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Oleoylethanolamide increases the expression of PPAR-A and reduces appetite and body weight in obese people: A clinical trial

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ABSTRACT

Obesity is a crucial public health problem worldwide and is considered as the main cause of many chronic diseases. The present study evaluated the effects of Oleoylethanolamide (OEA) supplementation on proximal proliferator-activated receptor-α (PPAR-α) gene expression, appetite sensations, and anthropometric measurements in obese people. This randomized, double-blind, placebo-controlled clinical trial was carried out on 60 healthy obese people in Tabriz, Iran, in 2016. The eligible subjects were divided into an intervention group (who received two 125 mg OEA capsules daily) and a placebo group (who received the same amount of starches) and treated for 60 days. Anthropometric measurements and body composition were assessed in a fasting state at baseline and at the end of the study. The visual analogue scales (VAS) were used to assess appetite sensations. Quantitative real-time PCR analysis targeting the 16S rRNA gene of PPAR-α was done. Analysis was done on 56 participants who continued intervention until the end of the study. A significant increase in PPAR-α gene expression was observed in the intervention group ($p < 0.001$). Weight, body mass index, waist circumference, and fat percent decreased significantly at the end of the study in the intervention group (all $p < 0.01$). Hunger, the desire to eat, and cravings for sweet foods decreased significantly and fullness increased significantly by the end of study in the intervention group at the end of study (all $p < 0.01$). The fullness item increased significantly by the end of study in the intervention group ($p < 0.001$). Use of OEA as a complementary approach could be effective in suppressing appetite and modulating energy balance in obese people.

1. Introduction

Obesity is a crucial public health problem worldwide (Tchernof & Després, 2013) and is considered to be the main cause of many chronic diseases (Bastien, Poirier, Lemieux, & Després, 2014). More than 20% of people in the world were obese in 2016 (NCD-RisC., 2017).

Control of appetite is considered as a safe approach in the management of obesity (Hopkins & Blundell, 2016). One potential means of the components to modulate appetite is pharmacotherapy based on Oleoylethanolamide (OEA) that has recently attracted more attention. OEA an endogenous ethanolamide fatty acid synthesized in cells of the small intestine, adipose tissues (Schmid, Wold, Krebsbach, Berdyshev, & Schmid, 2002), neurons (Hu & Mackie, 2015), and astrocytes (Herrera, Kölliker-Frers, Barreto, Blanco, & Capani, 2016). Dietary sources of OEA are oatmeal, nuts, and cocoa powder; however, the amount of OEA found in them is low (less than 2 μg/g) (Astarita et al., 2006; Premkumar, 2014, pp. 1–246).

In addition to the protective effects of OEA in many metabolic diseases (e.g., neurodegenerative and inflammatory-related disorders), it also induces apoptosis, and relieves pain (Gonzalez-Aparicio et al., 2014; Lueneberg et al., 2011). Furthermore, the experimental studies showed that OEA can be involved in eating and energy balance and feeding behaviors (Astarita et al., 2006; Lambert, Vandevoorde, Jonsson, & Fowler, 2002). Indeed, animal experiments indicate that

