5	0.0 ± 2.3	−1.4; 1.8
High t10c12	15	22.9 ± 4.6	21.9 ± 4.0	−0.9 ± 1.7	−2.2; 0.3
Lean body mass (kg)					
Placebo	15	56.1 ± 9.9	55.9 ± 10.0	−0.2 ± 1.2	
Low c9t11	18	56.7 ± 10.3	56.6 ± 10.5	−0.1 ± 1.1	−0.8; 0.9
High c9t11	18	57.4 ± 10.9	56.3 ± 10.7	−1.1 ± 1.4	−1.7; 0.0
Low t10c12	15	59.2 ± 11.4	58.7 ± 11.0	−0.5 ± 1.3	−1.2; 0.6
High t10c12	15	55.5 ± 8.4	55.2 ± 8.4	−0.3 ± 1.1	−1.0; 0.8

Results are expressed as mean ± SD. 95% Confidence intervals (CIs) have been calculated relative to the change in the placebo group. These data were obtained from DXA.

Daily energy intake was not significantly altered during the study (Table 3). Only a nonsignificant trend for a reduction in food intake was observed in the low-t10c12 group.

Discussion

The present placebo-controlled, double-blind, randomized trial carried out at the same time in two different centers is the largest published trial on the effects of CLA on body composition. Because recent animal (3) and in vitro studies (21) have suggested that effects of CLA on body composition are isomer specific, we decided to evaluate in overweight middle-aged subjects the specific impact on body composition of each of the two most common dietary CLA isomers (i.e., c9t11-CLA and t10c12-CLA) at two different doses (1.5 and 3 g/d). Body fat mass, as primary

Table 3. Daily energy intakes (kiloJoules) of the subjects at the end of the run-in and intervention periods

	n	Run-in period	Intervention period	Change	95% CI
Placebo	15	8562 ± 2026	8613 ± 2316	51 ± 1557	
Low c9t11	18	8978 ± 2025	8630 ± 2014	−348 ± 1193	−1403; 605
High c9t11	17	8160 ± 2180	8361 ± 2005	146 ± 1492	−922; 1113
Low t10c12	15	9353 ± 2405	8326 ± 1950	−1026 ± 1473	−2126; −28
High t10c12	15	9001 ± 1936	8230 ± 2032	−771 ± 1509	−1871; 227

Results are expressed as mean ± SD. 95% Confidence intervals (CIs) have been calculated relative to the change in the placebo group.

endpoint, was assessed by DXA, which is considered an accurate, precise, and reproducible method to assess body composition in adults and especially in obese patients (22). Although there was a slight decrease in fat mass after consumption for 18 weeks of 3 g per day of either isomer, differences with the control group did not reach statistical significance. Also, there was no relationship between changes in fat mass and the percentage of CLA detected in plasma phospholipids. Therefore, our results do not suggest that c9t11- or t10c12-CLA effectively lowers body fat mass in overweight humans within a period of 4 months. This extends the findings of Riserus et al. (23), who showed that a daily consumption of 3.4 g of t10c12 CLA for 12 weeks has no effect on body weight and fat mass in obese subjects. However, it cannot be excluded that higher intakes of CLA or longer supplementation periods are required.

The fact that our observations are not consistent with the convincing and consistent data on the effects of CLA on body composition in animals may be due to several reasons. In animals, the intake of CLA expressed as either percentage of total energy intake, percentage of total fat intake, or grams per kilogram body mass per day are, in general, much higher than in humans. For example, a daily intake of 0.70 g/kg body mass was effective in mice (3), whereas daily intakes in humans of 0.019 g/kg (12) or 0.04 g/kg (present study) were not. A value of 0.70 g/kg body mass in humans would correspond to a daily intake of ~56 g of CLA. In this respect, however, it has to be considered that CLA may also have negative effects in humans because a pro-oxidant effect of t10c12-CLA has been suggested (24). Moreover, as recently reviewed in detail by Larsen et al. (17), results from animal studies that high intakes of CLA may cause liver hypertrophy and may lead to insulin resistance has raised some concerns. With liver echography, however, we did not see any differences in liver ultrastructure among the five groups, and no indications of hepatic lipodystrophy were observed. Therefore, our results do not support the findings from animal studies, although our period of supplementation may have been too short or the doses used too low to observe any changes at all. Likewise, we could not demonstrate any effects on plasma insulin or glucose concentrations.

