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Review Article

Intake of Conjugated Linoleic Acid, Creatine Monohydrate and Green Tea in a Milk Matrix and Variations in Body Composition in Human Volunteers

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Abstract

Overweight and obesity are increasingly frequent in the world. This fact has a high cost for health and excess body fat increases the risk of chronic and cardiovascular diseases. Proper dietary guidelines along with an active life can promote the maintenance of a healthy weight. It is also possible that some functional foods may serve as a fast. Conjugated linoleic acid is a well-studied substance, and in different studies it has been suggested that it can help to modify the body composition, so it could avoid that it is interesting to include it, only in the company of other substances such as creatine monohydrate or green tea, which have also suggested certain effects on body composition, in certain food matrices for specific functional foods to help reduce the percentage of body fat.

Keywords: Overweight; Obesity; Green Tea

Introduction

Obesity and excess weight, a problem with a social dimension

According to the Spanish Society for the Study of Obesity (SEEDO)'s obesity prevalence study [1], the prevalence of obesity in Spanish adults is 15.5%, 13.9% in children and young people and 35% in the elderly. If we add the prevalence of excess weight to that of obesity, we can say that as a whole, more than 50% of the Spanish population has excess body fat. In other words, it could be called a true plague.

The relationship between excess body fat and the increased risk of certain diseases (hypertension, diabetes, and some types of cancer, etc.) is well known [2]. The modification of body composition with respect to the loss of fat and increase in the lean mass of individuals offers great benefits. In sedentary people and overweight people, etc., the reduction of fat mass can reduce cardiovascular risk and other types of diseases, and so it is essential that healthcare professionals have the tools with which to fight this problem.

Optimal diet and adequate physical training can contribute to modifying body composition, reducing the percentage of body fat, increasing lean mass and reducing cardiovascular risk [3]. However, some nutritional supplements such as conjugated linoleic acid (CLA) have at times been related to the ability to significantly increase this effect (any help in this regard is beneficial) [4,5]. If this ability were confirmed, it would have direct effects on health and quality of life. But its effect be not be limited only to the population with excess body fat: in athletes, a reduction in fat can help improve performance in certain cases [3].

In both cases (people with excess fat or athletes) the increase in lean mass (attributed to creatine and conjugated linoleic acid) would be a significant factor. When following a hypocaloric diet, the loss of body fat is usually accompanied by a reduction in lean mass due to loss of muscle mass. Muscle is highly active tissue that is full with mitochondria and consumes a significant amount of energy, even at rest. Therefore, a significant loss of muscle mass will cause a reduction of the basal metabolism and therefore a greater tendency to regain the lost weight (the so-called rebound effect). In athletes, increasing muscle mass can improve performance [3].

The incorporation of these substances in foods of habitual consumption would allow for the creation of functional foods: products which are increasingly popular. According to the FUFOSE (Functional Food Science in Europe) consensus document prepared by the International Institute of Life Sciences in Europe (ILSI), which is an organisation made up of scientists and industry professionals, a food can be considered functional if it has been satisfactorily demonstrated that it has a beneficial effect on one or more specific functions in the body, beyond the usual nutritional effects, this being relevant for improving health and well-being and/or reducing the risk of becoming ill.

Supplements with the ability to modify body composition

It is clear that by modifying the total caloric expenditure by means of a hypocaloric diet and/or by increasing energy expenditure by increasing physical activity, body fat can be lost [2], likewise, through adequate and constant training (in addition to an adequate diet) you can increase lean and muscle mass [3]. But any help in this regard is beneficial, and in this sense there are some supplements or substances that have sometimes been attributed a certain ability to help modify body composition through different mechanisms.

Conjugated linoleic acid (CLA)

Michael W. Pariza and his collaborators [6], identified a compound, conjugated linoleic acid (CLA), as a component with anticarcinogenic properties and, in fact, were the first to report information that pointed to the physiological benefits derived from the consumption of CLA.

The term CLAs refers to a mixture of linoleic acid isomers with conjugated double bonds. There are 28 different isomers, but the most important ones are cis9, trans11 and trans10, cis12. Mixtures with these two aforementioned isomers have been shown to be more effective and safer [7]. CLA is found as a dietary source for humans in ruminant meat and in dairy products. Linoleic acid is converted to CLAs by the microorganisms present in your intestine, therefore this compound is found naturally in meat and dairy products [8]. The diet of the livestock and seasonality influence the CLA-richness of their products [9].

It is really difficult to achieve the levels of CLA required for the desired effects described through diet alone; the dose used to study the loss of body fat in humans is 3 grams a day, an effective, safe and tolerated dose [10]. However, it is estimated that in western diets, the average intake of CLA is 1.5 to 2 grams per day, with fluctuations between 0.3 and 0.6 grams per day [10]. To achieve this dose, it would be necessary to increase total and saturated fats found in the diet, since 88% of the dietary CLA comes from the intake of fatty dairy and ruminant meat [8]. Eating more saturated fat is not recommended from the point of view of cardiovascular health and is far from current nutritional recommendations, since as a consequence of these changes in diet, LDL-cholesterol levels could rise [11]. Therefore, there are only two suitable alternatives to increase CLA dietary intake. The first would be to manipulate the diet of cattle to increase levels of CLA in their fat fraction, without increasing total fat [12], and the second would be to enrich foods for regular consumption with this product.

Currently excess weight and obesity are becoming a true epidemic. In Spain, more than half of the population is overweight or obese [1], therefore any tool that is useful in the fight against excess body fat must be considered. Excess weight and obesity are not only an aesthetic problem, but are above all a health problem as they increase the risk of suffering from various diseases, such as diabetes or cardiovascular illnesses [2].