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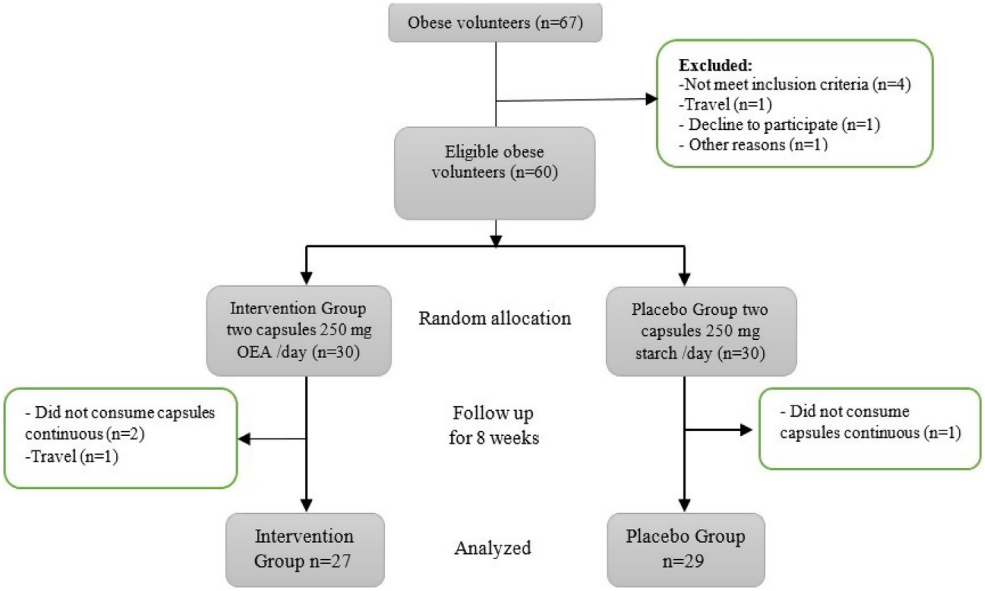

Fig. 1. Diagram of the protocol of study from baseline until the end of study.

OEA regulates body weight and satiety by delaying meal initiation and reduces meal size, and reduce body weight apart from increasing intervals between meals by the activation of PPAR-α receptors (Fu et al., 2003; Gaetani, Oveisi, & Piomelli, 2003; Oveisi, Gaetani, Eng, & Piomelli, 2004). PPAR-α is a group of ligand-activated nuclear receptor that involved in the gene expression of lipid metabolism and energy-homeostasis pathways (Fu et al., 2003; Guzmán et al., 2004).

Considering high affinity of OEA to bind PPAR-α and due to the lack of clinical studies assessinged OEA's potential in the management of obesity, the present study was aimed to investigate the effects of OEA supplementation on PPAR-α gene expression, appetite sensations, and obesity-related anthropometric measurements in obese people were assayed.

2. Materials and methods

This randomized, double-blind, controlled clinical trial was carried out on 60 healthy obese people from November to May 2016 in Tabriz, Iran. Individuals between the ages of 18 and 59 years and with a body mass index (BMI) between 30 and 40 kg/m^2 were recruited from clinics and healthcare centers of Tabriz University of Medical Science. Participants with current clinical problems including kidney diseases, liver and heart failure, gastrointestinal and rheumatic disorders, cigarette smoking, pregnancy, and breastfeeding and menopause in women were excluded from the study; in addition, participants taking antibiotics, probiotics and prebiotic supplements, weight loss drugs, omega 3 supplements, and multivitamin and mineral supplements during one month prior to the study were also excluded from the study.

The regional ethics committee of the Tabriz University of Medical Science approved the protocol of the study and allocated the number code IR.TB MED.REC.1395.618. The study was registered in the Iranian Registry of Clinical trials center with number IRCT201607132017N30, and with URL: www.IRCT.IR.

Demographic questionnaires including age, sex, occupational status, and educational level were completed by participants at baseline. The sample size was calculated according to the same variable (weight) as a previous study (Mangine et al., 2012). By considering the confidence interval 95%, power 90%, two-tailed test, and taking into account the 0.9% changes, the minimum sample size was calculated to be 26 healthy obese people in each group. Given that losses were possible in the follow-up period, 30 individuals were included in each group.

