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ORIGINAL CONTRIBUTION

Consumption of extra virgin olive oil improves body composition and blood pressure in women with excess body fat: a randomized, double-blinded, placebo-controlled clinical trial

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Abstract
Purpose Despite the fact that extra virgin olive oil (EVOO) is widely used in obese individuals to treat cardiovascular diseases, the role of EVOO on weight/fat reduction remains unclear. We investigated the effects of energy-restricted diet containing EVOO on body composition and metabolic disruptions related to obesity.
Methods This is a randomized, double-blinded, placebo-controlled clinical trial in which 41 adult women with excess body fat (mean ± SD 27.0 ± 0.9 year old, 46.8 ± 0.6% of total body fat) received daily high-fat breakfasts containing 25 mL of soybean oil (control group, $n = 20$) or EVOO (EVOO group, $n = 21$) during nine consecutive weeks. Breakfasts were part of an energy-restricted normal-fat diets (−2090 kJ, ~32%E from fat). Anthropometric and dual-energy X-ray absorptiometry were assessed, and fasting blood was collected on the first and last day of the experiment.
Results Fat loss was ~80% higher on EVOO compared to the control group (mean ± SE: −2.4 ± 0.3 kg vs. −1.3 ± 0.4 kg, $P = 0.037$). EVOO also reduced diastolic blood pressure when compared to control (–5.1 ± 1.6 mmHg vs. +0.3 ± 1.2 mmHg, $P = 0.011$). Within-group differences ($P < 0.050$) were observed for HDL-c (−2.9 ± 1.2 mmol/L) and IL-10 (+0.9 ± 0.1 pg/mL) in control group, and for serum creatinine (+0.04 ± 0.01 µmol/L) and alkaline phosphatase (−3.3 ± 1.8 IU/L) in the EVOO group. There was also a trend for IL-1β EVOO reduction (−0.3 ± 0.1 pg/mL, $P = 0.060$).
Conclusion EVOO consumption reduced body fat and improved blood pressure. Our results indicate that EVOO should be included into energy-restricted programs for obesity treatment.

Keywords Extra virgin olive oil · Soybean oil · Body fat · Blood pressure · Adiposity · Monounsaturated fatty acid

Introduction

Obesity results from complex interactions between genetic and lifestyle factors. The consumption of high-fat diets has been considered one of the main factors predisposing fat gain [1–3]. However, the role of dietary fat on obesity pathogenesis remains unclear.

Extra virgin olive oil (EVOO) is a high-quality oil rich in monounsaturated oleic acid (55–85% of fatty acid content), which contains more than 230 chemical constituents with antioxidant activity such as vitamin E, carotenoids, and phenolic compounds [4]. Due to the well-established beneficial effects of that oil over CVD risk [5–8] and the strong association between CVD and excess body fat, the consumption of energy-restricted diet containing EVOO has been adopted in weight loss programs. However, the benefits of EVOO over CVD have been inadvertently extrapolated for weight/fat loss promotion without adequate scientific evidence [9, 10].

The current hypothesis that EVOO could also contribute to weight/fat loss is mostly based on observational evidence, demonstrating that the consumption of Mediterranean diet rich in olive oil was significantly less likely to favor obesity

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[11–13]. Results from these observational studies are difficult to interpret because habitual use of olive oil in salads and vegetable-based dishes within the Mediterranean diet is also associated with the consumption of other functional low-density foods [10, 14]. Furthermore, randomized clinical trials about this topic are scarce, and presented inconclusive and controversial results [10, 15]. In some clinical trials, the great discrepancy in the dietary intervention applied to the control and test groups may have favored the reduction in body weight/fat in response to olive oil consumption [16, 17]. On the other hand, other clinical trials reported no influence of olive oil on body weight/fat [18] or even an increase in abdominal obesity [19] when it was incorporated into Mediterranean diet. When consumed associated with an energy-restricted non-Mediterranean diet, olive oil reduced less body weight than medium-chain triacylglycerol—MCT [20].

Despite the fact that the incorporation of good fat source into energy-restricted diets can improve palatability and favor compliance of the traditional energy-restricted low-fat diet [21], there is no clear evidence supporting the effect of EVOO to improve body weight/fat loss. Therefore, we investigated the effect of the consumption of EVOO into an energy-restricted non-Mediterranean diet on body weight/fat. Additionally, we assessed the role of EVOO on systemic inflammation, cardiovascular, hepatic and renal functions, which can be impaired due to lipotoxicity.