It is not only total fat which is important, but also its distribution, as those with the greatest accumulation of abdominal fat have a higher risk of suffering from this type of disease. An excess of visceral fat is related to a higher risk of this type, and in this regard CLA appears especially effective in reducing said accumulation [13], which may be very useful for people at cardiovascular risk.

CLA is part of dietary fat, and a daily intake of fat is vital as it fulfils essential metabolic functions such as the transport of fat-soluble vitamins, forming part of the structure of cell membranes, and participating in the synthesis of hormones, etc. It is not only total fat which is important, however, but also the type of fat ingested. In this sense, oleic acid, medium chain fatty acids and omega 3 fatty acids have at times been attributed some influence on the structure and amount of body fat. However, from this point of view (amount of body fat), it is CLA that has the greatest influence on the amount of total body fat, with several studies supporting this [14].

Traditionally, however, one of the main criticisms against its ability to reduce body fat was the absence of any meta-analysis that concluded the physiological efficacy of CLA. In 2007, an important change occurred in this regard, with the publication of meta-analysis in the American Journal of Clinical Nutrition [15]. It concludes that conjugated linoleic acid helps to reduce body fat in a statistically significant way.

From the point of view of its usefulness in the treatment of obesity and excess weight, CLA can be effective, not only because of its ability to reduce body fat, but also because it is likely to be able to increase muscle mass [16]. This is very interesting, since often when a diet plan is followed, a loss of muscle mass occurs. Muscle is a very active tissue, therefore a loss of muscle mass can cause a significant decrease in basal metabolism and therefore facilitate a subsequent rebound effect with a gain in body fat. There exists meta-analysis in which it is also concluded that CLA causes this effect, although it seems that the time of treatment and the dose are not influential [17].

From the point of view of studies that reflect a loss of body fat after CLA intake, most were performed on healthy individuals, although some were performed on individuals with metabolic syndrome [18]. In most cases there is no strict control of dietary intake [19], but in other studies, individuals were confined in order to be able to control dietary intake [16]. Although most of the studies have a duration of a few months, there is a double-blind study carried out over 24 months which shows a reduction in body fat, and in the Body Mass Index (BMI), with an increase in lean mass [20]. In most of the published works, CLA is administered as a supplement (capsules, sachets, etc.), but there are studies that show that when it is incorporated into a dairy matrix (that is, in milk, as is the case in this doctoral study), it is also effective [21,22].

Whenever reference is made to studies with CLA, it must be borne in mind that there are 28 isomers, which is why it is especially important to know which ones are present in the commercial product used. The reality is that from the point of view of body composition, the isomers that have been shown to be the most active and effective are c9, t-11 and t10, c-12 [23,24].

The effectiveness in reducing body fat is verified both in individuals who engage in physical activity on a regular basis [13] and in individuals who do not practice regular physical activity [25-27], although it is true that with physical activity the effect on reducing body fat can be very significant, far exceeding a loss of 10% of body fat [13]. From this point of view, the intake of products such as CLA combined with physical activity, could not only reduce body fat, but also increase lean mass [16,17,28,29]. In fact, an experimental work carried out in adult rats concludes that the ingestion of the 10 trans 12 cis isomer stimulates muscle protein synthesis [30].

With regard to the safety of CLA, there are studies in animals such as rats [31] or pigs [32], but also in humans [33-35], that show that there are no adverse health effects. In the case of humans, these are carried out with high-quality preparations and doses of between 3 and 6 grams daily.

Possible CLA mechanisms of action

Action involving apoptosis, causing cell death of the preadipocyte; Action involving the differentiation from preadipocyte to adipocyte, blocking this process; Inhibition of Glut-4 transporter expression and activity, thus reducing the entry of glucose into the adipocyte; Possible decrease in caloric intake; Action involving li-

polysis; Increased activity of the enzyme carnitine palmitoyltransferase (CPT); Facilitation of the binding of fatty tissue to the gamma receptor (PPAR), with a consequent reduction of the hormone leptin and of triacylglycerol within the adipocyte; Change of energy expenditure; Blocking of lipogenesis: by inhibition of the enzyme stearoyl desaturase delta 9 desaturase and enzyme lipoprotein lipase [36-38].

In summary, it seems that the daily intake of CLA (in doses of about 3 grams per day) could reduce the percentage of body fat from 1.60% to 22.22% [39].

The differences between studies are caused by various reasons: the duration of the study, the dose of CLA, the type of isomers used, whether physical exercise is practiced or not, and, if practiced, the type of physical exercise carried out, whether or not diet is controlled, and the body composition measurement technique (infrared, BIA, or plestimography, etc.).

Creatine monohydrate

Creatine monohydrate (Cr), is a natural nitrogen compound very similar to amino acids, which is found in muscle and nerve cells and combines with phosphate, creating phosphocreatine (PCr). It is synthesised endogenously in the liver, pancreas and kidneys from the amino acids arginine, glycine and methionine.

Creatine supplementation seems to cause an increase creatine and phosphocreatine levels, muscle mass and intracellular water volume [40,41].

The intake of Creatine monohydrate, either in the form of about 20 grams for 5-6 days (loading phase) and 2-3 grams in the following days or about 3-5 grams for 11-28 days, allows for an increase of intramuscular creatine and phosphocreatine levels, and in some way increases lean and muscular mass without affecting fat mass.

Green tea

Green tea is a non-fermented drink and is produced from the fresh leaves of the *Camellia sinensis* plant, in which we find: water, proteins, carbohydrates, minerals, vitamins and polyphenols of the flavonoid type. The main flavonoids in green tea are catechins, which make up about a third of its total dry weight. The most abundant catechin, with greater than 50% of the total, is epigallocatechin gallate (EGCC).

From some studies, it can be concluded that green tea and its catechins have a reducing effect on body weight [42,43] possibly through their interference with the adreno-sympathetic system and enzymes that act in the synthesis of fatty acids.