Eligible participants completed a written consent form at baseline. All participants were randomly allocated to an intervention or a placebo group based on the random block procedure produced by Random Allocation Software (RAS) (Saghaei, 2004). The intervention group received two 125 mg OEA capsules daily for 60 days. OEA synthesized at the Nutrition Research Center, Tabriz University of Medical Science, Iran. Briefly, a ratio of 1–10 mol of oleic acid (Merck, Germany) and mono ethanolamine (Sisco Research Laboratories, India) was mixed with 50% potassium methoxide as a catalyzer. The reaction was carried out in a volume of 20 ml of ethanol per 20 g of oleic acid under reflux for 5 h at 65 °C. The solvent was removed using a rotary evaporator at low pressure. The mixture gradually precipitated and the temperature decreased to 4 °C. After 24 h, the crystals were separated. The oleic acid used was assayed by gas by gas chromatography (Buck Scientific, model 610, USA) with flame ionization detection and a long capillary column (0.25 mm × 0.20 μm × 60 m, #TR-CN100, Teknokroma, Spain) and found to contain 84.3% oleate, 8.5% linoleate, 4.8% palmitate, and smaller amounts of other fatty acids. The synthesized OEA was subjected to gas chromatography-mass spectrometry and found to contain ~85% (2-Hydroxyethyl) octadec-9-enamide, ~8% linoleoyl ethanolamide, ~5% palmitoleyl ethanolamide, and ~2% myristoyl ethanolamide. Available literature indicates that these compounds have some beneficial biological activity and are non-toxic (Çimen et al., 2016; Ezzili, Otrubov, & Boger, 2010; Ishida et al., 2013; Lucanic et al., 2011; Micale et al., 2009). The synthesized OAE dissolved in DMSO was subjected to nuclear magnetic resonance spectography in a Bruker (USA) ultrashield Avance 400 spectrometer at 400 MHz. The data corresponded to the structure of OEA generated by Chemdraw software (Perkin Elmore, USA). Finally, 24 h culturing of the synthesized OEA failed to indicate pathogenic contamination.

The placebo group received the same amount of placebo (starch). All capsules with identical shapes and colors were placed into bottles by a third person who labeled the bottles with 2 codes that remained unknown to the researchers until the end of the assays. Fig. 1 shows how people were recruited and followed until the end of the study.

All subjects were advised not to change their physical activity or usual intakes during the study. To assay compliance, all subjects were followed by weekly phone call to confirm that they consumed the capsules regularly, and the data on those who consumed more than 90% of the capsules were analyzed.

Table 1
The demographic characteristics of obese people in the onset of study (n = 56).

Variables	OEA group (n = 27)	Placebo group (n = 29)	t (df)/χ^2 (df)/F (df, Error), ηp2	P[b]
Age (year)	37.4 (8.7)	38.1 (9.2)	t (54) = 0.318	0.752
Sex [a]				
Female	15 (55.6)	19 (65.5)	χ^2 (1) = 0.58	
Male	12 (44.4)	10 (34.5)		0.446
Education level [a]				
- Illiterate	13 (48.1)	15 (51.7)		
- Diploma	3 (11.1)	11 (37.9)	χ^2 (2) = 9.22	0.010
- Bachelor degree	11 (40.7)	3(10.3)		
Occupation [a]				
- Clerk	8 (29.6)	4 (13.8)		
- Housewife	14 (51.9)	19 (65.5)	χ^2 (2) = 2.11	0.348
- Worker	5 (18.5)	6 (20.7)		
Weight (kg)	93.0 (13.2)	91.2 (13.6)	F (1, 50) = 0.017, < 0.001	0.628[&]
Height (cm)	163.3 (9.4)	160.9 (8.9)	t (54) = −0.963	0.340
BMI (kg/m^2)	34.7 (2.4)	35.1 (2.8)	F (1, 50) = 0.359, 0.007	0.552[&]

Data presented as Mean (SD).
& ANCOVA test (Adjusting on Baseline values, age, sex, education, and occupation variables).
Supplementary data for Chi2 test presented as χ^2 (df).
Supplementary data for Independent sample *t*-Test presented as t (df).
Supplementary data for ANCOVA test presented as F (df, Error), Partial Eta Squared or ηp2.
[a] Presented as frequency (percent).
[b] Independent sample *t*-Test/Chi2 test.