Methods

Subjects

Seven hundred fifty-three women were assessed for eligibility through local advertisements and seventy-seven apparently healthy middle-aged women (19–41 years, BMI between 26 and 35 kg/m^2) met the inclusion criteria and were allocated to study groups (Fig. 1). Potential subjects had excess body fat (>32%); habitually used soybean oil as cooking oil; were nonsmoker, non-pregnant, and non-lactating. The exclusion criteria were the followings: alcohol consumption (>15 g of ethanol/day), elite athletes (>10 h of exercise/week), habitual consumption of olive oil (more than 8 mL/day), recent changes (<3 months) in diet or physical activities habits, use of supplements or drugs except contraceptive ones, the presence of food allergy/intolerance or aversion to tested ingredients, gastrointestinal diseases or other acute or chronic diseases besides obesity.

From the 77 initially recruited women, 16 dropped out before starting the intervention. Sixty-one eligible women were included in the study, 51 completed the adopted protocol, and 41 were included in the analyses. The reasons by which ten women were not included in the final analyses were the following: pregnancy ($n = 1$), secondary pathological events not related to the intervention ($n = 6$) and drop out ($n = 3$). Because all subjects which finished the study follow the entire study protocol, there was no exclusion due to lack of compliance in this study. Power calculation was performed retrospectively [22] and indicated that 21 subjects were necessary to detect an increment of 1.09 kg in total body fat loss presented by EVOO group (mean ± standard deviation of change in body fat loss of overall subjects; 1.9 ± 1.8 kg; statistical power = 90%; $\alpha = 5\%$). An increment of ~1 kg in body fat loss is relevant considering the duration of this study and this preventive nature [23].

All recruited participants gave written consent after receiving verbal and written information about the experiment. The study protocol was approved by the Ethics Committee of Universidade Federal de Viçosa (protocol number: 892.467/2014), conducted in accordance with 1964 Declaration of Helsinki and its latter amendments, and registered at http://www.ensaiosclinicos.gov.br/ (identifier: RBR-7z358j).

Experimental design

This was a double-blinded, randomized, parallel, placebo-controlled clinical trial for nine consecutive weeks (±5 days), in which subjects were randomly assigned to control (soybean oil) or interventional (EVOO) groups. The tolerance of ±5 days to end the experiment was required to prevent impairment on anthropometric/body composition parameters assessments due to hormonal changes. The allocation on the control or interventional groups was made using the block randomization technique [24] and was concealed from the investigators. High-fat drinks were served into colored cups to avoid visual identification of the type of drink tested. There was no description or dietary information about the breakfasts on those cups. Therefore, neither subjects nor investigators were aware of the treatment assignments.

One week before beginning the trial, selected women refrained from eating olive oil, were instructed to not consume alcohol beverages and to maintain their usual dietary and physical activity habits. A standard dinner (2508 kJ, carbohydrate: 62 E%, fat: 29.4 E%, protein: 8.5 E%) was consumed the night before the test day. Women were reported to laboratory in a fasting state for anthropometric, body composition, and blood pressure assessments at baseline and on the last day of the experiment. Inclusion in the study was postponed if women presented any symptoms of inflammation or intestinal disorder. After the assessments, subjects underwent blood collection and consumed a high-fat breakfast containing 25 mL of soybean oil or EVOO for breakfast. The amount of oil (25 mL) added to the drinks was based on the range of olive oil usually consumed by Mediterranean population (25–50 mL/day) [25] without exceeding the

