Asian Journal of Scientific Research

ISSN 1992-1454

science alert
http://www.scialert.net

ANSInet
an open access publisher
http://ansinet.com

Asian Journal of Scientific Research 6 (4): 679-690, 2013
ISSN 1992-1454 / DOI: 10.3923/ajsr.2013.679.690

Dietary Supplementation of Conjugated Linoleic Acid, Added to a Milk Drink, in Women

[1]Daniella Cristine Fialho Lopes, [1,2]Marialice Pinto Coelho Silvestre, [2]Viviane Dias Medeiros Silva, [1]Thais Garcia Moreira, [1]Emerson Silami Garcia and [2]Mauro Ramalho Silva

[1]Universidade Federal de Minas Gerais, Belo Horizonte, Brazil
[2]Edetec Indústria Alimentícia S/A, Belo Horizonte, Brazil

Corresponding Author: Marialice Pinto Coelho Silvestre, EDETEC Food Industry S.A Avenida José Cândido da, Silveira, 2100, sala 24, 31035-536, Belo Horizonte, MG, Brazil Tel: +55 31 3486 4167 Fax: +55 31 3486 1733

ABSTRACT

Some reports in the literature associate Conjugated Linoleic Acid (CLA) with several health benefits. However, the results are inconclusive and further studies are needed to evaluate the effect of CLA on body composition and biochemical parameters in humans. This study concerns the evaluation of the effect of the dietary supplementation of Conjugated Linoleic Acid (CLA) added to a milk drink in Brazilian women with body fat greater than 30%. Twenty-eight volunteers participated in this study and the experiment was randomized, double-blind and placebo controlled. The volunteers consumed 400 mL of a milk drink added of 4 g of CLA or canola oil (placebo) during 16 weeks. The dual energy x-ray absorptiometry (DXA) was used to assess body composition, a three day food diary was used to calculate the total energetic value and the resting metabolic rate (RMR) was measured by open-circuit indirect calorimetry. The measured biochemical parameters were fasting glucose, total cholesterol and its fractions, triglycerides, some liver enzymes and C-reactive protein. Symptoms related to the digestive system or signs of allergy were used to evaluate an adverse effect. The results showed no significant differences between the values obtained at months 0 and 4 for both groups, concerning body composition, resting metabolic rate and biochemical parameters. Also, the milk drinks were well tolerated and no adverse effect was reported by the volunteers during the clinical trial.

Key words: Conjugated linoleic acid, body composition, resting metabolic rate, biochemical parameters, body fat

INTRODUCTION

Beneficial health effects have been attributed to Conjugated Linoleic Acid (CLA) such as antiatherogenic and anticarcinogenic activities as well as modulation of body composition (reduction of fat mass and increase of lean body mass). These properties of CLA have been hypothesized to arise from an increase in basal metabolic rate, a reduction of the daily energetic intake and an increase of lipolysis and fat oxidation (Carvalho *et al*., 2010).

Previous clinical studies have examined the effect of CLA on body composition, energy intake, Resting Metabolic Rate (RMR) and several biochemical parameters. However, the results of these studies have been inconsistent; some authors found no change in the components of body

composition (Larsen *et al*., 2006; Malpuech-Brugere *et al*., 2004; Nazare *et al*., 2007; Zambell *et al*., 2000), Energy Intake (EI) (Laso *et al*., 2007; Nazare *et al*., 2007; Tricon *et al*., 2006) or RMR (Lambert *et al*., 2007; Petridou *et al*., 2003; Whigham *et al*., 2004) while others reported a reduction of body fat (Blankson *et al*., 2000; Gaullier *et al*., 2004; Laso *et al*., 2007; Raff *et al*., 2009) and a significant change in EI (Larsen *et al*., 2006; Raff *et al*., 2009) or RMR (Nazare *et al*., 2007).

Measurements of the biochemical parameters are associated with the safe administration of CLA. In some experiments, an increase in fasting glucose (Riserius *et al*., 2001; Tricon *et al*., 2004) and changes in serum lipids (Gaullier *et al*., 2004; Lambert *et al*., 2007; Tholstrup *et al*., 2008; Tricon *et al*., 2004) were observed. However, in most studies, dietary supplementation with CLA did not affect fasting glucose, serum lipids or inflammation markers (Berven *et al*., 2000; Blankson *et al*., 2000; Gaullier *et al*., 2005; Laso *et al*., 2007; Nazare *et al*., 2007; Petridou *et al*., 2003; Tricon *et al*., 2006). It can be inferred from these inconclusive results that further studies are needed to evaluate the effect of CLA on body composition and biochemical parameters in humans.

Thus, this study aimed to evaluate the effect of the dietary supplementation of CLA added to a milk drink on the body composition, resting metabolic rate, caloric intake and biochemical parameters of a group of women with fat percentage above 30%.

