Original Article
CLINICAL TRIALS: BEHAVIOR, PHARMACOTHERAPY, DEVICES, SURGERY

Obesity

Effects of α-Lipoic Acid and Eicosapentaenoic Acid in Overweight and Obese Women During Weight Loss

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Objective: To evaluate the potential body weight-lowering effects of dietary supplementation with eicosapentaenoic acid (EPA) and α-lipoic acid separately or combined in healthy overweight/obese women following a hypocaloric diet.

Methods: This is a short-term double-blind placebo-controlled study with parallel design that lasted 10 weeks. Of the randomized participants, 97 women received the allocated treatment [Control, EPA (1.3 g/d), α-lipoic acid (0.3 g/d), and EPA + α-lipoic acid (1.3 g/d + 0.3 g/d)], and 77 volunteers completed the study. All groups followed an energy-restricted diet of 30% less than total energy expenditure. Body weight, anthropometric measurements, body composition, resting energy expenditure, blood pressure, serum glucose, and insulin and lipid profile, as well as leptin and ghrelin levels, were assessed at baseline and after nutritional intervention.

Results: Body weight loss was significantly higher ($P < 0.05$) in those groups supplemented with α-lipoic acid. EPA supplementation significantly attenuated ($P < 0.001$) the decrease in leptin levels that occurs during weight loss. Body weight loss improved lipid and glucose metabolism parameters but without significant differences between groups.

Conclusions: The intervention suggests that α-lipoic acid supplementation alone or in combination with EPA may help to promote body weight loss in healthy overweight/obese women following energy-restricted diets.

Obesity (2015) **23**, 313–321. doi:10.1002/oby.20966

Introduction

Obesity is considered as an important public health disease that predisposes people to a wide range of other chronic pathologies such as type-2 diabetes, coronary heart disease, stroke, obstructive-sleep apnea and certain forms of cancer (1).

α-lipoic acid (thioctic acid, 5-(1,2-dithiolan-3-yl) pentanoic acid) is a naturally occurring antioxidant and co-factor for mitochondrial enzymes (2). Several studies have suggested important anti-obesity properties for α-lipoic acid. Thus, in rodents, α-lipoic acid supplementation promotes the reduction of body weight and fat mass (3-5) not only by decreasing food intake, but also by reducing feed efficiency (3) and stimulating energy expenditure (6). Furthermore, some trials in overweight, obese and/or diabetic subjects have also suggested that supplementation with α-lipoic acid could in some cases promote weight loss, reduce fat mass and increase satiety (7,8). However, studies in humans with α-lipoic acid supplementation are limited and with controversial outcomes, and it is difficult to reach firm conclusions regarding the proper dose and its potential role in the treatment of obesity.

Although several studies have demonstrated that n-3 polyunsaturated fatty acids (n-3 PUFAs) could improve cardiovascular health, the use of these fatty acids on obesity treatment remains unclear (9). Thus, some studies described that n-3 PUFAs could have positive effects on

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Funding agencies: This research was supported by a grant from Ministerio de Economía y Competitividad, Spain, AGL 2009–10873/ALI; and by Línea Especial de Investigación "Nutrición, Obesidad y Salud," University of Navarra-Spain. AE Huerta was supported by a predoctoral grant from Asociación de Amigos de la Universidad de Navarra.

Disclosure: The authors declared no conflict of interest.

Author contributions: Designed research, raised grants, and coordinated the complete project: MJM-A and JAM; conducted laboratory research: AEH and PLPH; performed the statistical analyses and wrote the manuscript: AEH; help in the implementation of the clinical research plan: SNC; critically reviewed the manuscript: SNC, PLPH, JAM, and MJMA. All authors have approved the final version of the manuscript.

Additional Supporting Information may be found in the online version of this article.

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Received: 2 June 2014; **Accepted:** 16 October 2014; **Published online 31 December 2014. doi:10.1002/oby.20966**

body weight reduction (10,11), whereas others have reported no effects on adiposity (12,13). Most of the studies in humans have been performed using a combination of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), but only a few trials have evaluated the effects of these n-3 PUFAs separately. Several investigations have suggested that EPA and DHA have different hemodynamic properties and actions on cell function (14,15). Furthermore, the effects of EPA supplementation in healthy overweight/obese subjects on body weight and fat mass loss have been scarcely investigated until now.

Hence, the aim of this trial was to evaluate the potential body weight-lowering effects of dietary supplementation with EPA and α-lipoic acid separately or in combination, in healthy overweight/obese women during a hypocaloric diet.

Methods

Participants

Women aged 20–50 years were recruited by advertisement in local newspapers and by calls for volunteers from the database of the Metabolic Unit (MU) of the University of Navarra. The inclusion criteria were: 1) female, 2) regular menstrual cycles, 3) BMI between 27.5 and 40 Kg/m^2, 3) unchanged weight (± 3 Kg) for the last 3 months and 4) all subjects should have an overall healthy physical and psychological condition.

Study design and intervention

This study was a parallel, short-term randomized double blind placebo-controlled trial performed in compliance with the Helsinki Declaration. The trial was approved by the Research Ethics Committee of the University of Navarra and registered at clinicaltrials.gov as NCT01138774.