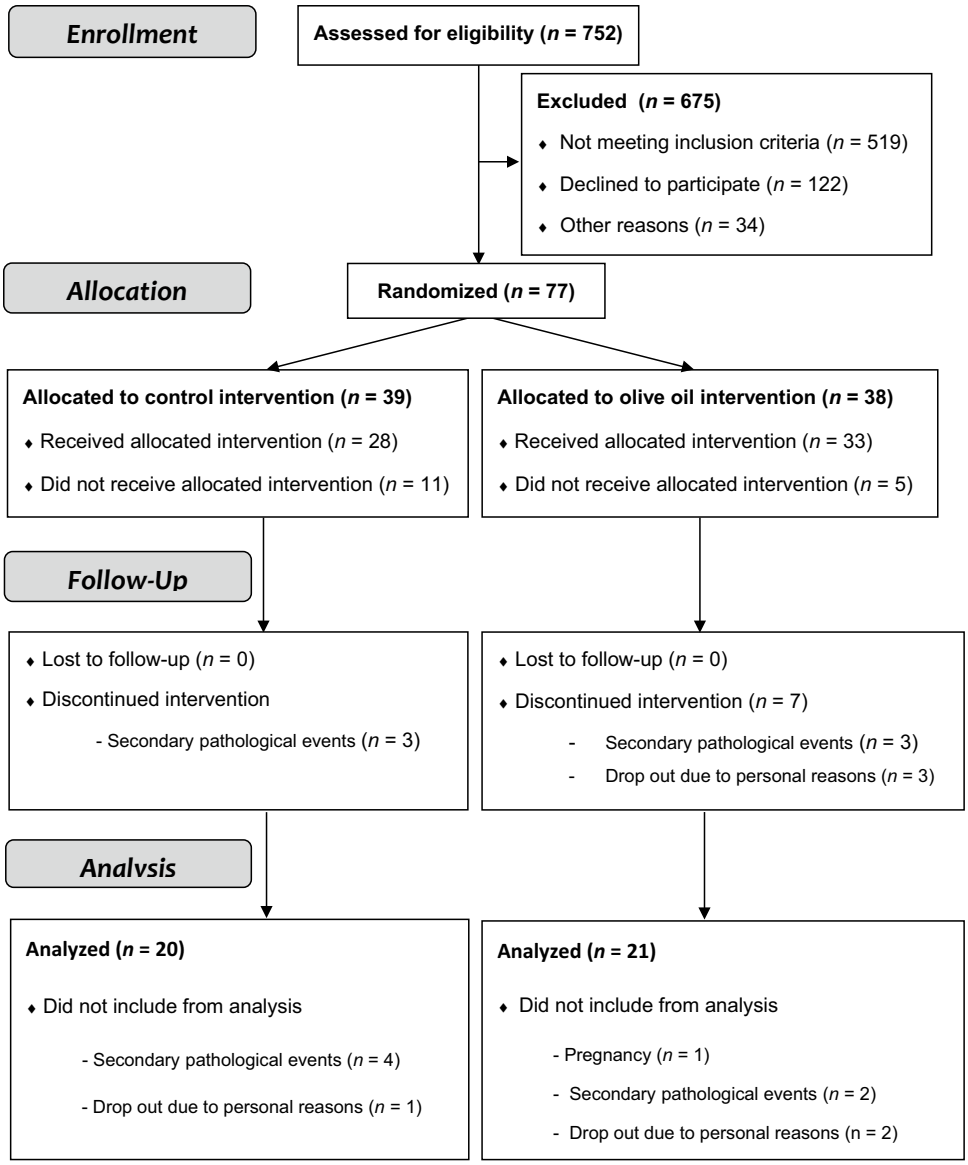

Fig. 1 CONSORT diagram showing the flow of participants through each stage of the trial. CONSORT Consolidated Standards of Reporting Trials

fat consumption recommendations [26]. During the other study days, high-fat breakfasts were daily provided in the laboratory as part of an energy-restricted non-Mediterranean diet and women were released from the laboratory to follow the prescribed diet in free-living conditions. Habitual food intake, physical activity level, and prescribed diet compliance were also assessed (Suppl. Figure 1).

Breakfasts

Extra virgin olive oil (Andorinha®, Sovena S.A., Algés, Portugal) and soybean oil (Corcovado, Archer Daniels Midland, Uberlândia, Brazil) were used to prepare the high-fat drinks (300 mL of a milk-derived flavored drink containing 25 mL of the previously mentioned oils) as part of a breakfast. Both oils were protected from light and heat until their consumption. The high-fat drinks were matched in all ingredients except for the type of oil used to prepare them. During all the experimental period, subjects attended the laboratory daily on week days to have the breakfasts according to the allocated group. On weekends, identical breakfasts containing the test oils were provided to be consumed at home. Besides the high-fat drinks, two low-fat cookies were also offered for breakfast. A rotating menu of six breakfast flavors, with very similar nutritional composition, was prepared to avoid monotony and to improve compliance to the study protocol (Suppl. Table 1). Protocol compliance on weekends was assessed by asking subjects about the breakfast consumption and by the return of the packages in which the breakfasts were taken. Subjects were not informed about the exclusion of the study if they did not follow the protocol to guarantee the confidence of the information.

EVOO and soybean oil fatty acids profile were performed in triplicate. Fatty acid composition of EVOO was assessed in laboratory after esterification [27] by gas chromatography (GC) [28] (Suppl. Table 1).