MATERIALS AND METHODS

Preparation of milk drinks: Two milk drinks were produced on an industrial scale at the milk manufacturing company Cemil and were packaged in 200 mL Tetra Pack boxes. One of these milk products was supplemented with 2 g of CLA (Clarinol™ G-80, Lipid Nutrition, Wormerveer, Netherlands) in the form of triacylglycerols and was named CLAd. The other milk drink contained 2 g of canola oil and was named CANd. The caloric and macronutrient distribution were the same in both drinks. Clarinol™ G-80 consists of a 50:50 mixture of cis-9, trans-11-CLA and trans-10, cis-12-CLA and 2 g of this product contains 80% CLA, which corresponds to 1.6 g (0.8 g of each isomer). Each subject consumed 2 drinks per day, which corresponds to the consumption of 1.6 g of each isomer or 3.2 g of CLA per day.

Aiming the double making, each lot of beverages, containing CLA or canola oil, was randomly enumerated in such a way to avoid the identification of oils in the products by the researcher. This information was disclosed only after the end of the clinical trial.

Study subjects: Initially, young obese and overweight women were recruited in two Universities of Belo Horizonte, MG, Brazil (Universidade Federal de Minas Gerais-UFMG and Centro Universitário de Belo Horizonte-UNI-BH). Among 58 registered volunteers, only 30 were invited to participate in the trial, because they matched the inclusion criteria, which included the following items: be women, be between 18 and 40 years old and have a fat percentage over than 30%. The exclusion criteria were the presence of chronic diseases such as hypertension, diabetes and dyslipidemia, as well as the use of controlled medication. Pregnant women and those in the stage of lactation were also excluded (Fig. 1).

The volunteers were informed about the clinical trial and its potential risks and benefits and provided their written consent before beginning the study. This study was approved by the Ethical Committee for Human Research of the Federal University of Minas Gerais-UFMG (Protocol No. 400/05).

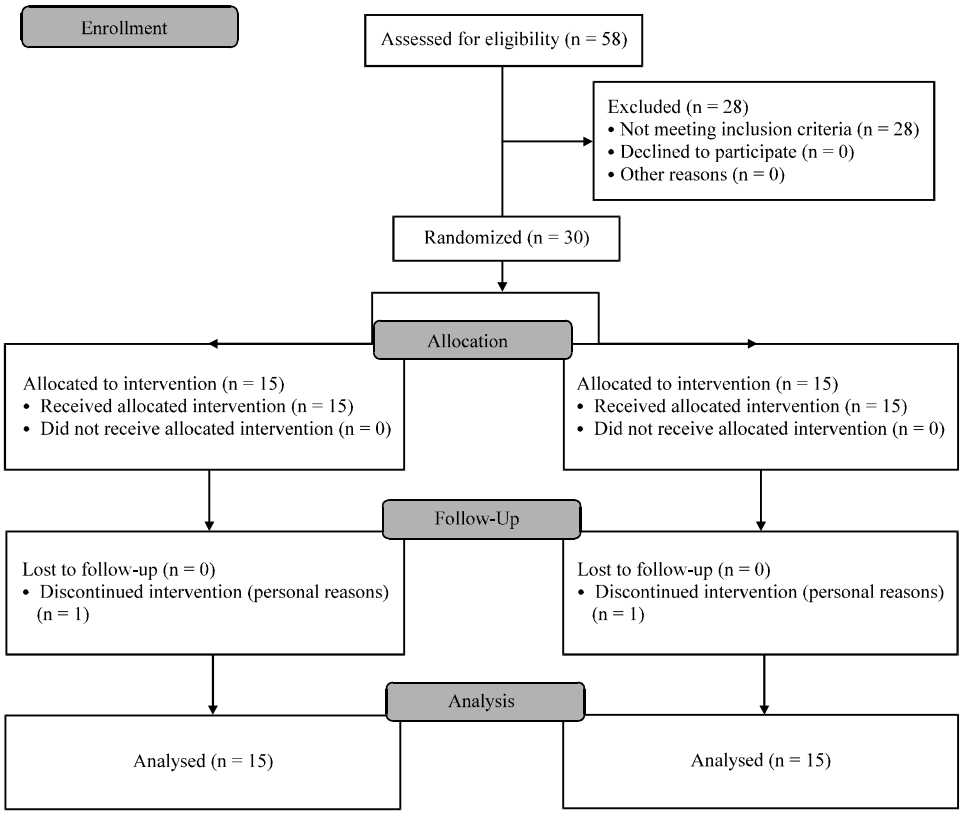

Fig. 1: Flow diagram (CONSORT 2010)

Experimental design: Primary outcome measures were the body composition (weigh, lean body mass, total body fat) and the CLA safety consumption. Second outcome measures were Resting Metabolic Rate (RMR), Energy Intake (EI) and biochemical parameters (glucose, total cholesterol and its fractions, triacylglycerols, liver enzymes and C-reactive protein) (Schulz *et al.*, 2010).

The study was a randomized, double-blinded and placebo-controlled design with two parallel groups. A restrict and sequential randomization technique was applied using tables with randomized numbers aiming at keeping the same number of volunteers in the groups. Each group received a drink from one of the produced lots, in such a way to avoid the identification of the oils in the products by the volunteers. At the moment of presenting the results, the group that had received the CLA drink was named CLAd (CLA group) and the other that had received the canola oil drink was named CANd (placebo group). Subjects were instructed to consume two milk drinks daily, with any meal, for four months.