Out of the 103 participants, who gave their signed consent and were assigned by a scientist of the MU to one of the four experimental groups by simple randomization using the Microsoft Excel Office 2003 Software (Microsoft Inc., USA) that generates random numbers, finally 97 women received the allocated treatment, with 77 finishing the trial (Figure 1). The intervention groups varied in the type of daily supplement: 1) Control group: 3 placebo-I capsules (containing sunflower oil) and 3 placebo-II capsules (containing same excipients as the α-lipoic acid capsules), 2) EPA group: 1300 mg/d of EPA distributed in 3 capsules of EPA 80 (provided by Solutex®, Madrid, Spain) containing 433.3 mg of EPA and 13.8 mg of DHA as ethyl-ester and 3 placebo-II capsules, 3) α-lipoic acid group: 300 mg/d of α-lipoic acid from 3 capsules containing 100 mg of α-lipoic acid (Nature's Bounty®, NY, USA) and 3 placebo-I capsules; and 4) EPA + α-lipoic acid group: 1300 mg/d of EPA (distributed in 3 capsules of EPA 80) and 300 mg/d of α-lipoic acid (from 3 capsules containing 100 mg of α-lipoic acid). Therefore, each daily dose was divided into three equal doses to minimize the impact of decline in plasma levels after oral supplementation, and each participant consumed a total of 6 capsules per day (2 at breakfast, 2 at lunch and 2 at dinner). Both placebo-I (sunflower oil) and EPA capsules were provided by Solutex, and were similar in shape and size. Placebo-II capsules were similar in appearance to the α-lipoic acid capsules.

All intervention groups followed a weight-reduction program consisting of a calorie-restricted balanced diet (55% carbohydrates; 30% lipids, 15% proteins) in accordance with the American Heart Association guidelines, and prescribed individually by a dietitian. During the baseline visit, each subject was instructed to follow an energy-restricted diet accounting for 30% less than her total energy expenditure, and to not change the physical activity pattern during the 10-week intervention period. Additionally, volunteers were advised to avoid any marketed omega-3 supplement and enriched products.

The main outcome of the study was the amount of weight loss. Follow-up visits were scheduled with each volunteer every two weeks to monitor weight, assess the compliance with the assigned diet, motivate participants and ensure that all capsules were consumed.

At baseline and at end point the volunteers went under 10–12 h fasting conditions to the MU and met with the physician, the dietitian and the nurse. Anthropometric measurements, body composition analysis, physical activity, food intake, respiratory exchange measurements by indirect calorimetry and blood pressure were evaluated (*See* Supporting information). A catheter was then inserted into the antecubital vein for a fasting blood sample extraction. Thereafter, all participants underwent an oral glucose tolerance test (OGTT). In order to evaluate the effect of supplementation, biochemical determinations of glucose and lipid metabolism as well as of leptin and ghrelin were assessed (*See* Supporting Information).

Statistics

The number of subjects per arm of intervention was estimated at sixteen subjects, which would allow a detection of approximately a difference of 5 kg with a dispersion of 5 kg (5 ± 5 kg) in weight loss between experimental groups, at a 0.05 level of significance, with a statistical power of 80%. Since a dropout rate of 25% was expected, the final sample size estimation was established in at least 20 participants per group to be recruited.

A per-protocol analysis was performed including all volunteers for which end-of–intervention values were available, however, intention-to-treat analysis using the last measured reported during at least one follow-up visit was also conducted without relevant differences between both analyses (See Supporting Information Table S1). For categorical variables, differences were examined by using the chi-square test. Normality was evaluated using the Shapiro-Wilk test. Differences were considered significant if P-value <0.05. Additionally for secondary outcomes the Benjamini-Hochberg approach (16) was used to correct for multiple comparisons in order to counteract the false discovery rate. The differences after the nutritional intervention trial within each group were analyzed by Student's t test or by Wilcoxon test, depending of the sample distributions. Comparisons at baseline and in the effects between the four experimental groups in subjects who completed the study were evaluated using the two-way ANOVA. When the differences were statistically significant at the interaction level (EPA x α-lipoic acid supplementation), a Student's t test was performed to compare the effects of each treatment. Also, some values affected by potential confounders were adjusted accordingly. Statistical analysis was performed using Stata Statistical Software (Release 12. College Station, TX: StataCorp LP).

Results

Effects on body weight and body composition

There were no significant differences between the four experimental groups in all subjects whom came to the first visit (Supporting