The results obtained in experimental animals suggest that in humans a daily consumption of green tea drinks equivalent to 2g or more of dry tea (or 4 cups per day of infusion) would be required to show the same effects [44].

Hypothesis and objectives

Hypothesis

Although there are no studies that combine the intake of CLA and green tea, by making a brief bibliographic review of each of them, verifying that they share some of their effects and studying their mechanisms of action, it could be hypothesised that simultaneously ingesting the optimal dose of CLAs together with the optimal dose of green tea would increase the loss of body fat and in this way, lead to body fat losses greater than those achieved by ingesting each of them separately: that is to say, that one could expect a synergistic effect.

The fact that there is evidence that both act at different levels in fat loss, sharing some mechanisms (intake, adipocytes, etc.) and not others (e.g. thermogenesis) could support this hypothesis.

There are some studies [45,46], though these are not very numerous, in which the intake of CLA and creatine are combined and this has a positive effect with regard to improvement in body composition. Based on these, and verifying that both substances seem to share some of their effects (increase in lean mass), it could be hypothesised that by simultaneously consuming the optimal dose of CLA together with the optimal dose of creatine, the gain of lean mass would be increased and greater benefits will be achieved than would be attained consuming each of them separately. This synergistic effect would be expected to be evident both at 3 and 6 months.

Objectives

The objectives of this research work are:

Objective 1: To confirm if CLA, within a balanced diet and with regular physical activity, is able to reduce body fat in a statistically significant way, both at 3 and 6 months, compared to a group of people on a balanced diet with regular physical activity.

Objective 2: To verify if CLA, within a balanced diet and with regular physical activity, is effective in increasing lean mass at 3 and 6 months.

On the other hand, it is also our objective to verify if there is a possible summation effect for the effects of Cr and green tea with CLA, including a possible synergistic effect both at 3 and 6 months.

In this regard:

Objective 3: To verify if the simultaneous intake of Green Tea and CLA, within a balanced diet and with regular physical activity, reduces body fat more effectively than CLA alone does in the same circumstances, both at 3 and at 6 months.

Objective 4: To verify if the simultaneous intake of Cr and CLA, within a balanced diet and with regular physical activity, causes a statistically significant increase in lean mass compared to that resulting from the intake of CLA alone, both at 3 and 6 months.

Objective 5: To verify if the simultaneous intake of Cr and CLA, within a balanced diet and with regular physical activity, causes a significant reduction in fat mass greater than that of taking CLA alone, both at 3 and 6 months.

The demonstration of all or some of the hypotheses described above would have numerous applications with regard to excess weight, sport, the elderly, etc., and in the food industry (creation of new functional foods).

Material and Methods

Subjects under study

60 volunteers participated in our study, recruited from various gyms in Asturias (Spain) through informative talks and poster placement.

The selection has been made among people who regularly practice sports (3-4 weekly sessions of 45 minutes - 1 hour long). The consistency of the volunteers' sports practice has been confirmed through interviews with their monitors.

Table 1 shows the characteristics of the people who participated in the study.

All study participants signed an informed consent form before beginning the process. The study was approved by the ethics committee of the Central University Hospital of Asturias.

Number of participants	60
Number of volunteers who withdrew	6
Number of volunteers who completed the study	54
Age range in years	19-53
BMI range in kilograms/m^2	22-30
Gender	31 men and 19 women

Table 1

Through a health questionnaire, it was verified that the volunteers did not suffer from chronic diseases (diabetes, heart disease, etc.), serious illnesses (AIDS, cancer, etc.) or psychiatric disorders. The participation of pregnant or lactating women and people undergoing treatment with adrenergic drugs was also ruled out.

The 60 individuals were divided into 4 groups of 15 people each:

- **Group 1:** 3 grams of CLA and 3 grams of creatine are administered daily.
- **Group 2:** Receive 3 grams of CLA daily. This is considered the control group.
- **Group 3:** 3 grams of CLA and 2 grams of green tea are administered daily.
- **Group 4:** Only fat-free milk is administered daily. This is considered the placebo group.

The study was concluded with 54 people, thus registering 6 withdrawals for different reasons (in two cases due to changes of residence, one case due to suffering from type B hepatitis, another two cases due to job changes and one due to family reasons).

Withdrawals were distributed as follows: group 1 (one withdrawal), group 2 (two withdrawals), group 3 (one withdrawal) and group 4 (two withdrawals).

The number of overweight people is not considered relevant since BMI is not a good indicator in this case (these were people who usually train with weights, and muscle also adds weight).

Tests carried out

- Evaluations of body weight, height, lean body mass and percentage of fat mass are carried out.
- Weight was measured with the Inbody 720 scale and height was measured using a SECA-brand wall height rod. Weight was measured every month (at each visit) and height only the first time.

- The percentage of body fat mass was calculated using multi-frequency electrical bioimpedance techniques (BIA Inbody 720 analyser); these measurements were taken once a month.
- Lean mass was obtained via the difference between body weight and fat mass obtained by BIA, likewise, the total amount of water obtained by BIA was considered.

Analysis of body composition

The accuracy of the analysis of Total Body Water with this system is acceptable since the correlation with the deuterium oxide dilution method is high [48].

It also allows us to estimate fat mass, which refers to the total amount of lipids that can be extracted from fat and other cells. Fat mass cannot be estimated directly by bioimpedance, but is calculated by subtracting weight from fat-free mass (FFM):

Fat Mass = Weight - Fat Free Mass (FFM).

Fat mass accumulates under the skin (subcutaneous fat) as well as in the abdomen and muscles (visceral fat).

Likewise, this allows for lean mass to be estimated, which can be calculated by subtracting bone mineral mass from fat-free mass, and in this analysis these are considered at both an overall and segmental level.

A comparative study between results obtained with DEXA and In Body 720 showed a high correlation [47].

