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Effects of milk supplementation with conjugated linoleic acid on weight control and body composition in healthy overweight people

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Abstract

Introduction: **Conjugated linoleic acids (CLAs) have shown beneficial effects in weight control therapy however this relation is not clear.**

Objetive: **The aim of the study was to examine the effects and safety of 3 g of a 1:1 mix of c9-t11 and t10-c12 on weight control and body composition in healthy overweight individuals.**

Methods: **A prospective, placebo-controlled, randomised double-blind, parallel clinical trial lasting 24 weeks was carried out in 38 volunteers (29w, 9m) aged 30-55 years and BMI ≥27-<30 kg/m² who consumed 200 ml/day of skimmed milk with 3g of CLAs or 3g olive oil (placebo). Anthropometric, biochemical and dual x-ray absorptiometry (DXA) tests were measured. Diet and physical activity were assessed.**

Results: **Subjects maintained their habitual dietary and exercise patterns over the study. Only CLA group showed a significant decrease in weight (74.43 ± 10.45 vs 73.54 ± 11.66 kg, p = 0.029) and waist circumference (91.45 ± 10.33 vs 90.65 ± 9.84 cm, p = 0.012) between baseline and end of the study. BMI and waist height ratio decreased (28.44 ± 1.08 vs 27.81 ± 1.43 kg/m², p = 0.030 and 0.57 ± 0.05 vs 0.56 ± 0.04 p = 0.013 respectively) in CLA group at the end. CLA group experienced a reduction in total fat mass after 24 weeks (38.62 ± 5.02 vs 36.65 ± 5.64%, p = 0.035). No decrease was observed in Control group. HOMA index had no changes.**

Conclusions: **The consumption of skimmed milk enriched with 3g of a 1:1 mixture of c9-t11 and t10-c12 for 24 weeks led to a decrease in body weight and total fat mass in healthy, overweight subjects who maintained habitual diets and exercise patterns. No adverse effects were observed. Registered under ClinicalTrials.gov Identifier No. NCT01503047.**

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Key words: *Conjugated linoleic acids. Overweight. Weight lost. Dual X-Ray absorptiometry. Clinical trials.*

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EFECTO DE UNA SUPLEMENTACIÓN LÁCTEA CON ÁCIDO LINOLEICO CONJUGADO SOBRE EL CONTROL DE PESO Y LA COMPOSICIÓN CORPORAL DE PERSONAS SANAS CON SOBREPESO

Resumen

Introducción: **Los ácidos linoleicos conjugados (ALC) han mostrado unos efectos beneficiosos en el tratamiento del control de peso; sin embargo, esta relación no está clara.**

Objetivo: **El propósito de este estudio fue examinar los efectos y la seguridad de 3 g de una mezcla 1:1 de c9-t11 y t10-c12 sobre el control de peso y la composición corporal en individuos sanos con sobrepeso.**

Métodos: **Se realizó un estudio clínico prospectivo, de grupos paralelos, de distribución aleatoria, a doble ciego y con control placebo, de 24 semanas de duración, en 38 voluntarios (29 mujeres, 9 hombres) con edades de 30-55 años y un IMC ≥27- < 30 kg/m² que consumieron 200 ml/día de leche desnatada con 3 g de ALC o 3 g de aceite de oliva (placebo). Se midieron datos de antropometría, bioquímica y absorciometría dual de rayos X (DXA). Se evaluaron la dieta y la actividad física.**

Resultados: **Los sujetos mantuvieron sus patrones habituales de dieta y ejercicio a lo largo del estudio. Sólo el grupo de ALC mostró una reducción significativa del peso (74,43 ± 10,45 vs 73,54 ± 11,66 kg, p = 0,029) y de la circunferencia de la cintura (91,45 ± 10,33 vs 90,65 ± 9,84 cm, p = 0,012) entre el periodo basal y el final del estudio. El IMC y el cociente cintura/talla disminuyeron (28,44 ± 1,08 vs 27,81 ± 1,43 kg/m², p = 0,030 y 0,57 ± 0,05 vs 0,56 ± 0,04 p = 0,013, respectivamente) en el grupo ALC al final del estudio. El grupo ALC experimentó una reducción de la masa grasa total tras 24 semanas (38,62 ± 5,02 vs 36,65 ± 5,64 %, p = 0,035). No se observó reducción en el grupo control. El índice HOMA no experimentó cambios.**