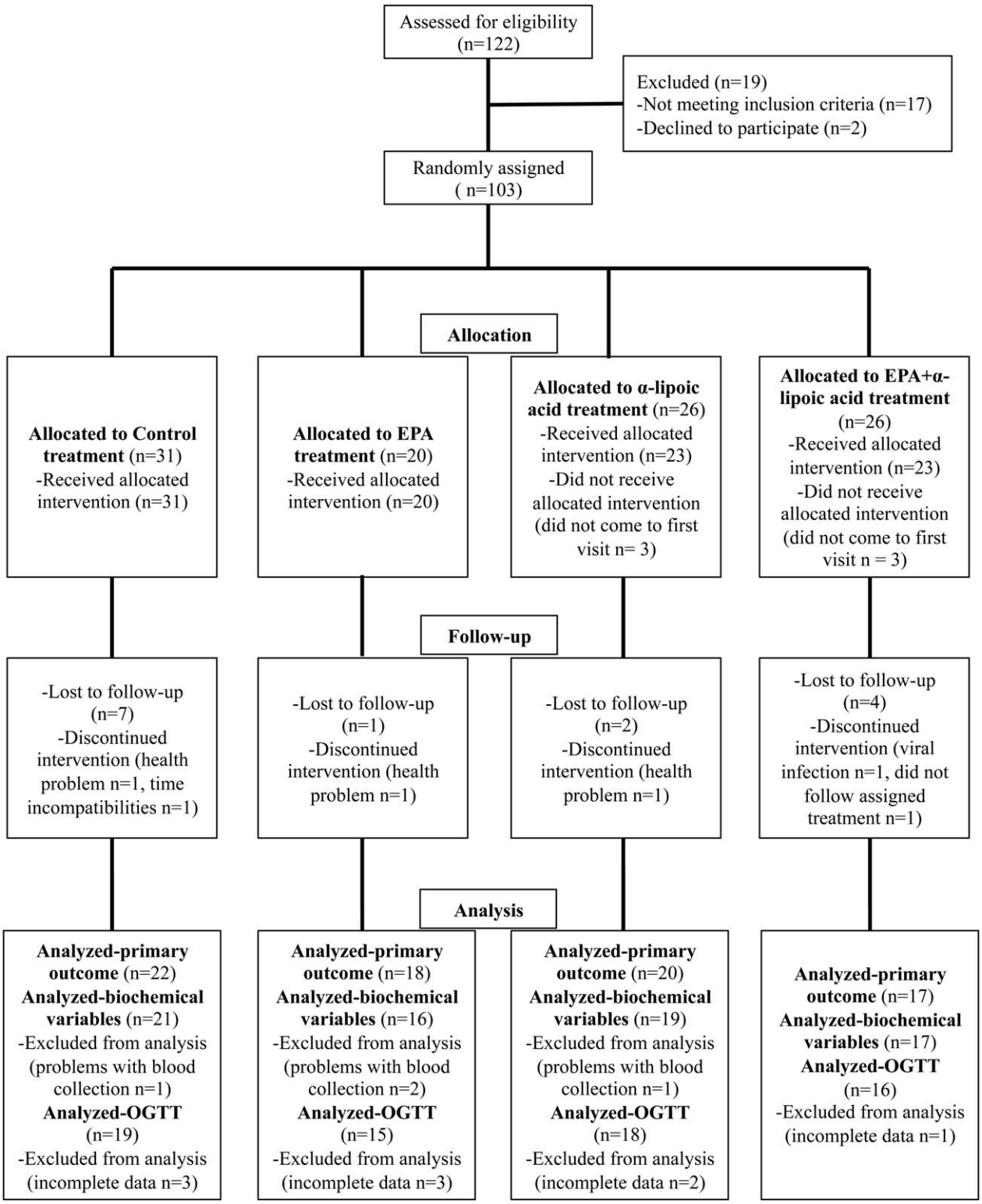

Figure 1 Flowchart of participants: Of the 103 randomized women who met the inclusion criteria, 97 started the allocated intervention; of these, 20 participants (21%) did not complete the study as they either discontinued the follow-up because of unexpected health problems ($n = 4$), withdrew from the study and did not came to all visits ($n = 15$), or were noncompliant with the assigned treatment ($n = 1$). The dropout rates were 29% ($n = 9$), 10% ($n = 2$), 13% ($n = 3$), and 26% ($n = 6$) for the control, EPA, α-lipoic acid, and EPA + α-lipoic acid groups, respectively, and no significant statistical differences were found in the dropout rates, after the chi-square statistic test. For the analysis of biochemical variables, 4 volunteers (1 control group, 2 EPA group, and 1 α-lipoic acid group) were excluded due to problems with blood collection. Similarly, in the analysis of the OGTT, 9 volunteers (3 control group, 3 EPA group, 2 α-lipoic acid group, and 1 EPA + α-lipoic acid group) were excluded because of incomplete data as a result of complications during the intravenous blood collection.

Information Table S1). On average, the physical activity factor was 1.40 ± 0.08, which corresponds to sedentary activity and was maintained during the study period; the rate of capsules intake was 99% in all the groups. In general no adverse effects were reported during the trial.

After the 10 weeks of nutritional intervention, all groups showed significant differences ($P < 0.05$) in the decrease in body weight, as well as in waist and hip circumference and the waist to hip ratio (WHR) as compared to baseline (Table 1). Additionally the changes in weight loss and anthropometric measurements were accompanied by a significant reduction of total fat mass, lean mass, android fat, gynoid fat and the android/gynoid ratio in all the intervention groups (Table 1). Weight loss was also accompanied by a statistically significant ($P < 0.001$) decrease in resting metabolic rate (RMR) in all intervention groups.