Product intake format

All the volunteers consumed a container (white tetra brik) daily with 200 millilitres of milk, which, depending on the study group, contained different ingredients:

- **Group 1:** 3 grams of CLA and 3 grams of creatine
- **Group 2:** 3 grams of CLA. This is the control group.
- **Group 3**: 3 grams of CLA and 2 grams of green tea.
- **Group 4:** Fat-free milk only. This is the placebo group.

In all cases, the cartons were completely white. The difference was a numerical code.

The volunteers in groups 2, 3 and 4 added a small teaspoon of sugar and those of group 1 added creatine. Purity is 99% (with 1% humidity).

Duration and specifications of the study, safety, and financial report

This was a randomised controlled study (the participating volunteers do not know what they are ingesting, but the researchers do), with dietary intervention and physical activity control. A diet model based on the food pyramid of the Spanish Community Nutrition Society (SENC) was proposed, with a structure of 6 daily intakes and carried out under the supervision of a Nutritionist.

Volunteers came to the clinic once a month and weight, BMI, and segmental multi-frequency BIA measurements were monitored (Inbody 720 from Inbody®).

Likewise, at each visit, the dietary guidelines were explained again and their importance was emphasised, as well as that of physical activity, to ensure that the habits remained constant, following the recommendations that had been given to them at the beginning.

The duration of the study was 6 months. Body composition measurements and clinic visits were therefore monthly (6 total).

Study safety

- The study is carried out with healthy people (a prior health questionnaire and a complete blood test was carried out).
- The volunteers were already practicing physical activity in gyms, and they were monitored by professionals.
- The dietary guidelines offered to the volunteers were based on the healthy eating pyramid (SENC): the way of eating that is recommended for the healthy, adult Spanish population.
- The products that were studied in the study (CLA, creatine and green tea) exist on the market and can be freely purchased by anyone without the advice of a healthcare professional and without a prescription. CLA and green tea were incorporated into a dairy matrix. In the market there are already prestigious companies that incorporate CLA or green tea in their dairy matrix. The novelty in this sense is to incorporate them both into the same dairy matrix.

With regard to Cr, this was supplied in powder form. In the market there are dozens of brands that for more than ten years have offered creatine monohydrate in the form of shakes, tablets, and sweets, etc. In short, CLA, green tea and Cr are dietary products

that are available to anyone in a multitude of stores, department stores, etc. and which have been approved for human consumption, making them completely safe products:

- Side effects can be (in the most serious cases) slight gastrointestinal complaints (discomfort, bloating and occasionally some diarrhoea) [20,23].
- The measurements were carried out by BIA, that is, a non-invasive method without risks, which does not require any special authorisation and which is usually carried out regularly in pharmacies, health food stores, and gyms, etc.

Diet plan

The current diet in Spain is too rich in fats and simple sugars. In this sense, the study sought to establish healthy eating habits based on the recommendations of the SENC food pyramid [49].

The dietary guideline was based on the premise of having 6 intakes a day. A breakfast was recommended that incorporated a serving of dairy products (milk, yogurts, etc.), a serving of fruit (oranges, pears, natural juice, etc.), a serving of cereals (breakfast cereals, toasts, wholemeal bread, etc.) and some extra food (a little ham or sausage, a handful of dried fruit, a little olive oil, for example).

A midmorning meal, a snack and a bedtime snack that consisted of a fruit, a low-fat yogurt or the study's own dairy mix.

A midday meal with a first course (rice, pasta, potatoes, legumes, vegetables-vegetables or a combination thereof) with a second protein course (meat, fish, eggs) and with an additional side to the first course (if there were already potatoes then the side would be vegetables, such as mushrooms, and not potatoes, for example). For dessert: fruit or fat-free dairy.

A dinner similar to lunch, but slightly lighter, including protein with vegetables, with the choice to add some rice, pasta or potatoes as a side. For dessert: fruit or fat-free dairy.

To season, they could add their chosen aromatic herbs and spices, but only one teaspoon of salt and 3-5 tablespoons of olive oil.

Physical activity plan

All the volunteers participating in the study go to the collaborating gym 4 times a week, with one hour per session. They perform a circuit that alternates aerobic exercises (bicycle, elliptical and treadmill) and exercises with light weights (bench press, behind the neck press, pull-up machine, bicep curl, triceps extension, squat machine and quadriceps extension machine).

Statistical analysis

Statistical analyses were carried out using the Statistical Program for Social Sciences (SPSS). One-way analysis of variance (ANOVA) was carried out to compare group values over time. Statistical significance was accepted at an alpha level $p < 0.05$. The values presented are the results of the means ± SD.

Results

Results at 3 months

At the third month, there are only statistically significant results regarding the variation in the percentage of total body fat. In the case of weight, lean mass and total water, there are no differences with statistical significance.

In addition, and with regard to body fat, there are only statistically significant differences when comparing the group that consumed milk with CLA with the placebo group (fat-free milk and nothing else) and the group that consumed milk with CLA + Cr with the placebo group.

Weight difference between the first and third month

Group	N	Subset for alpha= .05
	1	1
Fat-free milk	13	.0000
CLA + green tea	14	2.0500
CLA + cr	14	2.0857
CLA	13	2.6308
Sig.		.115

Table 2

At 3 months the control group maintained its weight, in the rest of the groups the loss of body weight was similar (the group that consumed milk with CLA stood out slightly, but without any statistical significance).

Percentage fat difference between the first and third month

Group	N	Subset for alpha = .05	
	1	2	1
Fat-free milk	13	.5538	
CLA + green tea	14	1.6714	1.6714
CLA + cr	14		2.9786
CLA	13		3.2946
Sig.		.298	.061

Table 3

The group that consumed milk with CLA stands out as the most effective for losing body fat (with 3.29%). A fairly close result to that above is obtained by the group that consumed milk with CLA + Cr, with 2.97%. In both cases the result is statistically significant with respect to the control group. The reduction in body fat in the group that consumed milk with CLA and green tea is two times less than in the group that only consumed CLA, and therefore has no statistical significance.