A difference in metabolic rate, which is much higher in animals, may also explain the differences observed between animal and human studies (25). Further, animal studies have been performed in growing animals, which may be more sensitive to dietary changes, whereas human studies have been performed in adults. In addition, large differences in responsiveness exist among animal species and even within some strains (26). Finally, CLA may affect energy intake in mice (27), but our study and other human studies (16,28) could not demonstrate a significant effect of CLA on spontaneous food intake in adults. Taken together, the differences show that the extrapolation of CLA effects observed in animals to the human situation should be done with caution.

So far, only a few human studies on the effects of CLA on body composition have been published as full articles in peer-reviewed journals (for recent review, see Larsen et al.) (17). These studies have used different mixtures of CLA isomers. Among these, five studies have suggested positive effects of CLA on body weight and/or on body composition (9,12–15). A number of reasons might explain the observed differences among these studies, including our own results.

First, several methods have been used to assess body composition. Three of the positive studies (12,14,15) have indirectly measured fat-mass using the skinfold thickness method (using either the classical method or near infrared light) or by BIA. Despite their reported effects on fat mass, none of these studies showed any effects of CLA on body weight. Another positive study (13) has also indirectly estimated fat mass by measuring the sagittal abdominal diameter. In fact, only one positive study has measured fat mass directly with DXA (9). In contrast, three of the seven studies that did not report an effect of CLA (including the present one) have used DXA (10,16), a highly reliable and precise method for determining body composition (22).

The general design of the studies and, more specifically, the intake and duration of CLA supplementation might have affected the outcomes. Although it is difficult to differentiate between the effects of amount and length of CLA supplementation, it appears that, in particular, the shorter studies showed positive effects of CLA treatment. For example, a daily supplement of CLA (4.2 g) for 4 weeks in 24 abdominally obese men induced a significant decrease in sagittal abdominal diameter without any change in body weight (13). Similarly, supplementation with CLA for 8 (12) and 12 (14) weeks in healthy nonobese men and women reduced the proportion of body fat mass as estimated by skinfold thickness and BIA. However, in our study, even when considering the mid-treatment evaluation at 8 weeks, no statistically significant treatment effects were evident. In the longest published study, which lasted 6 months, Atkinson et al. (7) did not show any significant changes in body weight or percentage body fat (DXA) in obese subjects who consumed 2.7 g of CLA daily. With respect to the effect of the amount of CLA ingested, the two different doses used in our study are comparable with those with either positive (9,12,15) or negative results (7,8,10,16,23,24). The discrepancies among the different studies might also be explained by the composition of the CLA preparation (17). In all studies, except those from Riserus et al. (23–24) and the present one, a mixture of CLA isomers was given, but the composition of the mixture was not always the same. Finally, the food matrix might also have played a role. All the published studies so far have used capsules, whereas we used a drinkable dairy-based

product. However, it is unlikely that this explains the lack of effect because both CLA isomers were well absorbed, as evidenced from their incorporation into plasma phospholipids.

Other factors that have been suggested to influence the impact of CLA include gender (14) and physical activity levels (10,29). In our study, however, and in the study of Thom et al. (15), effects were not related to gender. Further, our subjects were instructed not to change their level of physical activity during the study. This does not, however, exclude the possibility that CLA is effective in combination with an increased level of physical activity. In fact, among the studies published where some level of exercise was combined with an increased CLA intake (7,9–11,15,16), only one study in normal weight exercising volunteers showed a decreased body fat mass after consumption of 0.6 g of CLA three times daily for 12 weeks (15). In addition, differences in initial body weight of the volunteers cannot explain the inconsistent findings because both positive and negative studies have been performed with lean (10,11,15,16), overweight (9, 12, this study), or obese subjects (7,9,12,13,23,24).

In conclusion, human studies on the effects of CLA on body composition, for which there is no clear explanation, are not consistent. In any case, effects are much smaller than those observed in animals, and our results were not in favor of a health claim for products specifically enriched with the c9t11 isomer or the t10c12-CLA isomer to reduce body fat mass in moderately overweight men and women.

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