When the differences between groups were evaluated, it was observed that those receiving α-lipoic acid supplementation showed a higher statistically significant reduction ($P < 0.05$) in body weight, hip circumference and fat mass comparing to the others at the end of the trial (Table 1). This pattern was observed in the α-lipoic acid-supplemented groups from the first weeks of treatment and became more prominent during the trial (see Figure S1 in Supporting Information). Supplementation with EPA did not significantly modify body weight but caused a moderate decrease in the WHR ($P < 0.05$), even though when the change in WHR was adjusted for body weight loss. Interestingly, EPA supplementation was able to partly prevent ($P < 0.05$) the drop in RMR secondary to weight loss. In the α-lipoic acid-supplemented groups, the fall in RMR was not significantly different from the RMR changes observed in the control group, besides the higher weight loss observed in α-lipoic acid groups (Table 1). Moreover, there were not differences between groups either in changes (Table 1) or end point values of energy intake (See Supporting Information Figure S2) and the decreases in weight loss were not significantly affected by the changes in energy intake ($R^2 = 0.007$ $P = 0.48$).

Effects on lipid and glucose metabolism profile

After the 10 weeks of nutritional intervention, the groups supplemented with EPA showed a significant increase ($P < 0.05$) in the β-hydroxybutyrate levels, even after adjusting by body weight loss (Table 2). Serum levels of total cholesterol were significantly ($P < 0.05$) reduced in all experimental groups, while the LDL-cholesterol only decrease significantly ($P < 0.05$) in the groups with EPA. Furthermore, there was a statistically significant drop, compared with baseline levels, in triglycerides ($P < 0.01$) and diastolic blood pressure (DBP) ($P < 0.05$) in the group supplemented with EPA + α-lipoic acid. Besides, no significant differences were found between groups for any of these variables (See Supporting Information Table S2).

Levels of fasting glucose were reduced in the four experimental groups at the end of the trial as compared with baseline, although statistically significant decreases ($P < 0.01$) were only displayed in the control and EPA + α-lipoic acid groups. Furthermore, insulin levels as well as the HOMA-IR were also significantly reduced at the end point in control and in both groups supplemented with α-lipoic acid (Table 2). However, when the differences between groups were evaluated, no significant changes were obtained, although a significant interaction between supplements ($P < 0.01$) was observed (Table 2).

The data of the OGTT revealed that the EPA + α-lipoic acid group had the major changes in glucose during the OGTT (Figure 2a) but without significant modifications at the end of the nutritional intervention in the glucose incremental area under curve (iAUC) (Figure 2b). Although all groups, excepting the group supplemented with EPA, appeared to have improvements in the changes in insulin during the OGTT (Figure 2c), no significant differences were found between groups in the insulin iAUC (Figure 2d).

Effects on leptin and ghrelin levels

After the nutritional intervention, a significant ($P < 0.01$) reduction in leptin levels was demonstrated in almost all groups, except in the EPA-supplemented group (Table 2). Thus, the groups supplemented with α-lipoic acid showed an important drop in leptin in parallel with the reduction of fat mass. Interestingly, the groups with EPA supplementation showed a significantly lower decline ($P < 0.001$) in leptin levels with respect to the others (Table 2); also it was observed that the changes in leptin had a positive significant association with the RMR modifications ($r = 0.42$, $P < 0.001$). The EPA + α-lipoic acid group showed a statistically significant ($P < 0.05$) increase in ghrelin at the end of the trial but without differences between groups (Table 2).

Adjustment for multiple comparisons

To control the false discovery rate and give more forcefulness to the results, the Benjamini-Hochberg adjustment for multiple comparisons was used, finding that in the secondary outcomes, only the change in leptin remains significantly different between groups (Tables 1 and 2).

Discussion

In this study we found that the supplementation with α-lipoic acid (alone or in combination with EPA) at lower doses (300 mg/day) could help to promote weight loss and fat mass reduction in healthy overweight/obese women following an energy-restricted balanced diet. In this context, Koh et al. (8) found that α-lipoic acid supplementation (1200–1800 mg/day) together with a calorie-restricted diet in Asian overweight and obese subjects promotes weight loss as well as BMI and waist circumference reduction in a dose-dependent manner, but this effect was only significant at the highest dose tested. Carbonelli et al. (7) described how supplementation with α-lipoic acid (800 mg/day) promotes the reduction of weight, fat mass and waist circumference only in overweight and obese Caucasian subjects, but not in normal-weight subjects, suggesting that these effects could be due to the increased satiety induced by α-lipoic acid. Besides the positive effects on weight loss observed in some trials, the anti-obesity properties of α-lipoic acid in humans remain controversial. Thus, other studies in overweight and obese subjects with impaired glucose tolerance or type-2 diabetes, following a regular diet, have observed no effect on anthropometric measurements after supplementation with α-lipoic acid (17,18). Thereby, the discrepancies between the different trials could be related to the specific features of the subjects, the diet assigned and the duration of the trials.