At the beginning and end of the experiment, the volunteers were subjected to the following tests: anthropometry, dietary intake and resting metabolic rate (RMR). These tests were conducted at the Laboratory of Exercise Physiology of a Brazilian University (Centro Universitário de Belo Horizonte, Uni-BH, Belo Horizonte, MG, Brazil). Dual energy x-ray absorptiometry (DXA) was conducted in a private laboratory (Hermes Pardini, Belo Horizonte, MG, Brazil). For these assessments (i.e., anthropometry, DXA and RMR), the volunteers were asked to adopt the following guidelines prior to the test: No exercise, food and drink for at least 4 h prior to the tests; no

urination for at least 30 min prior to the tests; no alcohol consumption for at least 48 h prior to the tests; and no consumption of diuretics for at least seven days prior to the tests (Heyward and Stolarczyk, 1996).

Additionally, the blood biochemical tests were performed in a private laboratory (LABCLIN, Belo Horizonte, MG) at the beginning of the experiment (zero month) and 1, 2, 3 and 4 months of treatment, giving a total of five measurements.

Anthropometric evaluation: Body mass and height was obtained in accordance with the procedures described by Jelliffe (1968), using a platform medical balance with a stadiometer (model 31, Filizola, São Paulo, Brazil).

The body mass index (BMI; in kg m^{-2}) was calculated and those with a BMI higher than 25 were considered overweight.

Evaluation of body composition by dual energy X-ray absorptiometry (DXA): The DXA (model DPX-IQ, Lunar Radiation Corporation, Madison, USA) was used for evaluating the following body components: lean body mass, total body fat, bone mineral density. Moreover, this technique allowed us to analyze the fat distribution by body region (e.g., arms, legs and trunk), according to anatomic marks using computer-assisted algorithmics (enCORE, 6.00.270 version).

Evaluation of energy intake: The volunteers were informed to eat normally and to make no change in their diet during the study. To confirm this recommendation, a three-day food diary was completed before starting the supplementation (month 0) and at the end of the experiment (month 4). The volunteers were instructed regarding how to take notes by using pictures of utensils to minimize errors. The portion sizes were converted into grams and milliliters and the energy and nutrient intake was calculated using a computer program (Diet Win, Brubins, Porto Alegre, Brazil).

Evaluation of resting metabolic rate: The resting metabolic rate (RMR) was measured between 6:30 and 8:00 a.m., by open-circuit indirect calorimetry (VO2000 gas analyzer, Medical Graphics Corporation, St. Paul, USA). The data collected were analyzed by the Aerograph software (Medical Graphics Corporation, St. Paul, USA) to calculate the RMR.

Evaluation of biochemical parameters: This evaluation included the determination of the following parameters: fasting glucose, total cholesterol and its fractions (i.e., HDL, LDL and VLDL), triacylglycerols, liver enzymes (alanine transaminase-AST, aspartate transaminase-ALT and gamma-glutamyl transpeptidase-gamma GT) and C-reactive protein (CRP). Blood collection was performed after fasting for approximately 12 h by venipuncture in the elbow by a trained professional. The CRP-positive cases refer to the tests with values higher than 6 mg L^{-1}. The CRP was measured according to Singer *et al.* (1957), which is a latex-agglutination method. The principle of this test is based on the immunological reaction between CRP as an antigen and the corresponding antibody coated on the surface of biologically inert latex particles.

Evaluation of adverse effect: An adverse effect was defined as any symptom reported by the volunteers or observed by the researchers related to the digestive system (e.g., heartburn, indigestion, diarrhea and flatulence) or a sign of allergy (e.g., dermatitis or symptoms related to respiratory system). This effect was evaluated from a questionnaire that was answered by the volunteers at monthly meetings.

Statistical analysis: The estimation of the sample size was based on a parallel design for the study of superiority, considering all analyzed variables and the sample homogeneity. In this way, at least eleven volunteers were needed in each group to satisfy the scientific validation of this work, taking into account the normal distribution (Fletcher and Fletcher, 2006) with 5% significance and 80% for the power of the test. The primary outcome was the percentage of body fat and the parameters used to calculate the test power were 44.28% of body fat with a standard deviation of 3.8.

The equation of Schulz and Grimes (2005) was used to calculate the sample size:

$$n = \frac{(\alpha + \beta)^2 \sigma^2}{d^2} \quad (1)$$

Where:

n = Number of volunteers needed in each group
α = Type 1 error (0.05%)
β = Type 2 error (0.2%)
σ = Expected variance
d = Expected variance between the means of the studied quantitative variable

Moreover, most studies in the literature have used proportional numbers of control and test groups. In the analysis of data the Intention to Treat (ITT) was used aiming to minimize the giving up effect of some volunteers. We assumed that all randomized participants completed the study in our ITT analysis. Descriptive statistics were used to analyze the variables. The differences within the groups, from baseline until the end of the experiment, were evaluated with an analysis of variance and a paired t-test ($p<0.05$) in each study variable. An analysis of covariance or a nonparametric Wilcoxon test was used to compare the mean change in each study variable between the two treatment groups ($p<0.05$) (Heyward and Stolarczyk, 1996). The SPSS 17.0 trial version (SPSS Inc., IL, USA) and Minitab (Minitab, Inc., PA, USA) statistical programs were used to analyze the data.