Dietary assessments

Energy-restricted nutritionally balanced diets were individually prescribed by a single dietitian. The type of foods prescribed and the macronutrient distribution were maintained during the intervention to reduce the influence of prescribed diets beyond fats on results. There were no differences on energy and macronutrient content of prescribed diet between groups (7836.7 ± 897.4 kJ, carbohydrate: 49.0 ± 2.8% E, fat: 31.8 ± 2.85% E, protein: 19.1 ± 2.4% E). No other high MUFA food besides the 25 mL of EVOO for the EVOO group was prescribed, and a food substitution list was used to subsidize food choices.

Total energy requirements were estimated according to total energy expenditure for overweight/obese women [26]. Then, caloric restriction (−2090 kJ/day) was applied. Physical activity levels [29] were used to obtain physical activity coefficients (1.00 for sedentary or 1.16 for low-active individuals) [26]. Three non-consecutive days (2 week days and 1 weekend day) 24-h food records were applied to assess food intake on the week before baseline, and during the experimental period. Macro- and micronutrient intakes were analyzed by a single dietitian using DietPro software (version 5.2i, Agromídia, MG, Brazil), and were based on reliable composition tables [30–32].

Anthropometric, body composition, and blood pressure measurements

Anthropometric measurements were assessed by a single investigator. Body weight was measured on a digital platform scale with a resolution of 0.5 kg (Toledo®, Model 2096PP/2, SP, Brazil), while subjects were barefoot and wearing lightweight clothing. Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer (Wiso, Chapecó, SC, Brazil). BMI was calculated by dividing body (kg) by height (m) squared. Waist, hip, neck, and thigh circumferences, as well as sagittal abdominal diameter, were measured in triplicate as described by Vasques et al. [33]. The average of the two nearest values of the three collected measurements was recorded. Waist circumference and sagittal abdominal diameter were measured in the midpoint between the last rib and iliac crest. Waist/hip, and conicity index (CI) were calculated following the formula: CI = [waist circumference (m)]/[0.109 $\sqrt{}$(body weight (kg)/height (m))] [34]. Blood pressure was measured by an automatic Omron HEM-7200 device (Omron Inc., Dalian, China) in both arms, according to Mancia et al. [35].

Dual-energy X-ray absorptiometry scan (DXA) (model Prodigy Advance, GE Healthcare Inc., Waukesha, WI) was performed to assess changes in body composition according to manufacturer's instructions. Values of lean mass, total body fat, and fat distribution (truncal, gynoid, and android regions) were obtained.

Metabolic biomarkers

Antecubital blood samples were collected in the fasting state (12 h). Serum (serum gel tubes) and plasma (EDTA tubes) samples were separated from whole blood by centrifugation (3500 rpm, 4 °C, 15 min) and immediately frozen at −80 °C until analyses. Serum glucose, triglycerides (TG), total cholesterol, high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), uric acid, urea, creatinine, alkaline phosphatase (AP), γ-glutamyltransferase (Gamma GT), aspartate amino transferase (AST), and alanine amino transferase (ALT) were quantified by an automated analyzer system (BS-200™ Chemistry Analyzer, Mindray) using available commercial colorimetric assay kits (K802, K117, K083, K071, K088, K139, K056, K067, K021, K080, K048, and K049, respectively; Bioclin®, MG, Brazil). The serum very-low-density lipoprotein cholesterol (VLDL-c) was calculated using Friedewald et al. equations [36]. Serum insulin was quantified using eletroquimioluminescence method (Elecsys-Modular E-170, Roche Diagnostics Systems). The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated to estimate insulin resistance according to the equation proposed by Matthews et al. [37]. atherogenic index (TG/HDL-c ratio) were also calculated [38].

Flow cytometry analysis was performed using a BD FACS Verse™ flow cytometer (BD Biosciences). Interleukin-8 (IL-8), interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-10 (IL-10), tumor necrosis factor-α (TNF-α), and interleukin-12p70 (IL-12p70) plasma concentrations were measured using commercial kit (Cytometric Bead Array CBA Human Inflammatory Cytokines Kit, BD Biosciences) according to the manufacturers' instructions. Data were analyzed using the FCAP Array Software v3.0 (BD Biosciences).