Total water difference between the first and the third month

Group	N	Subset for alpha
	1	1
CLA + cr	14	-.9071
CLA	13	-.5308
Fat-free milk	13	-.2385
CLA + green tea	14	.1143
Sig.		.072

Table 4

The group with the highest total body water loss was the group that consumed milk with CLA + green tea, however, there is no statistical significance.

Lean mass difference between the first and the third month:

Group	N	Subset for alpha= .05
	1	1
CLA + cr	14	-1.2429
CLA	13	-.6308
Fat-free milk	13	-.5692
CLA + green tea	14	.1500
Sig.		.073

Table 5

It can be seen that the group that consumed milk with CLA + Cr has the greatest increase in the lean mass. The group that consumed milk with CLA and green tea lost some lean mass. However, these results do not show statistically significant differences.

Results at 6 months

At the sixth month, there are statistically significant results regarding the variation in weight and percentage of total fat.

When comparing the group that consumed milk with CLA with the placebo group, and the group that consumed milk with CLA + Cr with the placebo group, there are statistically significant differences in terms of body weight and percentage of total body fat.

Weight difference at the sixth month

Group	N	Subset for alpha= .05	
	1	2	1
Fat-free milk	13	-.5923	
CLA + green tea	14	2.4500	2.4500
CLA + cr	14		2.9500
CLA	13		3.0846
Sig.		.112	.963

Table 6

The group which consumed CLA achieved the greatest loss of body weight. The group that consumed milk with CLA + Cr presented quite similar results. The group that consumed milk with CLA + green tea reduced their weight by 2.45 kilograms. In all three cases, there are statistically significant results. The group consuming the placebo, despite following healthy dietary guidelines and practicing physical activity, did not reduce their weight.

Difference of fat percentage at the sixth month

Group	N	Subset for alpha= .05	
	1	2	1
Fat-free milk	13	-.1615	
CLA + green tea	14		2.4429
CLA + cr	14		3.3571
CLA	13		4.2046
Sig.		1.000	.131

Table 7

The group that consumed milk with CLA lost 4.2% of body fat. This group, like the one that consumed milk with CLA + Cr (with a loss of 3.35% in body fat percentage) and the one that consumed fat-free milk with CLA + green tea (2.44%) present statistically significant results in terms of fat loss compared to the placebo group, though there are no significant differences between these two groups. The group taking the placebo (slightly) increased their body fat.

Total water difference at the sixth month

Group	N	Subset for alpha= .05
	1	1
CLA	13	-1.0077
Fat-free milk	13	-.9923
CLA + cr	14	-.5643
CLA + green tea	14	-.1929
Sig.		.533

Table 8

Although there are no statistically significant results, the group that consumed fat-free milk with CLA + green tea still lost the most total water.

Difference of lean mass at the sixth month

Group	N	Subset for alpha= .05
	1	1
CLA	13	-1.2462
CLA + creatine	14	-.7786
Fat-free milk	13	-.5308
CLA + green tea	14	-.2429
Sig.		.314

Table 9

Without any statistical significance, the group that consumed fat-free milk with CLA had the greatest increase in its lean mass.

In short

- The results at 3 months indicate that the group that consumed fat-free milk with CLA lost 3.29% of fat and the group that consumed fat-free milk with CLA + Cr lost 2.97% of total body fat. In both cases there are statistically significant differences ($p < 0.05$).
- At 3 months there are no statistically significant differences regarding body weight, lean mass or body water.
- The results obtained at 6 months show that individuals who consumed milk with CLA have reduced their body fat percentage by 4.20%, those who consumed CLA + creatine reduced this by 3.35%, and those who consumed CLA + tea green by 2.44%, while in the group which consumed the placebo, the percentage of fat increases very slightly (+0.16%). These are statistically significant results ($p < 0.05$).
- Similarly, the results at 6 months show that individuals who consumed milk with CLA have reduced their body weight by 3.08 kilos, the group that consumed milk with CLA + creatine does so by 2.95 and the group that ingested milk with CLA + green tea lost 2.45 kilograms. Comparing these with the placebo (the placebo group increased their body weight slightly, by 0.56 kilos) they are statistically significant results.

However, there are no statistically significant differences regarding total body water and lean mass.

Discussion

Numerous studies have shown the ability of CLA to reduce body fat in a statistically significant way. In this study, which includes the practice of regular physical activity and dietary control through the promotion of a healthy diet, it was expected that in the group that exclusively consumed milk with CLA, the effect of loss of body fat would range from 1 - 8%, which is the most common value found in studies between 1 and 6 months. Indeed, the result has been as expected: the loss of fat in the group consuming CLA has been within said expected range (1 - 8%), confirming the results obtained by most of the studies carried out. Although it should be noted that there are some studies that report much greater fat losses in equivalent time intervals, most of the authors, as we have already pointed out, obtain similar results to those we have obtained in our experimental study.

Precisely because in the bibliography there are few studies in which CLA is combined with Cr [50] and none with green tea (or at least none that we have found), it was decided that this experimental work would investigate such a combination. The fact that some studies in which these compounds were taken alone, without combining them with CLA, showed positive results regarding their possible effectiveness in reducing body fat mass and/or increasing lean mass, led us to hypothesise that the possible existence of a synergistic or at least a summation effect should not be ruled out, in such a way that the group which consumed green tea in addition to CLA may lose more body fat than if they only consumed CLA.