Studies in animal models have suggested that α-lipoic acid could promote weight loss by reducing food intake and stimulating energy expenditure (3,19). However, our current data do not support a role of increased satiety for α-lipoic acid at the dose used in this trial,

TABLE 1 Effects of 10-week intervention with EPA and α-lipoic acid on anthropometry, body composition, RMR, energy intake, and energy balance[a]

	Control	EPA	α-lipoic acid	EPA + α-lipoic acid	Two-way ANOVA[h]		
N	22	18	20	17	EPA	α-lipoic acid	EPA × α-lipoic acid
Age (years)	39 ± 8	38 ± 8	38 ± 7	39 ± 7	ns	ns	ns
Body weight (Kg)							
Before	84.6 ± 14.4	88.4 ± 10.9	83.5 ± 11.4	84.9 ± 13.6	ns	ns	ns
Change	−5.2 ± 2.7[b]	−5.4 ± 1.9[b]	−7.0 ± 3.1[b]	−6.5 ± 3.6[b]	ns	0.032	ns
Waist circumference (cm)							
Before	99.1 ± 14.5	101.4 ± 7.3	95.6 ± 8.9	97.5 ± 9.0	ns	ns	ns
Change	−5.3 ± 2.5[b]	−6.6 ± 3.4[b]	−6.2 ± 3.3[b]	−6.3 ± 3.0[b]	ns	ns	ns
Hip circumference (cm)							
Before	115.4 ± 9.6	117.7 ± 8.1	115.0 ± 9.1	114.8 ± 8.6	ns	ns	ns
Change	−4.3 ± 2.4[b]	−3.6 ± 1.7[b]	−5.5 ± 2.5[b]	−5.3 ± 2.7[b]	ns	0.010	ns
Adjusted change[c]	−4.9 (0.3)	−4.0 (0.3)	−4.8 (0.3)	−5.0 (0.3)	ns	ns	ns
Waist to hip ratio							
Before	0.85 ± 0.08	0.86 ± 0.06	0.83 ± 0.07	0.85 ± 0.06	ns	ns	ns
Change	−0.014 ± 0.02[d]	−0.033 ± 0.026[b]	−0.015 ± 0.021[d]	−0.018 ± 0.020[d]	0.032	ns	ns
Adjusted change[c]	−0.015 (0.004)	−0.033 (0.005)	−0.014 (0.004)	−0.017 (0.005)	0.028	ns	ns
Fat mass (kg)							
Before	39.1 ± 9.3	41.2 ± 5.8	38.6 ± 8.7	39.3 ± 8.7	ns	ns	ns
Change	−4.3 ± 2.2[b]	−4.4 ± 1.5[b]	−5.6 ± 2.5[b]	−5.4 ± 2.8[b]	ns	0.029	ns
Adjusted change[c]	−4.8 (0.2)	−4.9 (0.2)	−4.8 (0.2)	−5.1 (0.02)	ns	ns	ns
Lean mass (kg)							
Before	42.6 ± 6.3	45.2 ± 5.2	42.1 ± 3.9	42.5 ± 5.1	ns	ns	ns
Change	−1.0 ± 1.1[b]	−0.6 ± 1.4	−1.4 ± 1.4[b]	−0.8 ± 1.3[e]	ns	ns	ns
Android fat (%)							
Before	57.1 ± 5.3	57.1 ± 5.1	56.1 ± 6.2	57.3 ± 6.1	ns	ns	ns
Change	−3.2 ± 2.7[b]	−3.7 ± 2.4[b]	−4.7 ± 3.1[b]	−4.7 ± 3.5[b]	ns	ns	ns
Gynoid fat (%)							
Before	57.0 ± 4.7	57.6 ± 3.8	58.0 ± 4.2	56.6 ± 4.0	ns	ns	ns
Change	−2.1 ± 1.8[b]	−2.3 ± 1.4[b]	−3.0 ± 1.9[b]	−3.2 ± 2.3[b]	ns	0.039	ns
Adjusted change[c]	−2.3 (0.4)	−2.5 (0.4)	−2.7 (0.4)	−3.1 (0.4)	ns	ns	ns
Android/gynoid ratio							
Before	1.00 ± 0.11	0.99 ± 0.09	0.97 ± 0.09	1.01 ± 0.09	ns	ns	ns
Change	−0.02 ± 0.04[e]	−0.03 ± 0.04[d]	−0.03 ± 0.04[d]	−0.03 ± 0.05[e]	ns	ns	ns
RMR (KJ/d)[f]							
Before	6576 (139)	6866 (164)	6479 (150)	6501 (154)	ns	ns	ns
Change	−399.2 (37.6)[b]	−305.0 (44.5)[b]	−448.8 (40.6)[b]	−370.3 (41.8)[b]	0.039	ns	ns
Adjusted change[c]	−416.2 (34.6)	−327.5 (41.0)	−421.2 (37.8)	−358.7 (38.3)	0.049	ns	ns
Energy intake (KJ/d)							
Before	7650 ± 1178	9072 ± 1865	8485 ± 2617	8346 ± 1704	ns	ns	ns
Change	−2543.6 ± 1713.2[b]	−3693.2 ± −1887.3[b]	−2731.6 ± 2594.0[b]	−3045.1 ± 2056.8[b]	ns	ns	ns
Adjusted change[g]	−3201.0 (260.3)	−3029.0 (282.8)	−2612.9 (269.8)	−3055.5 (287.0)	ns	ns	ns

[a]Means ± SDs (all unadjusted such values). For all secondary outcomes, the *P*-values were adjusted by the Benjamini-Hochberg multiple-testing correction (16). Data from all subjects for whom baseline and follow-up measurements were available were included.
[b]Significantly different from baseline (paired samples *t* test): $P < 0.001$.
[c]Means (SEMs): adjusted for the changes in body weight.
[d]Significantly different from baseline (paired samples *t* test): $P < 0.01$.
[e]Significantly different from baseline (paired samples *t* test): $P < 0.05$.
[f]Means (SEMs): adjusted by the age and lean mass.
[g]Means (SEMs): adjusted by energy intake at baseline.
[h]Differences between groups at baseline and in changes (10 weeks – before) were evaluated by two-way ANOVA ($P < 0.05$; ns, nonsignificant). No significant differences between groups were found in secondary outcomes after the adjustment by Benjamini-Hochberg.