RESULTS

Sample characterization: Among the twenty-eight volunteers who started the clinical trial, four dropped out during the course of the experiment because of personal reasons, two from each group (CLA and placebo). Thus, twenty-four women completed the experiment, twelve in each group. The main initial characteristics of the volunteers are presented in Table 1. Homogeneity of the data can be observed, with no significant differences between the results obtained from the two groups ($p<0.05$). For instance, the results for the placebo and CLEA groups were, respectively: Age (27.86 and 29.37); height (1.66 and 1.63), weight (74.42 and 75.69), body mass index (27.10 and 28.72), body fat (43.38 and 45.17); fat mass (31.46 and 33.35) lean body mass (40.43 and 39.96) and bone mineral density (1.16 and 1.24).

Body composition, energy intake and resting metabolic rate: The values obtained for body composition, dietary intake and resting metabolic rate in the period of study for the placebo and CLA groups are shown in Table 2. No changes in any of these parameters were observed because

Table 1: Physical characteristics and body composition of obese women, in CLA and placebo groups

Characteristics	Placebo group (n = 12)	CLA group (n = 12)	p-value
Age (years)	27.86±40.74	29.37±7.80	0.78
Height (m)	10.66±00.05	10.63±0.06	0.54
Weight (kg)	74.42±11.91	75.69±9.64	0.89
BMI (kg m^{-2})	27.10±40.12	28.72±3.93	0.65
Body fat (%)	43.38±30.58	45.17±4.02	0.59
Fat mass (kg)	31.46±70.36	33.35±6.68	0.76
Lean body mass (kg)	40.43±40.97	39.96±3.89	0.90
Bone mineral density (g cm^{-2})	10.16±00.05	10.24±0.28	0.65

CLA: Conjugated linoleic acid, BMI: Body mass index, No difference was found between groups (ANCOVA or Wilcoxon test, $p<0.05$), Values are Mean±SD

Table 2: Effect of dietary supplementation with CLA, added to a milk drink, versus placebo on body composition, dietary intake and resting metabolic rate

	Placebo group		CLA Group	
Parameters	Month 0	Month 4	Month 0	Month 4
Body mass (kg)	72.72±14.870	76.40±14.280	75.69±9.6400	75.69±10.300
Fat mass (kg)	31.46±7.3600	31.24±7.1000	33.35±6.6800	33.55±7.2900
Arms (kg)	30.06±0.5800	3.32±0.8200	30.27±0.6900	30.44±0.7900
Legs (kg)	12.20±2.8600	13.21±4.3400	13.39±2.9300	13.15±3.0100
Trunk (kg)	15.32±4.6300	16.66±4.6700	15.79±3.6100	16.13±3.9400
Lean mass (kg)	40.43±4.9700	40.07±5.0000	39.96±3.8900	40.48±3.8100
Body fat (%)	43.38±3.5800	43.45±3.7800	45.17±4.0200	44.91±4.2200
Bone density (g cm^{-2})	10.16±0.0500	1.16±0.0500	10.24±0.2800	10.14±0.0600
Food intake (Kcal)	1767.77±501.98	1722.21±429.46	1853.09±244.13	1810.54±400.06
Resting metabolic rate (Kcal)	1560.95±126.10	1408.02±202.65	1555.04±890.86	1443.70±221.27

CLA: Conjugated linoleic acid, No difference was found within the group between 0 and 4 months (analysis of variance and paired t-test, $p<0.05$) and among groups (ANCOVA or Wilcoxon test ($p<0.05$), Values are Mean±SD

cholesterol (172.67 and no significant differences were found between the results obtained at 0 and 4 months for both groups. In addition, a comparison of the results obtained in the fourth month between the CLA and placebo groups showed no significant differences for any of these parameters. For instance, the results of the month 0 and 4 for the placebo and CLEA groups were, respectively: Body mass (72.72 and 76.40; 75.69 and 75.69), lean mass (40.43 and 40.07; 39.96 and 40.48), body fat (43.38 and 43.45; 45.17 and 44.91), bone density (1.16 and 1.16; 1.24 and 1.14), food intake (1767.77 and 1722.21; 1853.09 and 1810.54) and resting metabolic rate (1560.95 and 1408.02; 1555.04 and 1443.70).

Biochemical parameters: The effects of the dietary supplementation of CLA on the levels of fasting glucose, serum lipids, liver enzymes and C-reactive Proteins (CRP) are presented in Table 3.