Conclusiones: **El consumo de leche desnatada enriquecida con 3 g de una mezcla 1:1 de c9-t11 y t10-c12 durante 24 semanas produjo un descenso del peso corporal y la masa grasa total en sujetos sanos con sobrepeso que mantuvieron sus patrones habituales de dieta y ejercicio físico. No se observaron efectos adversos. Registrado con el identificador núm. NCT01503047 en ClinicalTrials.gov.**

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Palabras clave: *Ácido linoleico conjugado. Sobrepeso. Pérdida de peso. Absorciometría Dual de Rayos X. Ensayos clínicos.*

Introduction

The conjugated linoleic acids (CLAs) form a family of 28 positional and geometric isomers with conjugated bonds of linoleic acid. CLAs are formed naturally via the biohydrogenation of fats by bacteria in the gastrointestinal tract of ruminants. This involves changing the position and configuration of the double bonds of PUFAs, thus producing, for example, trans-10, cis-12 (t10-c12) or cis-9, trans-11 (c9-t11) octadecadienoic acid[1], the CLA isomers of greatest biological activity[2].

Milk, milk products and meat products from ruminants are the major food sources of CLAs; humans can synthesise them but only in small amounts[3]. The c9-t11 isomer, which accounts for nearly 90% of all naturally occurring CLAs, has been attributed anti-carcinogenic effects while t10-c12, found in much smaller amounts (around 10%), appears to be responsible for changes in lipid metabolism and body composition[4]. The health benefits of these isomers might be obtained via dietary supplementation with the appropriate quantities and proportions. Early studies suggested that the intake of 3.4 g/d CLAs is effective in helping body weight control, with higher intakes achieving no greater effect[5].

Over the last 30 years, the prevalence of overweight and obesity has doubled across the globe, and the world is now home to some 500 million obese adults and nearly a billion more who are overweight[6]. Both are associated with an increased risk of death[7] and more efficient overweight treatment should be a priority in public health. The possible beneficial effects of CLAs on coadjuvants in weight control therapy have therefore attracted much attention. A number of studies have been performed to determine the effects of CLAs on body composition, with special emphasis on their effects on fat and lean mass[8]. With respect to weight control, trials involving the consumption of skimmed milk supplemented with the 1:1 mix of c9-t11 and t10-c12 for 12 weeks[9] suggest such behaviour to be beneficial. However, some authors also reported CLAs have no effects on body composition[10] or increase the risk of insulin resistance and cardiovascular disease, to increase in inflammation-mediating molecules such as C-reactive protein, TNF-α, IL6, adiponectin and leptin[11]. The reasons for such discrepancies probably lie in differences in the characteristics of the different trials, which have involved different isomers, doses and anthropometric techniques, as well as the background food matrix in which these CLAs were provided, besides of gender and characteristics of the subjects, from here that trials using a standardised methodology are needed.

Due to lack of evidence on previous stages to obesity the aim of the present study was to examine the effects and safety of consuming 200 ml/day of skimmed milk supplemented with 3 g of a 1:1 mix of c9-t11 and t10-c12 (Tonalin®) on weight control and body composition over 24 weeks in healthy overweight individuals who maintained their habitual dietary and exercise patterns.

Subjects and methods

Approval

Study subjects

Subjects were recruited by the Clinical Nutrition and Dietetic Department, (La Paz University Hospital, Madrid, Spain) via information pamphlets, press releases, and/or e-mails. The study included 42 volunteers (32 women and 10 men) aged 30-55 years and suffered grade II overweight (body mass index, BMI≥27 but <30 kg/m^2). The requirements of inclusion were that subjects should not be adhered to any calorie restriction diet, be taking any weight control medication, or have lost more than 5 kg in the three months prior to the study; these conditions were met by all subjects. The exclusion criteria were the suffering of serious concomitant disease such as diabetes mellitus type II, metabolic syndrome (according to the criteria of the Adult Treatment Panel III), cancer, kidney disease, HIV, tuberculosis, cardiovascular disease, chronic obstructive pulmonary disease, eating disorders, having undergone bariatric surgery and/or intestinal resection, and pregnancy. All subjects gave their signed, informed consent to be included in the study, as required by the latest version of the Helsinki declaration[12]. The study was approved by the Scientific Research and Ethics Committee of the La Paz University Hospital. This trial was registered at clinical trials.gov as NCT01503047.http://clinicaltrials.gov/.