TABLE 2 Effects of 10-week intervention with EPA and α-lipoic acid on glucose metabolism, β-hydroxybutyrate, leptin, and ghrelin[a]

	Control	EPA	α-lipoic acid	EPA + α-lipoic acid	Two-way ANOVA[k]		
N	21	16	19	17	EPA	α-lipoic acid	EPA × α-lipoic acid
Glucose (mmol/L)							
Before	5.0 ± 0.4	5.0 ± 0.3	5.0 ± 0.4	5.2 ± 0.5	ns	ns	ns
Change	−0.23 ± 0.31[c,1,2]	−0.04 ± 0.28[1]	−0.04 ± 0.32[1]	−0.25 ± 0.26[c,2]	ns	ns	0.005*
Adjusted change[h,i]	−0.25 (0.06)[1,2]	−0.06 (0.07)[1]	−0.01 (0.07)[1]	−0.23 (0.07)[2]	ns	ns	0.004*
Insulin (pmol/L)							
Before	52.6 ± 47.0	42.4 ± 29.4	43.4 ± 18.9	62.3 ± 53.4	ns	ns	ns
Change	−17.5 ± 31.7[f]	−3.4 ± 34.6	−12.7 ± 20.2[g]	−22.6 ± 21.8[e]	ns	ns	ns
HOMA-IR							
Before	1.89 ± 1.78	1.55 ± 1.11	1.54 ± 0.68	2.36 ± 2.06	ns	ns	ns
Change	−0.67 ± 1.24[f]	−0.13 ± 1.28	−0.46 ± 0.70[f]	−0.92 ± 0.85[e]	ns	ns	0.047
Adjusted change[h]	−0.68 (0.23)	−0.14 (0.26)	−0.44 (0.24)	−0.91 (0.26)	ns	ns	0.047
Adjusted change[h,i]	−0.66 (0.15)	−0.34 (0.17)	−0.53 (0.15)	−0.63 (0.16)	ns	ns	ns
β-Hydroxybutyrate (mmol/L)							
Before	0.36 ± 0.25	0.27 ± 0.23	0.38 ± 0.22	0.32 ± 0.31	ns	ns	ns
Change	−0.08 ± 0.25	0.16 ± 0.38	−0.02 ± 0.30	0.08 ± 0.38	0.033	ns	ns
Adjusted change[h]	−0.05 (0.07)	0.18 (0.08)	−0.06 (0.07)	0.06 (0.07)	0.018	ns	ns
Leptin (ng/mL)							
Before	23.6 ± 7.6	21.7 ± 8.0	22.5 ± 10.0	20.4 ± 7.0	ns	ns	ns
Change	−6.4 ± 6.4[b]	−0.9 ± 5.2	−8.7 ± 8.3[b]	−3.5 ± 5.4[d]	0.0009*	ns	ns
Adjusted change[h]	−7.2 (1.2)	−1.8 (1.4)	−7.6 (1.3)	−2.9 (1.3)	0.0003*	ns	ns
Ghrelin (ng/mL)							
Before	230.1 ± 103.3	251.2 ± 98.0	240.8 ± 136.3	170.1 ± 91.3	ns	ns	ns
Change	15.2 ± 117.5	11.4 ± 79.9	8.8 ± 76.7	56.7 ± 56.4[c]	ns	ns	ns
Adjusted change[h,j]	18.3 (17.9)	21.6 (21.4)	13.0 (19.0)	38.2 (21.1)	ns	ns	ns

[a]Means ± SDs (all unadjusted such values). For all secondary outcomes, the *P*-values were adjusted by the Benjamini-Hochberg multiple-testing correction (16).
[b]Significantly different from baseline in normally distributed samples (paired samples *t* test): $P < 0.001$.
[c]Significantly different from baseline in normally distributed samples (paired samples *t* test): $P < 0.01$.
[d]Significantly different from baseline in normally distributed samples (paired samples *t* test): $P < 0.05$.
[e]Significantly different from baseline in non-normally distributed variables (Wilcoxon's test): $P < 0.001$.
[f]Significantly different from baseline in non-normally distributed variables (Wilcoxon's test): $P < 0.01$.
[g]Significantly different from baseline in non-normally distributed variables (Wilcoxon's test): $P < 0.05$.
[h]Means (SEMs): adjusted for the changes in body weight.
[i]Means (SEMs): adjusted for the changes for the insulin levels at baseline.
[j]Means (SEMs): adjusted by ghrelin levels at baseline.
[k]Differences between groups at baseline and in changes (10 weeks – before) were evaluated by two-way ANOVA ($P < 0.05$; ns, nonsignificant).
*Statistically significant differences between groups after the adjustment by Benjamini-Hochberg. When a significant interaction between groups was found ($P < 0.05$), an unpaired samples *t* test was performed; means that do not share a common superscript number in a horizontal line were significantly different ($P < 0.05$).

since differences in energy intake were not detected. Moreover, our data suggest that the RMR changes are not differentially affected by α-lipoic acid supplementation, since after adjusting for body weight loss, the RMR decrease in the α-lipoic acid groups was similar to control group. Adipose tissue is a target organ whereby α-lipoic acid exerts its anti-adiposity effects by inhibiting lipogenesis (20), increasing lipolysis (21) and inhibiting adipogenesis (2). Therefore, these mechanisms could be also contributing to the body weight- and fat mass-lowering actions of α-lipoic acid, without necessarily involving an increment of energy expenditure.