Statistical analysis

Data were typed by two independent investigators to ensure data reliability. Group data were coded before the data analyses for blindness. Per-protocol analyses were performed due to the large number of participants who did not complete the intervention after being randomized. Statistical analyses were carried out on SPSS 20 for Windows (SPSS, Inc., Chicago, IL, USA). Data are expressed as mean ± standard deviation (SD) for descriptive variables or mean ± standard

error (SE) and median (interquartile range) for comparative data. Individual outlier values were excluded before analyses. The thresholds for lower and upper outliers were defined as follows: lower thresholds = lower quartile—(1.5 × interquartile range) and upper threshold = upper quartile + (1.5 × interquartile range). Data normality and homoscedasticity were assessed by Shapiro–Wilk and Levene's tests, respectively. Paired Student's *t* test or Wilcoxon signed-rank test were used to assess within group differences. Differences between groups were assessed over absolute delta (Δ) values (9 weeks—baseline) by Student's *t* test or Mann–Whitney *U* signed-rank test. Pearson's or Spearman's correlation coefficients were used to assess the relation between fat reduction and metabolic biomarkers. A 5% α level of significance was adopted.

Results

Subjects

Forty-one women completed the study protocol and were included in the analyses. Participants were 27.0 ± 0.9 years old, presented 46.8 ± 0.6% of total body fat, and 30.2 ± 0.4 kg/m^2 of BMI (overweight: $n = 23$ or 56.1%; obese: $n = 18$ or 43.9%). There were no significant between-group differences in baseline food intake and in all anthropometric, body composition, blood pressure, and metabolic variables assessed in this study, except for diastolic blood pressure and TNF-α which EVOO presented higher values (Table 1). None of the participants had systolic blood pressure higher than 139 mmHg and only one EVOO group participant had diastolic blood pressure ranging from 90 to 99 (first state of hypertension). Despite the fact that none of the participants showed symptoms of acute inflammation during the test days, five of them presented a clear inflammatory cytokines profile (TNF-α and IL-6 values were twice as higher as the highest values showed by the total sample) and were excluded from final analysis. Eight participants from both groups presented TNF-α concentration below the detection limits of the assay kit. Six participants from the control group and five from the EVOO group had no detectable concentrations for IL-1β. That did not occur for the other cytokines.

Dietary assessments

As expected, food intake analyses during experiment period showed reduction in energy and macronutrients intake values compared to baseline in both groups due to energy restriction. Dietary intake during the experiment only differed between groups for C18:1, C18:2, total monounsaturated fatty acids, and total polyunsaturated fatty acids ($P < 0.001$), reflecting the differences in the fatty acid profile of the supplemented oils (Table 2).

Table 1 Baseline characteristics of study subjects according to experimental groups

	Control	Extra virgin olive oil
Subjects (*n*)	20.0	21.0
Age (years)	27.2 ± 6.1	26.8 ± 5.0
Physical activity (S/LA)	6.00/14.0	3.00/18.0
Systolic blood pressure (mmHg)	109 ± 2.10	115 ± 2.40
Diastolic blood pressure (mmHg)	67.5 ± 1.50	74.5 ± 1.90
Body weight (kg)	77.6 ± 2.00	77.6 (13.2)
BMI (kg/m^2)	29.7 ± 0.60	30.5 ± 0.60
Waist circumference (cm)	97.7 ± 1.60	98.9 ± 1.60
SAD (cm)	19.6 ± 0.50	19.7 ± 0.40
Total body fat (kg)	37.0 ± 1.40	34.4 (11.2)
Total body fat percentage (%)	46.6 ± 0.70	47.0 ± 0.90
Total lean mass (%)	49.4 ± 0.84	49.0 ± 0.98

Values are mean ± SE or median (interquartile range). Waist circumference values were measured at umbilical level

BMI body mass index, *S/LA* number of sedentary and low-active individual ratios (28), *SAD* Sagittal abdominal diameter, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids, *SFA* saturated fatty acids