2.1. Anthropometric, body composition, and appetite assessments

Anthropometric measurements were taken in the fasting state at baseline. Body weights and heights were measured without shoes and with light clothing using a Seca scale (Seca, Hamburg, Germany) and a stadiometer (Seca), respectively. The BMI was calculated by dividing weight/height^2 (kg/m2). Hip circumferences were measured at the site of the anterior superior iliac spine, where this could be felt, otherwise at the broadest circumference below the waist. Waist circumferences were measured at the narrowest point below the ribs or halfway between the lowest ribs and the iliac crests. Body composition including fat mass, fat present, and fat-free mass was measured by bioelectrical impedance analysis (TANITA BC-418, Co. Tokyo, Japan). To assess appetite sensations, 10–15 min after giving blood samples participants completed a 10 cm visual analog scales (VAS) were used to measure hunger, fullness, the desire to consume food, and the desire to eat sweet/salty/fatty foods. (Flint, Raben, Blundell, & Astrup, 2000).

2.2. PPAR-α gene expression assessment

2.2.1. Isolation of peripheral blood mononuclear cells (PBMC)

Blood samples (5 mL) were collected in vacuum collection tubes containing EDTA (Vacutainer K2E) in the fasting state (10–12 h, water permitted) at baseline and at the end of intervention. PBMCs were isolated through density gradient centrifugation using Ficoll-Histopaque solution gradient (Ficoll-paque, Miltenyi Biotec GmbH, and Germany) and immediately stored at −80 °C until use.

2.2.2. Isolation of RNA for PPAR-gene

Total RNA purification was extracted using Ambion Trizol LS reagent (Thermo Fisher Scientific, USA), according to the manufacturer's protocol. After the extraction of total RNA, the quality and quantity of the isolated RNA were evaluated with NanoDrop spectrophotometry (NanoDrop One^c, Thermo Scientific). Then, total RNA was converted to complementary DNA (cDNA) using a random hexamer primer and reverse transcriptase according to the manufacturer's protocol (Thermo Scientific Revert Aid First Strand cDNA Synthesis Kit, USA). The integrity of total RNA was shown by the gel electrophoresis of individual samples on a 1% agarose gel.

2.2.3. Real-time PCR for PPAR-α gene expression

The PPAR-α gene sequence was from the National Center for Biotechnology Information (NCBI), and Ensembl (http://asia.ensembl.org/) databases. For the PPAR-α mRNA sequence, the primer pairs were designed using OLIGO7 software, (Molecular Biology Insights, Inc., Cascade, CO., USA).

The level of PPAR-α mRNA was examined by SYBR Green Master mix (Thermo Fisher Scientific, USA). The primer sequences for the human PPAR-gene were forward, 5′-TTCGACTCAAGCTGGTGTATG- 3′ and reverse, 5′-GTGTGACATCCCGACAGAAA- 3′; for the β-actin gene they were forward, 5′-GGTGAAGGTGACAGCAGT- 3′ and reverse, 5′-TGGGGTGGCTTTTAGGAT- 3′ designed with PrimerBank. Data was normalized to β-actin expression by the ΔΔCT comparative method. All samples were run in triplicate. The fold change of the PPAR-α mRNA was calculated as a relative expression of post-intervention/placebo.

2.3. Statistical analysis

Data were analyzed using SPSS software (version 20; SPSS Inc., Chicago, IL). The distribution of variables was assessed by the Kolmogorov-Smirnov test. Numerical data was presented as mean (standard deviation) or median (25th-75th percentile), and categorical data was presented as frequency (percentage). The baseline characteristics were assessed by independent sample *t* tests and the chi-squared test in both groups. The Mann-Whitney test was performed to compare the non-normal variables in the intervention and placebo groups. The within-group changes of variables were assessed by paired sample *t*-test, and the Wilcoxon sign test was used for within-group, non-normal items. Considering the nature of the design of the study and the specific aims to be assessed in this study, we used analysis of covariance (ANCOVA) to detect difference between the intervention and placebo groups after adjusting for baseline measurements and confounder factors including age, sex, occupational and educational status (Fleiss, 2011). $p < 0.05$ was defined as statistically significant.

3. Results

Of 60 obese people recruited to the study, 4 were excluded during the study. Analysis was performed on data from 56 participants who completed the intervention. Mean ± SD ages of participants in the intervention and placebo groups were 37.4 ± 8.7 and 38.1 ± 9.2 years, respectively. About 55% and 65% of participants in the intervention and placebo groups, respectively, were females. Participants reported no side effects or symptoms either during OEA treatment or at the end of intervention. Table 1 shows the demographic characteristics of participants at baseline.