Studies evaluating the effects of n-3 PUFAs on weight loss in obese humans have reported contradictory outcomes (10,22,23), which largely depend on the different amounts and ratios of n-3 PUFAs type (EPA or DHA), as well as the phenotypical characteristics of the recruited subjects. Most of the studies analyzing the effects of n-3 PUFAs have used supplements that include both EPA and DHA. In fact, several studies have suggested that EPA and DHA have different effects on adipocyte function (24). In agreement with the present study, Itoh et al. (22), who used highly purified (>98%) EPA in obese subjects, showed that supplementation with this n-3 PUFA (1.8 g/day) for 3 months had no effects on waist circumference reduction and BMI loss.

A remarkable finding of the present trial was the fact that the reduction of leptin secondary to fat mass loss was significantly lower in

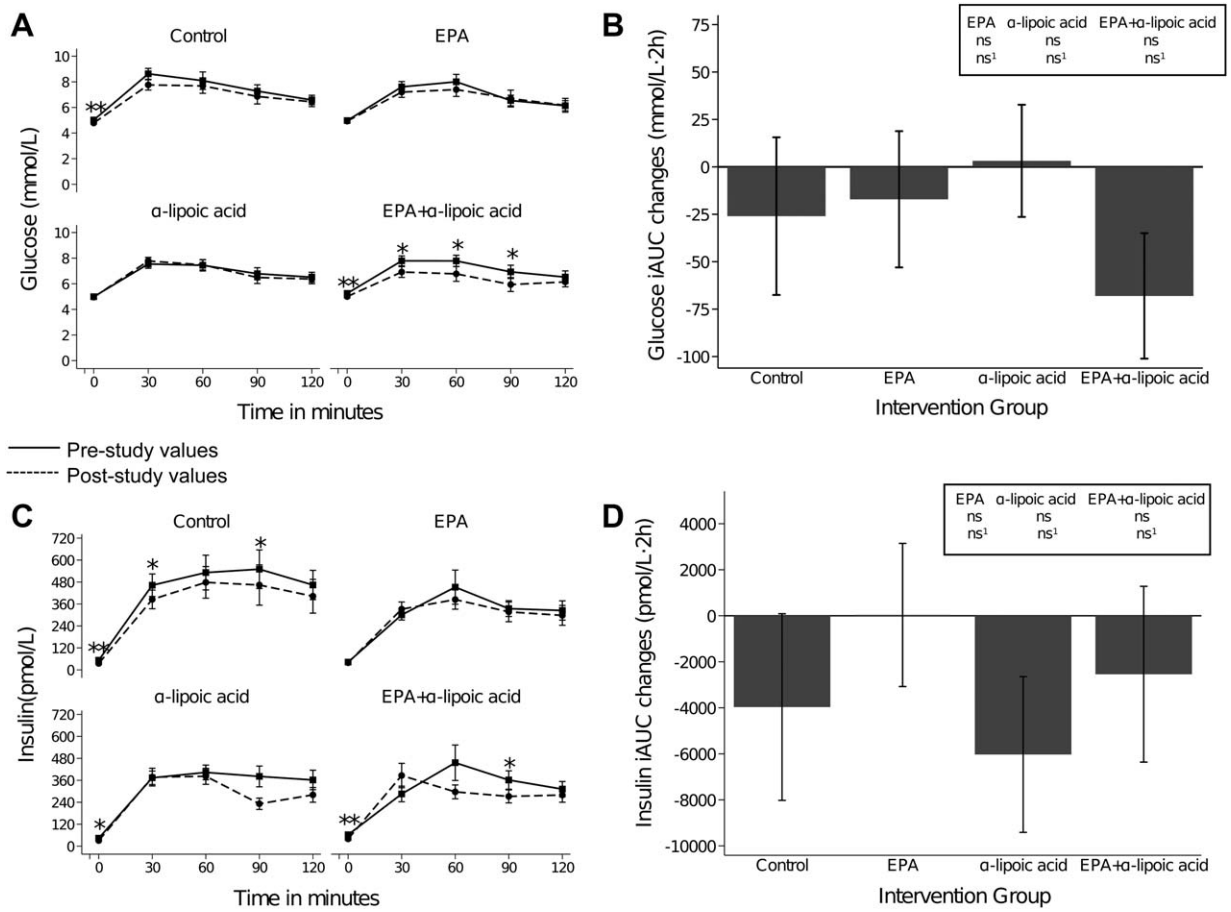

Figure 2 Mean (±SEM) plasma glucose (**A, B**) and insulin (**C, D**) during the 2-hour 75 g OGTT. Data from all subjects for whom baseline and follow-up measurements were available are included (in the glucose and insulin incremental area under curve (iAUC): control, $n = 19$; EPA, $n = 15$; α-lipoic acid, $n = 18$; EPA + α-lipoic acid, $n = 16$). (**A, C**) Solid lines represent pre-study values; dotted lines represent post-study values. The comparisons in each group before and after the nutritional intervention were assessed by Wilcoxon's test (**$P < 0.01$, *$P < 0.05$). (**B, D**) Differences between groups in the changes of the iAUC for glucose and insulin were evaluated by two-way ANOVA, and no significant differences were observed ($P > 0.05$). [1]Adjusted by body weight loss and baseline values.