Table 3 shows no significant differences were observed for any parameters during the period of study for both groups (placebo and CLA). Moreover, when comparing the values obtained between the two groups (placebo and CLA) each month, no significant differences were observed in any of the months in the study. For instance, the results of the month 0 and 4 for the placebo and CLEA groups were, respectively: glucose (80.22 and 69.18; 85.33 and 74.14), total

Table 3: Effect of dietary supplementation with CLA, added to a milk drink, versus placebo on fasting glucose, serum lipids, liver enzymes and C-reactive protein

	Placebo group					CLA group				
Parameters	Month 0	Month 1	Month 2	Month 3	Month 4	Month 0	Month 1	Month 2	Month 3	Month 4
Glucose (mg dL^{-1})	800.22±8.870	760.8±15.150	79.00±15.67	78.91±13.56	69.18±12.93	85.33±9.890	74.11±11.15	82.11±11.95	86.89±12.97	74.14±10.57
Total cholesterol (mg dL^{-1})	172.67±33.81	173.9±30.010	173.91±26.44	161.18±25.45	194.55±38.88	148.11±32.01	159.89±31.35	167.67±48.54	146.44±26.53	183.14±27.72
HD cholesterol (mg dL^{-1})	59.13±12.01	61.10±10.87	56.64±13.28	55.45±14.38	56.09±12.04	54.11±13.36	55.22±11.48	52.22±12.46	53.00±13.78	57.00±17.87
LDL cholesterol (mg dL^{-1})	78.15±22.71	84.57±21.87	91.78±28.33	78.67±21.26	108.35±29.48	75.89±23.80	83.36±26.03	86.31±23.39	74.38±28.42	103.89±37.13
VLDL cholesterol (mg dL^{-1})	27.73±11.24**	28.06±17.93**	24.58±10.63**	27.02±9.010**	30.11±17.13**	18.11±6.630**	21.27±8.190**	18.24±3.700**	19.07±8.090**	22.26+7.500**
Triglycerides (mg dL^{-1})	129.82±50.03**	138.82±85.21**	127.55±51.21**	135.27±45.02**	153.82±82.11**	90.56±33.16**	106.33±40.95**	91.22±18.48**	95.33±40.43**	109.06±36.65**
AST (IU L^{-1})	12.5±3.0200	13.00±03.86	13.18±2.920	14.27±4.490	14.45±6.130	12.22±2.730	14.44±2.600	14.11±3.950	13.00±2.690	17.57±4.230
ALT (IU L^{-1})	13.5±4.9600	15.5±6.4200	13.81±3.060	15.36±4.800	13.27±2.410	11.67±2.920	15.67±3.390	13.44±4.880	14.11±3.790	13.00±2.940
Gamma GT (IU L^{-1})	24.0±6.1200	23.2±6.3700	21.27±5.130	21.54±4.270	21.45±4.270	24.89±4.450	26.33±6.240	25.44±6.500	22.55±5.610	20.42±4.150
CRP (positive cases)	7	2	1	1	4	4	1	0	0	2

CLA: Conjugated linoleic acid, HDL: High density lipoproteins, LDL: Low density lipoproteins, VLDL: Very low density lipoprotein, SD: Standard deviation, *No difference within the group between 4 and 0 months was observed (analysis of variance and paired t-test, $p<0.05$), **Difference between groups (ANCOVA or Wilcoxon test, $p<0,05$), Gamma GT: Glytamyl transpeptidase, ALT: Alanine transaminase, AST: Aspartate transaminase, CRP: C-reactive protein, Values are Mean±SD

194.55; 148.11 and 183.14), triglycerides (129.82 and 153.82; 90.56 and 109.06), aspartate transaminase (12.5 and 14.45; 12.22 and 17.57), alanine transaminase (13.5 and 13.27; 11.67 and 13), Gamma GT (24 and 21.45; 24.89 and 20.42) and C-reactive protein (7 and 4; 4 and 2).

Adverse effects: The milk drink was well tolerated and no adverse effects were reported by the volunteers during the clinical trial, either in the placebo or in the CLA group.

DISCUSSION

The results obtained in the present study showed that supplementing a diet with CLA, through the addition of CLA to a milk drink, for 4 months did not affect body mass or total fat mass. The lean mass of the arms, legs and trunk, as well as the lean body mass and the bone mineral density of the volunteers who participated in the clinical trial were also not affected. These results are in agreement with those reported by other authors, who also used food as a matrix to test the effects of CLA (Malpuech-Brugere *et al*., 2004; Tricon *et al*., 2006; Nazare *et al*., 2007).

Despite the similarity of the results and the use of the same method to assess body composition (DXA), these previous studies had experimental designs that differed from our study, regarding the type of food and the population. Malpuech-Brugere *et al*. (2004) used fermented milk supplemented with CLA (3 g of a mixture of isomers and 1.5 g of purified isomer) in a group of men (n = 5) and women (n = 45). Nazare *et al*. (2007) tested yogurt supplemented with CLA (3.76 g of a mixture of isomers), also in a population of men (n = 22) and women (n = 22). Tricon *et al*. (2006) evaluated, in only men (n = 32), the consumption of UHT milk, cheese and butter, all naturally enriched with CLA (1.421 g x day^{-1}), by modifying the diet of cows.