In this sense, it could also be expected that the group that consumed Cr in addition to CLA would increase their lean mass more and even reduce their body fat mass more than if they consumed CLA alone. Given that the intake of Cr seems to have demonstrated its efficacy in increasing lean mass, this led us to hypothesise a possible advantage when it comes to reducing body fat in people who consume Cr, as lean mass is a more active tissue and consumes more energy than fat mass. Therefore, an increase in lean mass

could perhaps slightly increase the basal metabolism and consequently help to disturb the energy balance and reduce the percentage of body fat.

However, from the results obtained in our study, we can verify that the group that achieved the best results in both cases (fat loss and lean mass increase) was that which consumed CLA alone. Thus, a synergistic or summative effect of both substances does not seem likely, although a longer-term study may be necessary.

In the case of loss of body fat, in the group that consumed CLA and green tea, the result is especially "disappointing" as, although at the sixth month there is a loss of fat that is statistically significant in this group compared to the control group (although to a much lesser degree when compared with the other two groups), there was no loss of fat at the third month (unlike the other two groups). This result could be due to some type of interaction between both products, in addition to a probable inactivation of the catechins on contact with the dairy matrix. In this sense, it is interesting to indicate that in the containers with CLA and green tea (and only in these containers), "lumps" formed and the product acquired a brownish coloration, which could indicate some type of chemical reaction that could damage or oxidise a part of the CLA and/or catechins. In any case, this is a strange result, as green tea alone can reduce the oxidation of fatty acids. The results that we have obtained, and the presence of the "lumps" previously described, suggest the need for an in-depth chemical study that explains the interaction between these products used for our study.

The group that consumed Cr and CLA also obtained worse results (regarding body fat loss) both at the third and the sixth month than the group that consumed CLA alone, although these were not differences with statistical significance. Perhaps this is because, contrary to expectations, this group (consuming Cr + CLA) did not increase the lean mass by the amount initially expected and therefore the increase in total energy expenditure is minimal.

In the case of lean mass gain, despite the practice of regular physical activity by the volunteers, no statistically significant results were achieved. However, it is curious to see how by the third month (as one might initially expect) the group which drank milk with CLA and Cr is the one that most increased their lean mass. This could be due to the initial intracellular drawing of water due to creatine intake and increased intracellular creatine deposits. However, at the sixth month (there are still no statistically significant results in any of the groups) it was the group that consumed CLA that stood out for its increase in lean mass. In this sense, a meta-analysis [17] underlines that indeed, CLA increases lean mass. However, its effect does not depend on time or dose, but causes an increase of about 0.3 kilograms. For this reason, we could expect that the group consuming milk with CLA would have the same results (regarding the increase in lean mass) at the third month as at the sixth month. However, the results we have obtained do not indicate this. However, this could be explained as a result of the sum of the effects of CLA and weight training.

In view of the existing scientific studies [47], as well as what might theoretically assumed, it was expected that when consuming milk with CLA and Cr the effects (at 6 months) in the increase in lean mass would be greater than when consuming CLA alone. However, our results show that the combination of CLA and Cr does not improve the effects of CLA on its own, which is perhaps because the studies described were carried out with the elderly (where perhaps phosphocreatine synthesis could be decreased) and whose protein intake is usually lower (meat is the main dietary source of creatine), while in this study we mostly consider young adults and habitual sports practitioners, whose protein consumption is higher.

In the case of the group that consumed CLA and green tea, at the third month of study there was a loss of lean mass and at the sixth, this group had the lowest increase in its lean mass. This could perhaps be due to some kind of catabolic effect derived from a possible increase in thermogenesis. In addition, it should be recalled that lean mass is also made up of body water, and that green tea (like all infusions) can have a certain diuretic effect, and so the group that consumed CLA and green tea had the greatest body water loss at the sixth month.

Also noteworthy is the fact that the group consuming the placebo and who also practiced regular physical activity and followed healthy eating guidelines did not manage to reduce their fat and body weight. These results agree with those of the study carried out in Murcia by Doctor Villegas and his team [22]. The explanation for this could be that if strict dietary control is not carried out (portion sizes, way of cooking, etc.), the energy intake compensates for the greater expense derived from physical activity. It is possible that although the dietary guidelines are healthier (less saturated fat, less simple sugars, less alcohol, etc.), the changes may be offset by an increase in the size of the portions consumed.

With regard to body water variation there are no statistically significant differences. This could be justified, as previously stated, by the diuretic effect attributed to green tea. A greater loss of body water could be expected, but it must be borne in mind that the amount of body water is influenced by a multitude of factors that are difficult to control (sweating, intensity of physical activity, temperature, meals outside of the home, salt added at home, canned food or prepared dishes, etc.).

Looking to the future, it would be interesting to carry out new studies in which tea is not incorporated into a dairy matrix (in order to avoid the possible inactivation, at least in part, of the catechins) nor mixed with CLA (to avoid the possible reaction of this with tea). Perhaps in this way, a synergistic effect could be achieved in the loss of body fat.

It would be of interest to combine CLA with other substances that "promise", at least theoretically, changes in body composition: such as caffeine, omega 3 fatty acids, medium chain triglycerides and forskolin, a diterpene which activates adenylate cyclase [50].

Improving the results that are currently achieved with the intake of CLA alone would allow for a new range of functional foods to launch on the market with real efficacy, which would be very useful for a large segment of the population (those who are overweight and obese).

Conclusion

At 3 months

- The daily intake of 3 grams of CLA, within a balanced diet and with constant physical activity, reduces body fat statistically significantly.
- The daily intake of 3 grams of CLA, within a balanced diet and with regular physical activity, does not increase lean mass in a statistically significant way.
- The simultaneous intake of CLA and green tea within a balanced diet and with regular physical activity, does not cause a statistically significant increase in the loss of body fat, with respect to the effect produced by the consumption of CLA alone, nor with respect to the group consuming the placebo.
- The simultaneous intake of CLA and creatine, within a balanced diet and with regular physical activity, does not cause a statistically significant increase in the gain of lean mass, with respect to that produced by the intake of CLA alone, nor with respect to the group consuming the placebo.
- The simultaneous intake of CLA and creatine, within a balanced diet and with regular physical activity, does not cause a statistically significant increase in the reduction of body fat, compared to that produced by the intake of CLA alone, though it does compared to the group consuming the placebo.