Study design and milk products

This study was designed as a prospective, placebo-controlled, randomised double-blind, parallel clinical trial lasting 24 weeks. Treatment consisted of exchanging the normal milk product consumed at breakfast for 200 ml of a skimmed milk with a lipid composition of 0.42 g saturated fatty acids (SFAs) and 0.72 g oleic acid, enriched with 3 g of a 1:1 mix of c9-t11 and t10-c12 (Tonalin®) (CLA group; n = 22), or a placebo drink, containing 200 ml of the same skimmed milk but without added CLAs, compensating for their absence with 3 g oleic acid (placebo, Control group, n = 20). Both milks provided 49 kcal/100 ml to the habitual diet. Special care was taken to ensure that both the CLA and P product had the same organoleptic qualities. The blinding of these products were undertaken by the supplying company, with both provided in identical 200 ml impermeable cartons.

Over the 24 weeks experimental period, the study subjects attended six appointments (one per month) at the Clinical Nutrition and Dietetic Department, La Paz University Hospital in Madrid. During the first visit they were randomly assigned a "treatment number" consisting of three digits, which placed them in either the CLA or Control group. Neither the subjects nor the researchers knew to which treatment group the subjects

had been assigned; the researchers were unblinded only at the end of the study. The subjects also underwent anthropometric, biochemical and dual X-ray absorsptiometry (DXA) tests (see below), their diets were examined, and their physical activity recorded. During visits 2-5 their adherence to the consumption of the supplemented milk was determined, as well as their tolerance of it. Any variation in the diet and body weight was also recorded, along with any adverse events. On the sixth visit the tests performed during the first appointment were repeated.

Diet and exercise

It was made very clear to the study subjects that they should keep to their habitual dietary and exercise habits during the experimental period. The diet of each subject was monitored at each of the six appointments. All food and beverages consumed were recorded in the week prior to each appointment using a food frequency questionnaire and a "3-day food and drink record" validated for the Spanish population[13]. All food and drinks consumed at home and away for three consecutive days, including a weekend day were recorded. Subjects were instructed to register the weights of food consumed if possible and to use household measurements (spoonfuls, cups, etc) if not. At each visit, every record was thoroughly reviewed by a nutritionist and the participant in order to complete the information and avoid the forgetfulness in the register of foods. The energy and nutritional content of the food consumed was then calculated using DietSOURCE® v.3.0 software. The values obtained were compared to the recommended values to determine dietary adequacy[14]. Physical activity was determined using an activity questionnaire, validated for the Spanish population[15], covering the seven days prior to each appointment.

Anthropometric measurements and body composition

Anthropometric measurements were made using standard techniques and adhering to international norms set out by the WHO[16]. All measurements were made by trained personnel, first thing in the morning, with the subjects barefoot and wearing only underwear. Body weight was determined using a single frequency body composition analyser (TANITA BC-420MA, Biologica Tecnología Médica S.L. Barcelona, Spain), with subjects standing upright and with heels together. Height was determined using a height meter with an accuracy of 1 mm (range 80-200 cm), again with the subject standing upright, and with the head in the Frankfurt plane.

Waist circumference (WC) was measured midway between the lowest lateral border of the ribs and the upper lateral border of the iliac crest, following normal expiration, using a non-stretchable metallic tape accurate to 1 mm. All measurements were made in triplicate by the same observer and the mean was calculated.

The values recorded were used to determine the BMI (body weight in kg/height in m^2) and the waist/height ratio (WHR; WC in cm/height in cm).

Body composition was determined by DXA (General Electric. Madison, Wisc, USA) a technique that distinguishes between fat and lean mass content. Subjects were examined in the supine position with their arms at their sides.

Blood samples analysis

Blood samples were obtained from fasting subjects at baseline and at 24 weeks, and the plasma glucose, fasting immunoreactive insulin, total cholesterol, HDL- and LDL-cholesterol, triglycerides, leptin, adiponectin, C-reactive-protein, plasminogen activator inhibitor-1, alanine amino transferase, aspartate amino transferase and creatinine concentrations determined. Insulin sensitivity was estimated using the HOMA-IR index [HOMA-IR = fasting glucose (mmol/l)/fasting immunoreactive insulin (mU/ml)/22•5]. Analyses were performed in the laboratories of Biochemistry of the La Paz University Hospital and Laboratory of Molecular Biology, Nutrition and Biotechnology (LBNB), Balearic Islands University.