PPAR-α gene expression was increased (mean ± SD) 2.41 ± 0.98 fold in the intervention group vs. 1.55 ± 0.644 fold in the placebo group, t = −3.886, p = < 0.001. Fig. 2 shows the difference in PPAR-α gene expression in both groups.

Mean ± SD weight and BMI values decreased significantly in the intervention group. At baseline, the mean ± SD values of waist circumference were 105.3 ± 13.8 cm; and decreased significantly to 100.6 ± 14.5 cm ($p < 0.001$). Fat mass decreased significantly in the intervention group by the end of study. Table 2 and Table 3 show the results of OEA supplementation on the anthropometric measurements and body composition.

In the intervention group, hunger and desire to eat and cravings to eat sweet foods decreased significantly ($p < 0.05$), and fullness increased significantly ($p < 0.001$). Table 4 depicts the results of OEA on appetite sensations.

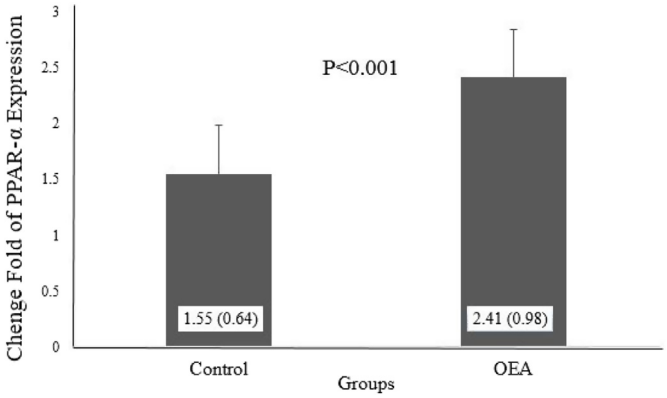

Fig. 2. Mean (SD) difference in fold-change of PPAR-α expression in OEA and control groups.

4. Discussion

The key role of obesity in the incidence of chronic diseases and the alarming increase in its prevalence has raised awareness of the need to identify molecular mechanisms involved in energy homeostasis and appetite sensations to modulate obesity (Glickman, Sim, Del Valle Cook, & Miller, 2012). Dietary modification is more acceptable in the successful prevention of obesity than other treatments because of fewer side effects (Wadden, Webb, Moran, & Bailer, 2012). Numerous dietary factors have been shown to have positive effects on energy balance and feeding behaviors through different cellular and molecular mechanisms (Spiegelman & Flier, 2001).