Anthropometric, body composition, and blood pressure measurements

Body weight (−1.70 ± 0.47 kg 95% CI −2.69 to −0.72 vs. −2.75 ± 0.38 kg 95% CI −3.54 to −1.95 for control and EVOO groups, respectively; $P_{inter} = 0.094$) and BMI (−0.64 ± 0.17 kg/m^2, 95% CI −1.00 to −0.28 vs. −1.06 ± 0.15 kg/m^2 95% CI −1.37 to −0.75; $P_{inter} = 0.072$) reduced with time in both groups due to energy restriction. However, EVOO presented a greater reduction on total body fat than control (−1.30 ± 0.40 kg 95% CI −2.21 to −0.44 vs. −2.4 ± 0.3 kg 95% CI −3.1 to −1.73, $P_{inter} = 0.037$). Fat loss was ~80% higher on EVOO compared to control group. In addition, EVOO reduced diastolic blood pressure (+0.25 ± 1.16 mmHg, 95% CI −2.18 to 2.68 vs. −5.05 ± 1.60 mmHg 95% CI −8.39 to −1.70; $P_{inter} = 0.011$). There were no differences between groups in systolic blood pressure (−3.65 ± 1.54 mmHg 95% CI −6.87 to −0.44 vs. −3.91 ± 1.88 mmHg 95% CI −7.83 to 0.02; $P_{inter} = 0.918$) (Fig. 2).

There was no difference between groups for the other variables. As expected all of the evaluated anthropometric variables, except waist/thigh ratio reduced with time in control and EVOO groups. In addition, both groups showed weight reductions on total fat and specific fat mass sites (truncal, gynoid, and android regions), but not on lean mass (Suppl. Table 2). Total body lean mass

Table 2 Dietary assessments at baseline and change from baseline (95% CI) according to experimental groups

Metabolic biomarkers	Control ($n = 20$)			Extra virgin olive oil ($n = 21$)			P_{Inter}
	Baseline	Δ values	95% CI	Baseline	Δ values	95% CI	
Energy content (kJ)	3565 (2775)	−794 ± 184	−1179 to −406	8343 ± 434	−1041 ± 179	− 1417 to − 665	0.342
Carbohydrate (g)	229 (66.7)	−24.5 ± 5.66	−36.4 to −12.6	261 ± 16.1	−36.1 ± 5.88	−48.4 to −23.7	0.165
(%E)	50.8 ± 1.58	51.8 (55.2)	21.9 to 159	52.2 ± 1.54	54.6 (74.5)	30.1 to 93.7	0.624
Fiber (g)	19.2 ± 1.50	−0.60 ± 0.72	−2.11 to 0.91	21.0 ± 1.50	−1.67 ± 1.09	−3.95 to 0.61	0.418
Protein (g)	78.2 ± 5.30	−9.66 ± 2.39	−14.7 to −4.65	81.8 ± 3.5	−11.1 ± 2.02	−15.3 to −6.84	0.654
(%E)	16.6 ± 0.79	15.8 (25.2)	−21.6 to 37.9	16.8 ± 0.72	18.8 (24.1)	7.06 to 60.7	0.840
Total fat (g)	61.8 (25.6)	−2.11 (12.2)	−7.96 to 1.82	67.7 ± 5.04	−2.80 (17.1)	−10.7 to 0.27	0.708
(%E)	30.1 ± 1.41	23.8 (52.4)	−73.2 to 44.3	30.2 ± 1.19	13.7 (59.4)	−37.2 to 27.5	0.773
Total SFA (g)	20.1 (12.3)	−2.41 (4.42)	−5.86 to −1.95	21.1 ± 1.50	−2.60 (4.72)	−4.90 to −2.00	0.954
Total MUFA (g)	16.4 (9.11)	−1.54 ± 0.64	−2.89 to −1.20	20.2 ± 1.46	4.56 (4.56)	1.53 to 6.04	<0.001
Total PUFA (g)	10.6 (5.18)	5.57 (3.96)	3.24 to 5.82	12.8 (11.2)	−2.84 (4.05)	−5.30 to −1.32	<0.001
C18:1 (g)	11.7 ± 0.99	0.85 ± 0.42	−0.03 to 1.72	13.8 ± 1.15	6.23 (3.28)	4.00 to 7.49	<0.001
C18:2 (g)	7.49 (3.64)	5.07 (2.99)	3.57 to 5.51	9.67 (5.22)	−1.73 (2.55)	−3.83 to −0.65	<0.001
Cholesterol (mg)	222 ± 17.0	−37.6 ± 10.0	−58.7 to −16.6	251 ± 20.8	−45.5 ± 13.4	−73.7 to −17.3	0.642
Sodium (mg)	2469 ± 159	−408 ± 79.8	−575 to −240	2168 (1662)	−404 (807)	−908 to −385	0.234

Values are mean ± SE or median (interquartile range)

SFA saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids, *C18:1* oleic fatty acid, *C18:2* linoleic fatty acid, P_{Inter} between-group Δ values (9 week—baseline) by Student's *t* test or Mann–Whitney *U* signed-rank test, $P > 0.050$