Contrarily to our results, Laso *et al*. (2007) evaluated the effect of CLA added to milk in men (n = 23) and women (n = 20) and found a reduction in total body fat as well in the trunk of the volunteers. These differences could probably be explained by the fact that most of the volunteers in the present study had sedentary lifestyles while those in the study of Laso *et al*. (2007) were physically active.

There are still several disagreements regarding the results about the effect of CLA on the body composition of volunteers when it is used in the form of capsules, *i.e.,* some authors did not find any changes while others described some modifications. In the first case, the supplementation of 3.4 g (Berven *et al*., 2000) and 3.9 g (Lambert *et al*., 2007) of CLA for 12 weeks did not alter any component of body composition in overweight men and women. Similarly, CLA supplementation in a larger dose (6 g×day^{-1}) and for a longer time (52 weeks) caused no changes in body fat or lean mass in a population of overweight men and women (Whigham *et al*., 2004). In another study, the authors tested two main isomers of CLA in a purified form (cis9, trans11 CLA and trans10, cis12 CLA) in different doses and found no changes in the body compositions of men and women (Tricon *et al*., 2004). Despite these differences in methodology and experimental design, the results of these studies corroborate the results of the present study.

Unlike the results obtained here, studies in published literature have found some effect of CLA intake (in the capsule form) on body composition. The supplementation of the diet with 3.4 or 4.5 g×day^{-1} of CLA in a mixed population (n = 83) of overweight adults over 6 months or a year was effective in reducing the fat percentage and body mass and increasing lean body mass (Gaullier *et al*., 2004, 2005; Larsen *et al*., 2006; Gaullier *et al*., 2005).

Using 5.5 g×day^{-1} of CLA over 4 months in a group of overweight post menopausal women, Raff *et al*. (2009) reported a reduction of fat mass and an increase of lean mass in the legs. A

reduction of body fat percentage was reported by Blankson *et al.* (2000) testing various doses of CLA (1.7 to 6.8 g×day^{-1}) and by Thom *et al.* (2001) using 1.8 g×day^{-1}of CLA over 12 week, in a mixed normal weight or overweight population of adults, respectively.

The differences between the results described above and those of the present study may be explained by differences in the experimental design, methodology and especially in the composition of the population, which in the current study consisted only of sedentary overweight young women (up to 39 years old).

Resting Metabolic Rate (RMR) and Energy Intake (EI) were evaluated in the present study, because according to some authors (Nazare *et al.*, 2007), one of the hypotheses used to explain the mechanisms of action of CLA in reducing adipose tissue and increasing lean body mass is associated with increased RMR and reduced EI. However, the experimental conditions used here did not influence these parameters during the 4 month study.

The effect of CLA on EI and RMR has also been evaluated by other authors and the results are somewhat inconsistent, similar to those mentioned above for body composition: Some of these studies showed a change (Larsen *et al.*, 2006; Raff *et al.*, 2009) while others indicated no effect (Lambert *et al.*, 2007; Laso *et al.*, 2007; Tricon *et al.*, 2006). Additionally, it is important to note that among the 20 studies found in the literature, this effect was estimated in only 13 for EI and 3 for RMR.

Among the experiments that used a food matrix to test the effect of CLA, a change in EI was not reported in any work (Laso *et al.*, 2007; Nazare *et al.*, 2007; Tricon *et al.*, 2006), as was found in the present work. In relation to the RMR, a significant increase was induced in subjects who consumed CLA for 98 days (Raff *et al.*, 2009), which differs from the current study.

In the case of studies using CLA in the capsule form, Lambert *et al.* (2007) and Whigham *et al.* (2004) found no change in EI or RMR, similar to the results of the present study.

In other experiments, only EI was assessed with no change in this parameter (Gaullier *et al.*, 2005; Gaullier *et al.*, 2004; Petridou *et al.*, 2003; Smedman *et al.*, 2005; Tholstrup *et al.*, 2008). However, Larsen *et al.* (2006) and Raff *et al.* (2009) found a significant reduction in EI in subjects who consumed CLA.

With respect to the biochemical parameters, it is important to note that the measurements of fasting glucose and liver enzymes are associated with the safety of CLA administration, because the intake of this substance may lead to an increase in insulin resistance (Riserius *et al.*, 2001) and changes in the liver (Tsuboyama-Kasaoca *et al.*, 2003). However, in this study, no significant changes were observed in these two parameters during the 4 month study.

In some reports from the literature, in which different food matrices and experimental designs from those of the present study were used, similar results were found, with no changes in these two parameters (Laso *et al.*, 2007; Nazare *et al.*, 2007; Tricon *et al.*, 2006). Even when CLA was ingested in the capsule form for a period of one year, the safety of its administration was confirmed by the maintenance of these parameters throughout the study (Gaullier *et al.*, 2004; Whigham *et al.*, 2004). However, in two studies in which CLA was provided in the capsule form to overweight men, an increase in the level of fasting glucose was observed, indicating a negative effect of CLA on this parameter (Riserius *et al.*, 2001; Tricon *et al.*, 2004). Additionally, the ingestion of the trans10, cis12 CLA isomer resulted in a greater increase in the fasting glucose than in the group that ingested the cis9, trans11 CLA isomer (Tricon *et al.*, 2004).