Blood pressure and heart rate

Blood pressure and heart rate were measured in the right arm using an Automatic Monitor Welch (Allyn Spot Vital Signs 420 series. Arizona, United States) (± 5 mmHg). Three measurements were taken at 5 min intervals and the mean was calculated.

Compliance and adverse events

Compliance was assessed every month by comparing the number of milk cartons provided and the number of unopened cartons returned. A subject was considered compliant when having consumed the contents of at least 75% of the cartons dispensed. Potential adverse events were asked on each visit and, if any recorded. An adverse event was defined as any unfavourable, unintended effect reported by a subject or observed by the investigator during the study. All were recorded along with the symptoms involved (nausea, vomiting, diarrhoea, bad breath, constipation).

Statistical analysis

Data are presented as means ± standard deviation (SD). The Kolmogorov-Smirnov test was used to check the normal distribution of the data. Atypical data

(i.e., lying more than two SDs from the mean) in asymmetric distributions were deemed to reflect true results; they were, therefore, not eliminated from the analysis. The Levene test was used to determine whether the variance presented by the measured variables was homogeneous. When the distribution of the results was normal, the Student t test was used to compare the mean values of the studied variables recorded for the two treatment groups. The Mann-Whitney U test was used when the distribution was not normal.

Differences within groups at the beginning and end of the study were examined using the paired Student's *t* test when the distribution of the results was normal, and the Wilcoxon test when it was not. Bonferroni correction was used since multiple comparisons were made. All tests were two-tailed. Significance was set at $p<0.05$. All calculations were performed using SPSS v.9.0 software (SPSS Inc.).

Results

Thirty-eight of the 42 volunteers (90.5%) completed the study. Four subjects (0 in CLA and 4 in Control groups) withdrew during the intervention for personal reasons unconnected with the study. Thus, the analyses were conduced with 38 participants (29 women and 9 men), being 22 subjects in CLA group and 16 in Control group (Fig. 1). The baseline characteristics of the two study groups are summarized in the table I. The mean age of the subjects was 44 ± 8 years, with no significant differences between gender or treatment groups (Table I). Analysis of dietary quality showed that habitual energy intake profiles of subjects were imbalanced; intake of lipids was above recommendations, at expenses of carbohydrate intake, which were lower than recommended. In fact, just one subject had an adequate dietary distribution of macronutrients.

The subjects successfully maintained their normal dietary and exercise patterns over the study period (Table II). At the beginning of the study, the anthropometric and dietary characteristics of the members of the two treatment groups were similar, with no significant differences between them (Tables II and III).

At the beginning of the study all the subjects had a physical activity minor to ≤60 min/week and they were classified as "sedentary"; there were no changes at the end of the intervention.