In the present study, OEA significantly increased the expression of the nuclear receptor PPAR-α. Overall appetite sensations improved, and anthropometric measures including weight, BMI, waist circumference, and fat mass decreased significantly. In agreement with the present study, Barbaro et al. (Barbaro, Baldini, Pasquini, & Lapi, 2011) showed that supplementation of the diet of 38 obese people (BMI = 32–41 kg/m2) with 2 capsules containing 170 mg N-oleyl-phosphatidylethanolamine (NOPE) and 120 mg epigallocatechin-3-gallate (EGCG) daily for 2 months resulted in significant weight loss and reductions in hip circumference ($p < 0.001$). In the study of Rondanelli et al. (Rondanelli et al., 2008), the administration of 2 NOPE–EGCG capsules (85 mg NOPE and 50 mg EGCG) for 2 months in 138 healthy overweight people increased the sensation of fullness and decreased weight significantly ($p < 0.05$). In contrast, Mangine et al. showed that supplementation of 50 healthy overweight subjects with 120 mg NOPE and 105 mg of EGCG for 8 weeks had no significant effects on BMI, body composition, feelings of hunger, or binge eating ($p > 0.05$) (Mangine et al., 2012). Similar to these clinical studies, many experimental studies have reported the same results. In a study by Guzman et al., dietary supplementation of rats and wild-type mice (n = 4–6) with 5 mg/kg OEA intraperitoneally for 4 weeks activated the nuclear receptor PPAR-α, stimulated lipolysis and fatty acid oxidation, and reduced body weight and food intake (Guzmán et al., 2004). In another study, Fu et al. (Fu et al., 2003), showed that supplementation of 32 mice (7–8/group) with 5 mg/kg OEA once daily for 4 weeks significantly enhanced the expression of targeted genes of PPAR-α, decreased the desire to feed, and inhibited weight gain ($p < 0.05$). In a study by Nielsen et al., oral supplementation of OEA (1, 10, and 100 mg/kg) in male Wistar rats 30 min before food presentation decreased food intake significantly (at doses of 10 mg/kg) (Nielsen, Petersen, Astrup, & Hansen, 2004). Oveisi et al. administered OEA as gavage (0, 50, 100, and 200 mg/kg) and as capsules (0, 25, and 50 mg/kg) for 24 h to rats, which resulted in a significant reduction in food intake during 24 h after doses of 200 mg/kg (gavage) and 50 mg/kg (capsules) ($p < 0.01$). Also, OEA decreased the number of meals and increased post-meal intervals ($p < 0.05$) (Oveisi et al., 2004). A study by Thabuis et al. supplemented the diet of male mice (n = 21) with OEA at different doses (0, 10, and 100 mg/kg) for 4 weeks and found decreased food intake and adipose tissue mass in a dose-dependent manner ($p < 0.05$). (Thabuis, Destaillats, Landrier, Tissot-Favre, & Martin, 2010).

Table 2
The effect of OEA supplementation on the anthropometric measurements and body composition in obese people (n = 56).

Variables	OEA group (n = 27)	Placebo Group (n = 29)	F (df, Error), ηp2	p**
Weight (kg)				
Before	93.0(13.2)	91.2 (13.6)	F (1, 49) = 14.512, 0.228	< 0.001
After	91.8 (13.1)	91.7 (13.5)		
t (df), p *	t (26) = 3.24, 0.003	t (28) = −1.93, 0.063		
BMI (kg/m^2)				
Before	34.7 (2.4)	35.1 (2.8)	F (1, 49) = 9.666, 0.165	0.003
After	34.4 (2.5)	35.4 (2.8)		
t (df), p*	t (26) = 1.91, 0.067	t (28) = −2.71, 0.011		
Waist circumference (cm)				
Before	105.3 (13.8)	102.5 (10.5)	F (1, 49) = 18.671, 0.276	< 0.001
After	100.6 (14.5)	103.0 (11.6)		
t (df), p *	t (26) = 5.03, < 0.001	t (28) = −0.59, 0.559		
Hip circumference (cm)				
Before	118.8 (9.0)	119.4 (7.6)	F (1, 49) = 2.979, 0.057	0.091
After	116.7 (9.2)	119.0 (7.6)		
t (df), p *	t (26) = 2.88, 0.008	t (28) = 0.63, 0.545		
Fat mass (kg)				
Before	36.3 (7.6)	34.5 (6.2)	F (1,49) = 14.089, 0.223	< 0.001
After	35.1 (7.5)	35.2 (6.6)		
t (df), p *	t (26) = 3.99, < 0.001	t (28) = −2.24, 0.033		
Fat-free mass (kg)				
Before	57.3 (14.3)	55.5 (13.2)	F (1, 48) = 3.023, 0.059	0.088
After	58.0 (14.6)	57.7 (13.0)		
t (df), p *	t (25) = −0.46, 0.646	t (28) = −2.08, 0.046		
Fat percent (%)				
Before	39.1 (6.9)	37.7 (8.4)	F (1,49) = 0.009, < 0.001	0.923
After	38.1 (6.9)	38.0 (5.7)		
t (df), p *	t (26) = 4.49, < 0.001	t (28) = −2.08, 0.766		

Data were presented as Mean (SD) *paired *t*-Test ** ANCOVA test after adjusting for baseline measurements and confounder factors including age, sex, occupational and educational status.
Supplementary data for Paired *t*-Test presented as t (dF), p.
Supplementary data for ANCOVA test presented as F (df, Residual or Error), Partial Eta Squared orηp^2.