Another point related to the security of dietary supplementation with CLA refers to the markers of cardiovascular diseases, such as serum lipids and C-reactive Protein (CRP). It is noteworthy that

no change in the plasma lipid profile and CRP was identified during the 4-month study in the present work. The reports of other authors concerning the effect of CLA on serum lipids and inflammatory markers may be found in the literature. In some of these works, the following observations were noted: A reduction (Lambert *et al*., 2007; Tholstrup *et al*., 2008) or an increase (Gaullier *et al*., 2004) of HDL cholesterol; an increase of LDL cholesterol (Gaullier *et al*., 2004; Tricon *et al*., 2004); no effect on total cholesterol and its fractions or in triacylglycerols (Laso *et al*., 2007; Tricon *et al*., 2006). No effect on total cholesterol and its fractions or in triacylglycerols was found in the present work as well.

Among the works in the literature that used food matrices to test the effect of CLA on these markers, none has evaluated the CRP and only 3 have studied the behavior of serum lipids (Laso *et al*., 2007; Nazare *et al*., 2007; Tricon *et al*., 2006). As in this study, no changes in these parameters were found, such as in the present work. Similarly, in a study using CLA in capsules, Gaullier *et al*., 2005) did not observe any changes in serum lipids and CRP, as in the present study. In other experiments, the researchers assessed serum lipids but not CRP. Blankson *et al*. (2000), Berven *et al*. (2000) and Petridou *et al*. (2003) did not observe any change in total cholesterol, its fractions and in triacylglycerols.

Finally, it is worth stating that this research corroborates previous studies, with low dropout of volunteers, indicating that dietary supplementation with CLA is well tolerated (Gaullier *et al*., 2005; Gaullier *et al*., 2004). Although, experiments with mice have revealed an increased deposition of fat in the liver in the group that consumed CLA (Clement *et al*., 2002; Tsuboyama-Kasaoca *et al*., 2003), no adverse effects were reported by the volunteers in the present study, indicating that the consumption of CLA as a supplement in a milk drink was safe under the conditions tested here. In other previous studies on humans, some symptoms were observed, although to a small degree, especially with regard to the digestive system (Blankson *et al*., 2000; Gaullier *et al*., 2004; Sokal and Rohlf, 1995). However, even in these cases, the adverse effect was observed in the CLA as in the placebo groups.

CONCLUSION

Consumption of a milk drink supplemented with 4 $g \times day^{-1}$ of CLA for 16 weeks produced no significant modification of body composition, energy intake, resting metabolic rate and biochemical parameters in women. Moreover, no adverse effects on the volunteers were observed during the period of study.

ACKNOWLEDGMENTS

The authors thank CNPq and FAPEMIG for their financial support.

REFERENCES

Berven, G., A. Bye, O. Hals, H. Blankson and H. Fagertum *et al*., 2000. Safety of Conjugated Linoleic Acid (CLA) in overweight or obese human volunteers. Eur. J. Lipid Sci. Technol., 102: 455-462.

Blankson, H., J.A. Stakkestad, H. Fagertun, E. Thom, J. Wadstein and O. Gudmudsen, 2000. Conjugated linoleic acid reduces body fat mass in overweight and obese humans. J. Nutr., 130: 2943-2948.

Carvalho, E.B.T., I.L.P. Melo and J. Mancini-Filho, 2010. Chemical and physiological aspects of isomers of conjugated fatty acids. Cienc. Tecnol. Aliment., 30: 295-307.

Clement, L., H. Poirier, I. Niot, V. Bocher and M. Guerre-Millo *et al.*, 2002. Dietary trans-10, cis-12 conjugated linoleic acid induces hyperinsulinemia and fatty liver in the mouse. J. Lipid Res., 43: 1400-1409.

Fletcher, R.H. and S.W. Fletcher, 2006. Epidemiologia Clinica: Elementos Essenciais. 4th Edn., Artmed, Porto Alegre.

Gaullier, J.M., J. Halse, K. Hoye, K. Kristiansen, H. Fargentun, H. Vik and O. Gudmundsen, 2004. Conjugated linoleic acid supplementation for 1 year reduces body fat mass in healthy overweight humans 1'2'3. Am. J. Clin. Nutr., 79: 1118-1125.

Gaullier, J.M., J. Halse, K. Hoye, K. Kristiansen, H. Fagertun, H. Vik and O. Gudmundsen, 2005. Supplementation with conjugated linoleic acid for 24 months is well tolerated by and reduces body fat mass in healthy, overweight humans. J. Nutr., 135: 778-784.

Heyward, V.H. and L.M. Stolarczyk, 1996. Evaluation of Body Composition. Human Kinetics, Champaign, IL., USA.

Jelliffe, D.B., 1968. Evaluation of the Nutritional Status of the Community. WHO, Geneva, Switzerland.