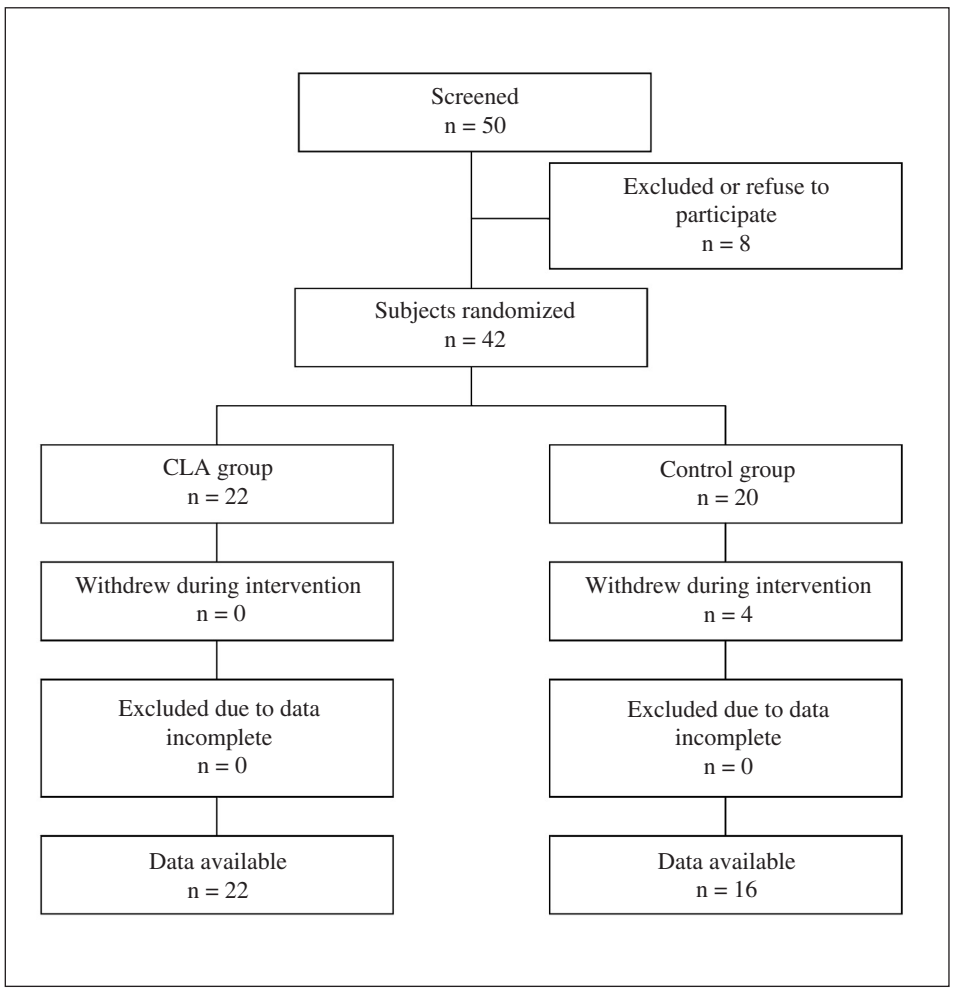

Fig. 1.—Study flow chart.

Table I
Subjects characteristics at the baseline

	CLA group (n = 22)	*Control group (n = 20)*	*P*
Sex *(female/male)*	17 / 5	12 / 4	NS
Age *(years)*	43.00 ± 8.30	44.35 ± 7.79	NS
Weight *(kg)*	74.43 ± 10.45	73.88 ± 7.73	NS
BMI *(kg/m²)*	28.44 ± 1.08	28.56 ± 0.95	NS
WC *(cm)*	91.45 ± 10.33	91.48 ± 5.41	NS
WHR	0.57 ± 0.05	0.57 ± 0.03	NS
FM *(kg)*	28.63 ± 4.21	28.14 ± 4.26	NS
FM *(%)*	38.62 ± 5.02	38.49 ± 5.27	NS
LM *(kg)*	43.22 ± 9.70	42.17 ± 6.01	NS
Total intake			
Kcal/d	1997.82 ± 385.44	1889.41 ± 341.62	NS

BMI: body mass index; FM: fat mass. LM: lean mass.WC: waist circumference. WHR: waist/height ratio. NS: no significant.

The initial mean BMI and WHR of the subjects in both groups was similar (Table III), as were their body fat mass in absolute values and as percentage of body weight (Table III).

CLA subjects showed a significant decrease in body weight ($p = 0.029$) and WC ($p = 0.012$) between baseline and the end of the study. The indices dependent on these variables —BMI and WHR— also showed significant reductions ($p = 0.030$ and $p = 0.013$ respectively) in the CLA group by the end of the study period. Indeed, after 24 weeks of treatment, CLA subjects had a significantly lower weight versus Control ($p < 0.05$).

At the beginning of the study, 100% of the subjects belonging to CLA group showed grade II overweight (BMI ≥27 but <30 kg/m²), but by the end 28.6% (6/21) had moved to grade I (BMI ≥25 but <27 kg/m²). Interestingly, the Control group showed no such trend: although 16.6% (2/12) moved down from grade II to grade I, another 16.6% moved up to obesity type I (BMI ≥30 but <35 kg/m²).

Changes in body weight associated with CLA treatment were also associated with significant changes in body composition, as determined by DXA between baseline and the end of the study (Table III). Subjects who consumed CLA presented a decrease in total fat mass measured by kg ($p = 0.046$) as well as by percentage ($p = 0.035$); while in the Control group no reductions were observed. A significant correlation was demonstrated between changes in body weight and fat mass in the CLA group ($r = 0.600$, $p = 0.03$), on the other hand no correlation was found in the Control group ($r = 0.464$, $p = 0.08$). No changes were observed in lean mass in either group.