the group supplemented with EPA. In accordance with this, *in vitro* and *in vivo* studies have evidenced the ability of EPA in stimulating the production of leptin in rodents (25,26). In this context, Hinkle et al. (27) have suggested that the decrease of leptin during weight loss could contribute to hunger, a lowered metabolic rate, and further weight regain; therefore, leptin replacement therapy could prevent subsequent RMR fall after weight loss and the weight regain in weight-reduced subjects. Moreover, the positive and significant association between RMR and leptin could suggest that the lower drop in the RMR found in the EPA-treated groups can be related with the effect of EPA on leptin. In this regard, Doucet et al. (28) have proposed that after weight loss, the decrease in RMR adjusted by fat mass and fat free mass, is explained mainly by changes in circulating plasma leptin. Although EPA supplementation did not promote weight loss, its actions on leptin seem to suggest that supplementation with this fatty acid could become important for weight loss maintenance. Few studies have addressed the beneficial effects of n3-PUFAs on weight maintenance after a weight loss program, and no significant effects have been described (13,23). However, it has been suggested that the effects of n3-PUFAs could be evident after a long time period and that their accumulation in the body could contribute to weight management (23,29).

The changes noted in serum metabolic profile in this study clearly confirm the benefits of weight loss and fat mass reduction by an energy-restricted diet on several metabolic alterations associated with the harmful effects of obesity, as previously demonstrated (30). The effects of the n-3 PUFAs on glucose and lipid profile are not consistent, depending mainly on the baseline metabolic characteristics. The main outcome observed in the studies with n-3 PUFAs in overweight/obese subjects is the reduction in triglyceride levels (23,31), however, the doses used in these trials range between 1.6 and 4 g per day, and are therefore higher than the dose used in this study (1.3 g/day). Furthermore, the effects of n-3 PUFAs on the cholesterol profile are not clear (32-35). Similar to our observations, other trials have described that the levels of LDL-cholesterol, HDL-cholesterol and total-cholesterol remain unchanged after n-3 PUFAs treatment (12,23,31). Although the increase in β-hydroxybutyrate levels was not significantly different between groups after the Benjamini-Hochberg correction, some effect of EPA in fatty acid

oxidation cannot be discounted, as has been previously reported in other studies in both humans (10) and rodents (36).

Several studies in type 2 diabetic and impaired glucose tolerance subjects have exhibited the properties of α-lipoic acid to ameliorate glucose metabolic complications (17,37). Furthermore, studies analyzing the effects of n-3 PUFAs in glucose metabolism have reached contradictory outcomes (12,38,39). In the current study, a significant interaction between both supplements in glucose concentration changes was found; however, it is important to note that the major decreases found in glucose and HOMA-IR in the control and EPA+α-lipoic acid groups may have been conditioned by the higher levels at baseline in these groups on insulin levels. Though, the effects of supplementation are influenced possibly by the metabolic status at baseline, it could be interesting to further evaluate the possible coadjuvant properties of n-3 PUFAs and α-lipoic acid on glucose metabolism.

The safety and toxicity of the α–lipoic acid have been tested by several authors at different doses and times, being the greatest dose administered 2400 mg/day during a 2 years period (40) with no reported adverse effects versus placebo. The dose used in our study is much lower than the dose considered safe.

However, it is important to take into account the limitations of this study, including the sample size, the short duration of the nutritional intervention, the lack of a maintenance weight loss period, the absence of blood samples of some volunteers and especially the fact that the outcomes could have been conditioned by the degree of adherence to the hypocaloric diet. Thus, the findings of our trial suggest the importance of performing longer randomized controlled trials followed by sustained weight maintenance periods.

In summary, our data suggest that α–lipoic acid supplementation at a dose of 300 mg/day combined with an energy-restricted diet might help to promote weight loss and fat mass reduction in healthy obese women. Although EPA supplementation did not have any additional effect on the reduction of body and fat mass, it prevents the fall of leptin during weight loss. It is essential that these observations be further explored and the underlying mechanisms better elucidated. O

Acknowledgments

EPA and sunflower oil capsules were generously provided by Solutex (Madrid, Spain). Solutex had no role in the study design, data collection, analysis and interpretation, or writing of the manuscript. The authors thank all participants of the study and the clinical investigators of the Metabolic Unit: Blanca Martínez de Morentin (M.D.), María Hernández (Dietitian), and Salomé Pérez (Nurse) for the execution of the study throughout and for taking care of study participants. They also thank María Zabala for her excellent technical assistance and Marta Cuervo for her support in diet design.

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