Lambert, E.V., J.H. Goedecke, K. Bluett, K. Heggie and A. Claassen *et al.*, 2007. Conjugated linoleic acid versus high-oleic acid sunflower oil: Effects on energy metabolism, glucose tolerance, blood lipids, appetite and body composition in regularly exercising individuals. Br. J. Nutr., 97: 1001-1011.

Larsen, T.M., S. Toubro, O. Gudmundsen and A. Astrup, 2006. Conjugated linoleic acid supplementation for 1y does not prevent weight or body fat regain. Am. J. Clin. Nutr., 83: 606-612.

Laso, N., E. Brugue, J. Vidal, E. Ros and J.A. Arnaiz *et al.*, 2007. Effects of milk supplementation with conjugated linoleic acid (isomers cis-9, trans-11 and trans-10, cis-12) on body composition and metabolic syndrome components. Br. J. Nutr., 98: 860-867.

Malpuech-Brugere, C., W.P.V.V. De Venne, R.P. Mensink, M.A. Arnal and B. Morio *et al.*, 2004. Effects of two conjugated linoleic acid isomers on body fat mass in overweigth humans. Obes. Res., 12: 591-598.

Nazare, J.A., A.B. Perriere, F. Bonnet, M. Desage and J. Peyrat *et al.*, 2007. Daily intake of conjugated linoleic acid-enriched yoghurts: Effects on energy metabolism and adipose tissue gene expression in healthy participants. Br. J. Nutr., 97: 273-280.

Petridou, A., V. Mougios and A. Sagredos, 2003. Supplementation with CLA: Isomer incorporation into serum lipids and effect on body fat of women. Lipid, 38: 805-811.

Raff, M., T. Tholstrup, S. Toubro, J.M. Bruun and P. Lund *et al.*, 2009. Conjugated linoleic acids reduce body fat in healthy postmenopausal women. J. Nutr., 139: 1347-1352.

Riserius, U., L. Berglund and B. Vessby, 2001. Conjugated Linoleic Acid (CLA) reduced abdominal adipose tissue in obese middle-aged men with signs of the metabolic syndrome: A rondomised controlled trial. Int. J. Obesity, 25: 1129-1135.

Schulz, K.F. and D.A. Grimes, 2005. Sample size calculations in randomised trials: Mandatory and mystical. Lancet, 365: 1348-1353.

Schulz, K.F., D.G. Altman, D. Moher and CONSORT Group, 2010. CONSORT 2010 statement: Updated guidelines for reporting parallel group randomised trials. J. Pharmacol. Pharmacother., 152: 726-732.

Singer, J.M., C.M. Plotz, E. Pader and S.K. Elster, 1957. The latex-fixation test. III. Agglutination test for C-reactive protein and comparison with the capillary precipitin method. Am. J. Clin. Pathol., 28: 611-617.

Smedman, A., S. Basu, S. Jovinge, G.N. Fredrikson and B. Vessby, 2005. Conjugated linoleic acid increased C-reactive protein in human subjects. Br. J. Nutr., 94: 791-795.

Sokal, R.R. and E.F.J. Rohlf, 1995. Biometry: The Principles and Practice of Statistics in Biological Research. 2nd Edn., W.H. Freeman, New York, USA.

Tholstrup, T., M. Raff, E.M. Straarup, P. Lund, S. Basu and J.M. Bruun, 2008. An oil mixture with trans-10, cis-12 conjugated linoleic acid increases markers of inflammation and *In vivo* lipid peroxidation compared with cis-9, trans-11 conjugated linoleic acid in postmenopausal women. J. Nutr., 138: 1445-1451.

Thom, E., J. Wadstein and O. Gudmundsen, 2001. Conjugated linoleic acid reduces body fat in healthy exercising humans. J. Int. Med. Res., 29: 392-396.

Tricon, S., G.C. Burdge, E.L. Jones, J.J. Russell and S. El-Khazen *et al.*, 2006. Effects of dairy products naturally enriched with cis-9, trans-11 conjugated linoleic acid on the blood lipid profile in healthy middle-aged men. Am. J. Clin. Nutr., 83: 744-753.

Tricon, S., G.C. Burdge, S. Kew, T. Banerjee and J.J. Russell *et al.*, 2004. Opposing effects of cis-9, trans-11 and trans-10, cis-12 conjugated linoleic acid on blood lipids in healthy humans. Am. J. Clin. Nutr., 80: 614-620.

Tsuboyama-Kasaoca, N., H. Miyazaki, S. Kasaoca and O. Ezaki, 2003. Incresing the amount of fat in a conjugated linoleic acid supplemented diet reduces lipodystrophy in mice. J. Nutr., 133: 1793-1799.

Whigham, L.D., M. O'Shea, I.C.M. Mohede, H.P. Walaski and R.L. Atkinson, 2004. Safety profile of conjugated linoleic acid in a 12-month trial in obese humans. Food Chem. Toxicol., 42: 1701-1709.

Zambell, K.L., N.L. Keim, M.D. Van Load, B. Gale, P. Benito, D.S. Kelley and G.J. Nelson, 2000. Conjugated linoleic acid supplementation in humans: Effects on body composition and energy expenditure. Lipids, 35: 777-782.