Table II
Dietary characteristics at the baseline and end of the experimental period depending on treatment

		CLA group (n = 22)	*Control group (n = 16)*
TEE *(kcal/d)*	Week 0	2397.77 ± 305.04	2346.44 ± 226.66
	Week 24	2413.06 ± 336.74	2384.05 ± 297.77
	Variation	-21.59 ± 43.97	-6.17 ± 28.16
Intake *(kcal/d)*	Week 0	1992.82 ± 385.44	1889.41 ± 341.62
	Week 24	1900.61 ± 286.11	1907.74 ± 280.25
	Variation	-97.20 ± 287.04	18.33 ± 254.47
Underestimation *(%)*	Week 0	16.7 ± 16.59	20.07 ± 15.52
	Week 24	19.08 ± 13.35	19.61 ± 12.04
	Variation	3.01 ± 13.19	-0.92 ± 11.16
Energy profile			
Proteins *(%)*	Week 0	19.46 ± 2.77	18.76 ± 2.33
	Week 24	18.92 ± 2.14	18.28 ± 1.98
	Variation	-0.54 ± 2.38	-0.48 ± 1.94
Carbohydrates *(%)*	Week 0	43.58 ± 7.52	40.71 ± 8.50
	Week 24	43.83 ± 5.64	43.82 ± 4.73
	Variation	0.26 ± 4.02	3.11 ± 6.69
Lipids *(%)*	Week 0	37.04 ± 6.84	40.47 ± 8.83
	Week 24	37.36 ± 5.33	38.00 ± 5.20
	Variation	0.32 ± 3.87	-2.48 ± 6.57

TEE, theoretical energy expenditure.
[a]significantly different with respect to values at the beginning of the study ($p < 0.05$).
[b]significantly different with respect to Control group ($p < 0.05$).

Table III
Anthropometric characteristics at the baseline and end of the experimental period depending on treatment

		CLA group (n = 22)	*Control group (n = 16)*
Body weight *(kg)*	Week 0	74.43 ± 10.45[ab]	73.88 ± 7.73
	Week 24	73.54 ± 11.66	74.01 ± 9.61
	Variation	-1.40 ± 2.82	-0.54 ± 1.95
WC *(cm)*	Week 0	91.45 ± 10.33	91.48 ± 5.41
	Week 24	90.65 ± 9.84 a	91.00 ± 5.52
	Variation	-2.60 ± 4.43	-0.46 ± 3.88
BMI *(kg/m²)*	Week 0	28.44 ± 1.08	28.56 ± 0.95
	Week 24	27.81 ±1.43[a]	28.51 ± 1.29
	Variation	-0.53 ± 1.06	-0.21 ± 0.81
WHR	Week 0	0.57 ± 0.05	0.57 ± 0.03
	Week 24	0.56 ± 0.04[a]	0.57 ± 0.03
	Variation	-0.02 ± 0.03	0.00 ± 0.03
FM *(kg)*	Week 0	28.63 ± 4.21	28.14 ± 4.26
	Week 24	27.51 ± 5.39[a]	26.72 ± 3.93
	Variation	-1.18 ± 2.74	-0.89 ± 1.89
FM *(%)*	Week 0	38.62 ± 5.02	38.49 ± 5.27
	Week 24	36.65 ± 5.64[a]	36.81 ± 6.20
	Variation	-1.02 ± 2.13	-0.44 ± 1.34
Lean mass *(kg)*	Week 0	43.22 ± 9.70	42.17 ± 6.01
	Week 24	44.69 ± 1.02	42.49 ± 7.73
	Variation	-0.66 ± 2.41	-0.56 ± 2.27

BMI: body mass index. FM: fat mass. LM: lean mass. WC: waist circumference. WHR: waist/height ratio.
[a]significantly different with respect to values at the beginning of the study ($p < 0.05$).
[b]significantly different with respect to Control group ($p < 0.05$).

No significant changes were demonstrated in blood analyses, except for glucose ($p = 0.002$) and plasma creatinine ($p = 0.001$) in the CLA group, both of which showed significant increases by the end of the study period. Nevertheless, insulin levels and HOMA index have no changes at the end of the intervention (Table IV).

The number of adverse events was low in both CLA and Control groups: nausea 4.2% vs 0%, diarrhoea 4.2% vs 13.0%, constipation 12.5% vs 13.3%, bloating 12.5% vs 20.0%, and bad breath 0% vs 13.3% (no significant differences for any comparison). No member of either group suffered stomach acidity.

Discussion

Many beneficial properties have been reported for CLAs since the firsts descriptions, including protection against cancer[17], diabetes[18] and obesity[19]. However, most of the intervention have been conducted in subjects with an established disease and there are no studies to observe CLAs effect on early stages as disease prevention.