Table 3
The supplementary data about the mean difference of the anthropometric measurements and body composition in obese people's supplemented with OEA (n = 56).

Variables	Sum of Squares	df	Mean square	F	ηp2	p[a]
Weight covariates:						
Weight before	5400.002	1	5400.002	1937.429	0.975	< 0.001
Age	8.522	1	8.522	3.058	0.059	0.087
Sex	0.196	1	0.196	0.070	0.001	0.792
Education	0.856	1	0.856	0.307	0.006	0.582
Occupational	0.080	1	0.080	0.029	0.001	0.866
Residual (Error)	136.573	49				
Explained (r Squared)	0.986					
BMI covariates:						
BMI before	306.570	1	306.57	883.043	0.947	< 0.001
Age	1.444	1	1.444	4.159	0.078	0.047
Sex	0.057	1	0.057	0.164	0.003	0.687
Education	0.048	1	0.048	0.138	0.003	0.712
Occupational	0.056	1	0.056	0.160	0.003	0.691
Residual (Error)	17.012	49				
Explained (r Squared)	0.958					
Waist circumference covariates:						
WC before	4377.681	1	4377.681	234.399	0.827	< 0.001
Age	33.275	1	33.275	1.782	0.035	0.188
Sex	11.629	1	11.629	0.623	0.013	0.434
Education	4.253	1	4.253	0.228	0.005	0.635
Occupational	14.929	1	14.929	0.799	0.016	0.376
Residual (Error)	915.134	49				
Explained (r Squared)	0.902					
Hip circumference covariates:						
HC before	2610.058	1	2160.058	236.441	0.828	< 0.001
Age	39.905	1	39.905	3.615	0.069	0.063
Sex	0.415	1	0.415	0.038	0.001	0.847
Education	2.886	1	2.886	0.261	0.005	0.611
Occupational	6.595	1	6.595	0.597	0.012	0.443
Residual (Error)	540.909	49				
Explained (r Squared)	0.863					
Fat mass covariates:						
Fat mass before	4377.68	1	4377.681	234.399	0.827	< 0.001
Age	33.275	1	33.275	1.782	0.035	0.188
Sex	11.629	1	11.629	0.623	0.013	0.434
Education	4.253	1	4.253	0.228	0.005	0.635
Occupational	14.929	1	14.929	0.799	0.016	0.376
Residual (Error)	915.134	49				
Explained (r Squared)	0.902					
Fat-free mass covariates:						
Fat-free mass before	2880.672	1	2880.672	156.941	0.766	< 0.001
Age	11.717	1	11.717	0.638	0.013	0.013
Sex	130.282	1	130.282	7.098	0.129	0.129
Education	2.607	1	2.607	0.142	0.003	0.003
Occupational	22.817	1	22.817	1.243	0.025	0.025
Residual (Error)	881.049	48				
Explained (r Squared)	0.913					
Fat percent covariates:						
Fat percent before	300.926	1	300.926	28.280	0.366	< 0.001
Age	7.547	1	7.547	0.709	0.014	0.404
Sex	114.026	1	114.026	10.716	0.179	0.002
Education	2.863	1	2.863	0.269	0.005	0.606
Occupational	13.568	1	13.568	1.275	0.025	0.264
Residual (Error)	521.404	49				
Explained (r Squared)	0.757					

Supplementary data for ANCOVA test (covariates) presented as Mean square, F, df, Error, Partial Eta Squared orηp2, p.

[a] ANCOVA test after adjusting for baseline measurements and confounder factors including age, sex, occupational and educational status. HC: Hip circumference. WC: Waist circumference.

Table 4
The effect of OEA supplementation on the appetite sensations in obese people (n = 56).