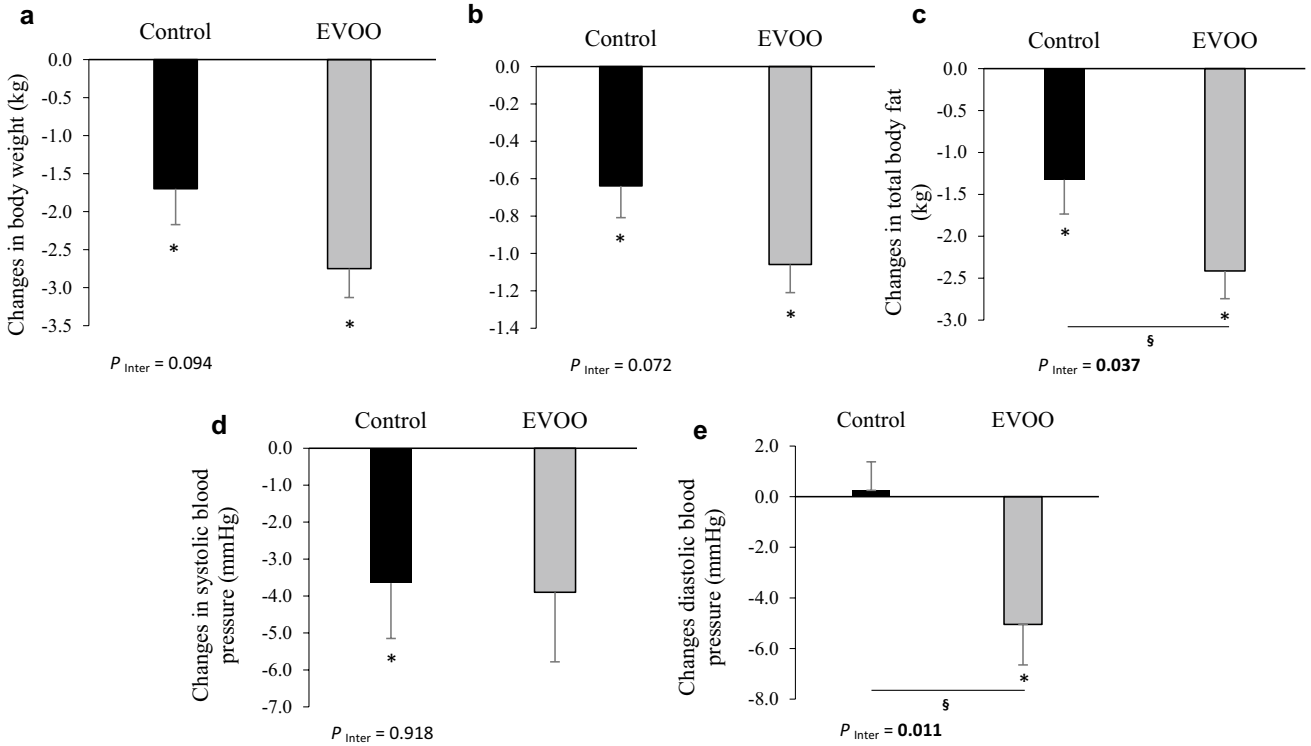

Fig. 2 Mean ± SE body weight (**a**), body mass index – BMI (**b**), total body fat (**c**), systolic blood pressure (**d**), and diastolic blood pressure (**e**) changes (Δ values = 9 week values – baseline values). Energy-restricted nutritionally balanced diets (−2090 kJ/d) containing 25 mL of soybean oil (control group, $n = 20$) or extra virgin olive oil – EVOO (EVOO group, $n = 21$) were prescribed. *Within-group significant differences (paired Student's *t* test or Wilcoxon signed-rank test, $P < 0.05$). $^{\S}P_{\text{Inter}}$ values indicate between groups differences (Student's *t* test or Mann–Whitney *U* signed-rank test, $P < 0.050$)

percentage was not affected in the control group, but there was an increase in EVOO group (0.68 ± 0.46% 95% CI −0.30 to 1.65 vs. 1.45 ± 0.36 95% CI 0.69–2.20; $P_{inter} = 0.195$).