Given the worldwide epidemic of obesity, and the millions of obesity-related deaths that occur every year, the potential beneficial effects of CLA consumption on body weight and body composition have received growing attention. CLA consumption has, for some time now, been associated with a decrease in body fat in overweight and obese individuals[5]. In later publication involving supplementation with 500 ml skimmed milk containing 3g of a 1:1 mixture of c9-t11 and t10-c12 administered to subjects with metabolic syndrome for 12 weeks, a significant reduction in total and trunk fat mass in overweight was observed, with no adverse effects[9]. Moreno et al[20] administered CLA-enriched milk to 572 subjects on a hypocaloric diet for 12 weeks, and reported significant improvements in body composition with no adverse effects. However, Nazare et al (2007)[21], who administered yoghurt containing 3,76 g of the Tonalin® mixture daily for 14 weeks to healthy, overweight subjects, observed no changes in body composition in terms of fat mass and fat percentage, although they did record an increase in basal metabolic rate. Thus after more than a decade of research, the effects of CLAs are yet to be fully characterised.

In the present study, a body weight reduction of 1.4 kg was demonstrated in the CLA group. This may seem to be clinically irrelevant, but body weight was strongly related to fat mass in these subjects ($r = 0.600$). The treatment therefore directly reduced the amount of total body fat. The rate of decrease (0.02 kg/week) was lower than that reported in a meta-analysis of 18 studies by Whigham et al (2007)[22] (0.09 kg/week). In the meantime, this is of interest when it is understood that, current trend for developed countries with high prevalence of obesity; gain an average of 0.4 kg total weight/year (0.009 kg/week)[23]. Thus, while the overall reduction in body weight was small among volunteers, the reduction

Table IV
Blood parameters at the baseline and end of the experimental period in members of both treatment groups

		CLA group (n = 22)	*Control group (n = 16)*
Chol-t *(mg/dL)*	Week 0	207.48 ± 30.77	196.94 ± 31.01
	Week 24	211.10 ± 33.94	205.70 ± 37.01
	Variation	3.62 ± 20.16	8.76 ± 24.58
LDL-chol *(mg/dL)*	Week 0	133.86 ± 29.20	148.04 ± 71.96
	Week 24	137.14 ± 23.70	135.56 ± 30.57
	Variation	3.29 ± 24.82	-12.46 ± 64.65
HDL-chol *(mg/dL)*	Week 0	57.19 ± 20.00	50.31 ± 7.88
	Week 24	54.24 ± 12.13	50.81 ± 6.60
	Variation	-2.95 ± 17.49	0.50 ± 4.10
TG *(mg/dL)*	Week 0	92.67 ± 28.70	106.13 ± 52.51
	Week 24	94.81 ± 30.04	96.81 ± 16.74
	Variation	2.14 ± 35.54	-9.31 ± 54.72
Glu-AC *(mg/dL)*	Week 0	91.05 ± 6.94	93.24 ± 6.60
	Week 24	93.71 ± 6.50a	93.82 ± 8.95
	Variation	2.76 ± 5.67	0.59 ± 9.57
Insulin *(mUI/mL)*	Week 0	7.89 ± 5.09	7.46 ± 3.28
	Week 24	7.17 ± 3.11	7.54 ± 5.13
	Variation	-0.72 ± 3.94	0.08 ± 4.16
HOMA index	Week 0	1.77 ± 1.13	1.72 ± 0.76
	Week 24	1.67 ± 0.79	1.78 ± 1.42
	Variation	-0.10 ± 0.81	0.07 ± 1.33
Leptin *(ng/mL)*	Week 0	19.04 ± 6.72	23.21 ± 11.67
	Week 24	15.73 ± 7.32	21.08 ± 16.28
	Variation	-3.31 ± 3.83	-2.13 ± 9.02
Adiponectin *(µg/mL)*	Week 0	10.85 ± 6.59	8.83 ± 4.76
	Week 24	10.56 ± 5.10	9.00 ± 5.50
	Variation	-0.29 ± 2.32	0.16 ± 1.19
CR-P *(mg/L)*	Week 0	1.93 ± 2.00	3.50 ± 3.12
	Week 24	2.83 ± 2.51	3.80 ± 3.35
	Variation	0.90 ± 1.33	0.30 ± 2.10
PAI-1 *(ng/mL)*	Week 0	4.52 ± 3.36	4.87 ± 3.12
	Week 24	4.83 ± 2.60	6.93 ± 4.30
	Variation	-0.14 ± 2.69	2.07 ± 2.55
ALT *(UI/L)*	Week 0	21.43 ± 10.98	17.75 ± 8.51
	Week 24	21.48 ± 12.57	18.31 ± 5.43
	Variation	0.05 ± 9.24	0.56 ± 6.69
AST *(UI/L)*	Week 0	20.67 ± 5.69	18.44 ± 5.24
	Week 24	23.71 ± 13.83	19.69 ± 3.96
	Variation	3.05 ± 13.68	1.25 ± 3.39
Creatinine *(mg/dL)*	Week 0	0.81 ± 0.17	0.86 ± 0.15
	Week 24	0.89 ± 0.16[a]	0.91 ± 0.17
	Variation	0.07 ± 0.09	0.06 ± 0.12