At 6 months

- The daily intake of 3 grams of CLA, within a balanced diet and with constant physical activity, reduces body fat and weight in a statistically significant way.
- The daily intake of 3 grams of CLA, within a balanced diet and with regular physical activity, does not increase lean mass in a statistically significant way.
- The simultaneous intake of CLA and green tea, within a balanced diet and with regular physical activity, does not cause a statistically significant increase in the loss of body fat, with respect to the effect produced by the consumption of CLA alone, though it does with respect to the group consuming the placebo.
- The simultaneous intake of CLA and creatine, within a balanced diet and with regular physical activity, does not cause a statistically significant increase in the gain of lean mass, with respect to that produced by the intake of CLA alone, nor in comparison with the group consuming the placebo.
- The simultaneous intake of CLA and creatine, within a balanced diet and with regular physical activity, does not cause a statistically significant increase in the reduction of body fat at 6 months, compared to that produced by the intake of CLA alone, though it does compared to the group consuming the placebo.
- The regular practice of physical exercise together with a balanced diet is not enough to reduce body fat or total body weight.

Bibliography

1. Aranceta-Bartrina J., *et al*. "Prevalencia de obesidad en España". *Medicina Clínica* (Barc). 125.12 (2005): 460-466.

2. Arrizabalaga JJ., *et al*. "Recomendaciones y algoritmo de tratamiento del sobrepeso y la obesidad en personas adultas". *Medicina Clínica* (Barc) 122.3 (2004): 104-110

3. Mcardle. Principios de Fisiología del Ejercicio. Editorial Mc Gra Hill. (2004).

4. Lamarch B and Desroches S. "Metabolic Syndrome and effects of conjugated linoleic acid in obesity and lipoprotein disorders: the Québec experience". *American Journal of Nutrition* 79 (2004): 1149S-1152S.

5. Kamphuis MMJW., *et al*. "The effect of conjugated linoleic acid supplementation after weight loss on body weight regain, body composition, and resting metabolic rate in overweight subjects". *International Journal of Obesity* 27 (2003): 840-847.

6. Pariza MW and HA YL. Conjugated dienoic derivaties of linoleico acid a new class of anticarcinogens". *Medical Oncology and Tumor Pharmacotherapy* 7 (1990): 169-171.

7. Gaullier JM., *et al*. "Clinical trial results support a preference for using CLA preparations enriched with two isomers rather than four isomers in human studies". *Lipids* 37 (2002): 1019-1025.

8. Chin SF., *et al*. "Dietary sources of conjugated dienoic isomers of linoleic acid, a newly recognized class of anticarcinogens". *Journal of Food Composition and Analysis* 5 (1992): 185-197.

9. Stanton C., *et al*. "Dietary influences on bovine milk cis-9, trans-11-conjugated linoleic acid content". *Journal of Food Science* 62 (1997): 1083-1086.

10. Gomez-Candela C. "El papel del CLA o ácido linoleico conjugado sobre la masa grasa corporal". *Nutrición y Dietética* 1 (2004): 75-83.

11. Villar Álvarez., *et al*. "Recomendaciones para el control de la colesterolemia en España". *Revista Española de Salud Pública* 74.5-6 (2000).

12. Bauman DE., *et al*. "The Biology of conjugated linoleic acid in rumiants". En: Advances in Conjugated Linoleic Acid Research, Volume 2. Sebedio JL, Christie WW, Adolf RO, eds. AOCS Press, Champaign, IL. (2003): 146-173.

13. López Román J., *et al*. "Actividad física e ingesta de leche con ácido linoleico connjugado (CLA) en personas sanas con sobrepeso". *Revista Espanola de Obesidad* 5.2 (2007): 109-118.

14. Martínez CV and Contiente AJC. "Grasa alimentaria y su papel en la regulación del peso corporal". *Revista Espanola de Obesidad* 1 (2004): 5-28.

15. Whigham DL., *et al*. "Efficacy of conjugated linoleic acid for reducing fat mass: a meta-analysis in humans". *The American Journal of Clinical Nutrition* 85.5 (2007): 1203-1211.

16. Terpstra AHM. "Effect of conjugated linoleic acid on body composition and plasma lipids in humans: an overview of the literature". *The American Journal of Clinical Nutrition* 79 (2004): 352-361.

17. Schoeller DA., *et al*. "A meta-analysis of the effects of conjugated linoleic acid on fat-free mass in humans". *Applied Physiology, Nutrition, and Metabolism* 34.5 (2009): 975-978.

18. Riserius U., *et al*. "Conjugated Linoleic acid (CLAS) reduced abdominal adipose tissue in obese middle-aged men with signs of the metabolic syndrome: a randomised controlled trial". *International Journal of Obesity* 25 (2001): 1129-1135.

19. Smedman A and Vessey B. "Conjugated linolenic acid supplementation in humans-metabolic effects". *Lipids* 36 (2001): 773-778.

20. Gaullier JM., *et al*. "Supplementation with Conjugated Linoleic Acid for 24 months Is Well Tolerated by and Reduces Body Fat Mass in Healthy, Overweight Humans". *Journal of Nutrition* 135 (2005): 778-784.

21. Laso N., *et al*. "Effects of milk supplementation with conjugated linoleico acid (isomers cis-9, trans 11 and trans-10, cis 12) on body composition and metabolic syndrome components". *British Journal of Nutrition* 11 (2007): 1-8.