Items	OEA group (n = 27)	Placebo group (n = 29)	U, Z	p
	Median (25th −75th)	Median (25th −75th)		
Hunger				
Before	7 (6–9)	9 (8–9.5)	114.5, −4.641	< 0.001
After	6 (5–7)	10 (7–10)		
Difference	−1	+1		
Fullness				
Before	3 (2–5)	1 (1–3)	73.0, −5.336	< 0.001
After	5 (3–6)	0 (0–2)		
Difference	+2	−1		
Feeling to eat				
Before	8 (6–9)	9 (8–9)	109.0, −4.703	< 0.001
After	6 (5–7)	9 (7–10)		
Difference	−2	0		
Desire to eat sweet				
Before	5 (3–7)	6 (5–9)	209.0, −3.017	0.003
After	4 (2–8)	7 (5–9)		
Difference	−1	+1		
Desire to eat salty				
Before	5 (2–6)	4 (3–5)	319.0, −1.200	0.230
After	3 (2–7)	5 (3–5)		
Difference	−2	+1		
Desire to eat fatty				
Before	6 (4–8)	5 (3–8)	326.0, −1.082	0.279
After	5 (3–7)	5 (3–8)		
Difference	−1	0		

Data were presented as Median (25th-75th).
Mann-Whitney *U* test.
$p < 0.05$ statistically significant.
Supplementary data for Mann-Whitney *U* test presented as U, Z, p.

Controlling food consumption is considered an important aspect of the management of lifestyle-related obesity (Volkow, Wang, & Baler, 2011), and OEA induces satiety and weight loss through various mechanisms. The main mechanism of OEA in weight management is related to PPAR-α gene expression (Fu et al., 2003). PPAR-α is involved in numerous metabolic pathways such as inflammatory processes, feeding behaviors, and the regulation of lipid metabolism, including the uptake of fatty acids and fatty acid oxidation (De Fonseca et al., 2001; Guzmán et al., 2004; Kersten, Desvergne, & Wahli, 2000). Activation of PPAR-α receptors following OEA exposure stimulates the genes involved in fatty acid oxidation and lipolysis in white adipose tissues (Contreras, Torres, & Tovar, 2013). Moreover, OEA induces fatty acid uptake in adipocytes by enhancing the expression of related genes such as FAT/CD36 (Yang, Chen, Georgeson, & Harmon, 2007), and OEA decreases gastric emptying and acts as a ligand of other genes involved in eating behaviors (Sarro-Ramírez et al., 2013). In addition to activating the nucleus of the solitary tract in the brainstem and the paraventricular nucleus in the brain, OEA suppresses food intake by releasing hypothalamic neuropeptides involved in appetite such as oxytocin and CART[1] (Sarro-Ramírez et al., 2013; Serrano et al., 2011).

The anorexic effects of OEA, also attributed to the activation of the GPR-119 gene, resulted in the production of anorexic hormones such as GLP-1. Indeed, GLP-1 reduces food intake and increases satiety through a combination of activating GLP-1 receptors in the central nervous system and on sensory neurons of the gastrointestinal tract (Hansen et al., 2011).

To the best of the authors' knowledge, this was the first clinical study to assess the unique effects of OEA on PPAR-gene expression, appetite sensations, and anthropometric measurements in obese people. However, this study has some limitations. The major limitation of this study was insufficient purity of the synthesized OEA (~85%). Others were the short duration of the study and not measuring OEA concentrations in serum

[1] Cocaine-and amphetamine-regulated transcript.

samples or evaluating other genes involved in lipid metabolism. Although OEA decreased overall appetite and increased satiety after 60 days of intervention, we could not measure its effect on eating after or during a meal and this was considered as another limitation of this study.

The main finding of this study is that supplementation with 2 × 125-mg OEA capsules for 8 weeks enhanced the expression of the PPAR-α gene, improved anthropometric measurements (weight, BMI, waist circumference, and fat mass) and appetite sensations (hunger, desire to eat foods, and cravings for sweets decreased, and fullness increased). Considering the many beneficial effects of OEA in various metabolic pathways, its use as a complementary approach to weight loss could be effective in suppressing appetite and controlling weight in obese people; however, further studies are needed to confirm the current results.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.appet.2018.05.129.

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