Chol-t: total cholesterol. TG: triglycerides. Glu-AC: fasting glucose. HOMA: insulin resistance index. CR-P: C-reactive protein. PAI-1: plasminogen activator inhibitor1. ALT: alanine aminotransferase. AST: aspartate aminotransferase.
[a]significantly different with respect to values at the beginning of the study ($p < 0.05$).

in fat mass could be considered a clinical benefit related to reduce of cardiovascular risk[24]. Mechanisms by which CLAs might reduce fat mass include the inhibition of adipogenesis, the suspension of lipogenesis[25], the stimulation of lipolysis, and the induction of apoptosis in white adipose tissue[26].

In addition, the CLA group also experienced a significant reduction in WHR, one of the variables most strongly related to cardiovascular risk[27]. Furthermore, CLA subjects experienced a significant reduction in WC, a phenomenon also reported by Risérus et al (2001)[28]. WC is a measure of central obesity an independent risk factor for metabolic syndrome and its decrease is therefore likely associated with a reduction in this risk. The CLA group members also experienced a significant decrease in WHR, one of the studied variables most strongly related to cardiovascular risk[27], as measured by specific blood markers such as oxidised LDL-cholesterol and triglycerides[29]. Another studies report that CLA consumption reduces fat mass differently in different parts of the body, with the largest reductions in the trunk of people with signs of metabolic syndrome and, in legs

of overweight/obese people[30]. Shu-Chiun et al (2012) demonstrated significantly decrease of body weight, BMI, fat mass, fat percentage and subcutaneous fat mass in overweight and grade I obese subjects supplemented with a milk beverage enriched CLA (3.4 g/d) for 12 weeks[31]. Nevertheless in the present study, no differences were observed (data not shown), probably because none of the subjects were obese.

One of the plausible mechanisms by which CLA may reduce body weight is via an increase in the metabolic rate due to increased muscular mass and its associated energy expenditure[32,33]. A number of studies have shown that, parallel to the reduction in fat mass, lean mass increase in subjects who consume CLAs. On the other hand, in the present study the lean mass remained stable in subjects.

It should be remembered that the present subjects maintained their normal dietary and exercise patterns. It is therefore to be expected that the reduction in anthropometric values and body composition be less than those seen in studies involving CLA and a hypocaloric diet and/or an exercise programme.

Some studies suggest that CLA c9-t11 and t10-c12 have a negative effect on HDL-cholesterol, plasma triglycerides, C-reactive protein, and plasminogen activator inhibitor-1, and therefore may increase the risk of cardiovascular disease and even glucose intolerance and diabetes mellitus type II[34]. In the present trial, hepatic function markers; cardiovascular risk markers, such as the blood lipid profile and the HOMA index; and the inflammatory markers did not underwent significant variation. The blood glucose and creatinine values showed a significant increase in the CLA group, but the values reached still remained within the normal range defined for this target population[34].

In summary, our results, in agreement with other trials performed with the same product show a net decrease of body weight, indicate a lack of adverse effects and contribute to support the safety of the CLA mixture (c9-t11 and t10-c12) as novel food ingredient recently recognised by the European Food Safety Authority in quantities up to 3.5 g day[35].

Conclusion

The daily consumption of skimmed milk enriched with 3 g of a 1:1 mixture of c9-t11 and t10-c12 for 24 weeks led to a slight decrease in body weight and total fat mass in healthy, overweight subjects who maintained habitual diets and exercise patterns. In this context, further clinical trials should be encouraged, including hypocaloric diet and regular physical exercise to the co-treatment of overweight.

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Conflict of interest

The authors have no conflicts of interest.

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