22. López Román J., *et al*. "Actividad física e ingesta de leche con ácido linoleico connjugado (CLA) en personas sanas con sobrepeso". *Revista Espanola de Obesidad* 5.2 (2007): 109-118.

23. Moreno B., *et al*. "Estudio clínico observacional en personas con sobrepeso con dieta y ejercicio controlados asociado a la ingesta de productos lácteos con ácido linoleico conjugado durante 12 semanas". *Revista Espanola de Obesidad* 5.4 (2007): 236-243.

24. Serra L and Bautista I. "Estudio clínico observacional. Realizado en el instituto canario de medicina y nutrición y en el Hospital Universitario Insular de las Palmas".

25. Pariza MW. "Perspective on the safety and effectiveness of conjugated linolenic acid". *American Journal of Clinical Nutrition* 79 (2004): 1132S-1136S.

26. Fernandez-Quintela A., *et al*. "Ácido linoleico conjugado y grasa corporal". *Revista Espanola de Obesidad* 2 (2004): 71-79.

27. Gaullier JM., *et al*. "Conjugated linoleic acid supplementation for 12 months and reduces body fat mass in healthy overweight humans". *The American Journal of Clinical Nutrition* 79 (2004): 1118-1125.

28. Lowery LM., *et al*. "Conjugated linoleic acid enhances muscle size and strength gains in novice bodybuilders". *Medicine and Science is Sport an Exercise* 30 (1998): S182.

29. Kreider RB., *et al*. "Effects of linoleic conjugated acid supplementation during resistance training on body composition, bone density, strength and selected haematological markers". *The Journal of Strength and Conditioning Research* 16.3 (2002): 325-334.

30. Arnal MA., *et al*. "Congrés comun Afrero, AFN, et SNDLF". *Nutrition et Obesité* (2001): 29-30.

31. O´Hagan S ad Menzel A. "A subchronic 90-day oral rat toxicity study and in vitro genotoxicity studies with a conjugated linoleic acid product". *Food and Chemical Toxicology* 41.12 (2003): 1749-1760.

32. Schulte S., *et al*. "Safety Assessment of conjugated linoleic acid (cla) esters in the future application field as a feed additive in growing pigs". 94th AOCS Annual Meeting and Expo (2004).

33. Albers R., *et al*. "Effects of cis-9, trans-11 and trans-10, cis-12 conjugated linoleic acid (cla) isomers on immune function in healthy men". *European Journal of Clinical Nutrition* 595 (2003): 595-603.

34. Wigham LD., *et al*. "Safety profile of conjugated linoleic acid in 12 a month trial in obese human". *Food and Chemical Toxicology* 42.10 (2004): 1701-1709.

35. McGuirre MK., *et al*. "Dietary sources of conjugated linoleic acid intake in humans". In: Conjugated linoleic acid, Yarawecz et al., eds. Champaign, IL: AOCS Press (1999): 369-377

36. Tsuboyama-Kasaoka N., *et al*. "Conjugated linoleic acid supplementation reduces adipose tissue by apoptosis and develop lipodystrophy in mice". *Diabetes* 130 (2000): 1920-1924.

37. Brodie AE., *et al*. "Conjugated linoleico acid inhibits differentiation of pre and post confluent 3T3-L1 preadipocytes but inhibits cell proliferaty only in PR confluent cells". *Journal of Nutrition* 129 (1999): 602-606.

38. Takahashi Y., *et al*. "Dietary conjugated linoleic acid reduces body fat mass and affects gene expression of proteins regulating energy metabolism in mice". *Comparative Biochemistry and Physiology B* 133 (2002): 395-404.

39. Park Y., *et al*. "Effect of conjugated linoleico acido n body composition in mice". *Lipids* 32 (1997): 853-858.

40. West DB., *et al*. "Effect of conjugated linoleico acido n body fat and energy metabolism in the Mouse". *American Journal Physiology* 275 (1998): R667-672.

41. Mújika I and Padilla S. "Creatine Supplementation as an Ergogenic aid for Sports Performance in Highly Trained Athletes: A critical Review". *International Journal of Sports Medicine* 18 (1998): 491-496.

42. Kreider R., *et al*. "Effects of creatine supplementation on body composition, strength and sprint performance". *Medicine and Science is Sport an Exercise* 30 (1998): 73-82.

43. Shixian Q., *et al*. "Green tea extract thermogenesis-induced weight loss by epigallocatechin gallate inhibition of catechol-O-methyltransferase". *Journal of Medicinal Food* 9.4 (2006): 451-458.

44. Dullo AG., *et al*. "Efficacy of a green tea extract Rich in Catechin poliprhenoles and caffeine in increasing 24-h energy expenditure and fat oxidation in humans". *The American Journal of Clinical Nutrition* 70 (1999): 1040-1045.

45. Liao S. "The medical action of androgens and green tea epigallocatechin gallate". *HKMJ* 7 (2001): 369-374.

46. Kao YH., *et al*. "Modulation of endocrine systems and food intake by green tea epigallocatechin gallate". *Endocrinology* 141 (2000a): 980-987.

47. Watanabe J., *et al*. "Isolation and identification of acetyl-CoA carboxylase inhibitors from green tea (Camellia sinensis)". *Bioscience, Biotechnology, and Biochemistry* 62 (1998): 532-534.

48. Tarnopolsky MA and Safdar A. "The potential benefits of creatine and conjugated linoleic acid as adjuncts to resistance training in older adults". *Applied Physiology, Nutrition, and Metabolism* 33.1 (2008): 213-227.

49. G Bedogni. "Accuracy of an eight-point tactile electrode impedance method in the assessment of total body wate". *European Journal of Clinical Nutrition* 56 (2002): 1143-1148.

50. Dapcich V., *et al*. Guía de la alimentación saludable. 2004: Editado por la Sociedad Española de Nutrición Comunitaria (SENC).