Metabolic biomarkers

Serum glucose reduced in both groups after the intervention without a significant difference between groups ($P = 0.811$). Despite no between-group changes in metabolic biomarkers, HDL-c reduced and IL-10 increased only in the control group. On the other hand, EVOO was the only group in which creatinine increased and alkaline phosphatase reduced (Table 3). There as positive correlation between changes in total body fat and changes in alkaline phosphatase ($R^2 = 0.488$, $P = 0.005$) and negative correlation between changes in total body fat and changes in serum creatinine ($R^2 = -0.360$, $P = 0.021$).

Discussion

This study was design to assess the effects of EVOO incorporated into an energy-restricted non-Mediterranean diet program on body weight, body composition and metabolic biomarkers in women with excess body fat. The main finding of the present study is that the consumption of EVOO increases total fat loss and reduces diastolic blood pressure compared to the control soybean oil group. To the best of our knowledge, this paper provides the first clinical evidence that EVOO consumption increases body fat loss due to energy-restricted program even when not incorporated into a Mediterranean diet. Analysis of food consumption during the experiment demonstrated that our high-fat breakfasts significantly changed daily consumption of dietary fatty acids. EVOO group increased body fat loss, which could be considered independent of a greater caloric restriction in EVOO than control group once difference between groups in energy intake was not significant and insufficient to explain

Table 3 Metabolic biomarkers at baseline and change from baseline (95% CI) according to experimental groups

Metabolic biomarkers	Control ($n = 20$)			Extra virgin olive oil ($n = 21$)			P_{Inter}
	Baseline	Δ values	95% CI	Baseline	Δ values	95% CI	
Glucose (mmol/L)	4.76 ± 0.09	−0.13 ± 0.05	−0.23 to −0.02	4.86 (0.50)	−0.11 (0.39)	−0.37 to −0.04	0.811
Insulin (pmol/L)	7.90 (3.80)	3.82 (35.6)	−0.56 to 27.0	8.10 (4.20)	−4.31 ± 5.90	−16.7 to 8.20	0.060
HOMA−IR	1.61 (0.76)	0.08 (1.15)	−0.08 to 0.76	1.92 (1.14)	−0.19 ± 0.22	−0.64 to 0.26	0.054
Triglycerides (mmol/L)	0.98 ± 0.09	−0.02 (0.28)	−0.24 to 0.06	1.27 ± 0.13	−0.07 ± 0.07	−0.23 to 0.09	0.579
Total cholesterol (mmol/L)	4.26 ± 0.19	−0.14 ± 0.08	−0.30 to 0.02	4.45 ± 0.20	−0.20 ± 0.12	−0.44 to 0.05	0.671
HDL-c (mmol/L)	1.19 ± 0.06	−0.07 ± 0.03	−0.14 to −0.01	1.31 ± 0.07	−0.03 ± 0.03	−0.10 to 0.03	0.385
LDL-c (mmol/L)	2.42 ± 0.15	−0.06 ± 0.06	−0.18 to 0.06	2.52 ± 0.15	−0.04 ± 0.08	−0.21 to 0.12	0.832
Triglycerides/HDL−c	0.90 ± 0.12	0.00 ± 0.13	−0.26 to 0.27	0.79 (0.55)	0.10 ± 0.10	−0.11 to 0.31	0.548
Uric acid (μmol/L)	206 ± 7.73	2.38 ± 7.14	−11.9 to 16.7	209 ± 8.92	−2.97 ± 5.95	−14.9 to 9.52	0.579
Creatinine (μmol/L)	51.3 ± 0.88	−0.00 ± 1.77	−2.65 to 2.65	50.4 ± 1.77	3.54 ± 0.88	1.15 to 5.75	0.057
AP (IU/L)	61.1 ± 3.47	−1.68 ± 2.05	−5.98 to 2.62	63.7 ± 4.89	−3.26 ± 1.78	−7.00 to 0.47	0.564
Gamma GT (IU/L)	21.9 ± 0.60	0.11 ± 0.52	−0.98 to 1.20	19.1 ± 1.43	−0.24 ± 0.70	−1.72 to 1.25	0.691
AST (IU/L)	34.0 ± 1.56	−0.95 ± 2.09	−5.35 to 3.45	30.0 (14.0)	−0.24 ± 1.51	−3.40 to 2.92	0.782
ALT (IU/L)	16.0 (7.25)	−2.06 ± 1.06	−4.30 to 0.19	17.7 ± 1.84	0.16 ± 1.38	−2.74 to 3.06	0.219
IL-8 (pg/mL)	6.83 ± 0.51	0.61 ± 0.51	−0.48 to 1.69	8.07 ± 0.78	0.27 ± 0.70	−1.22 to 1.77	0.706
IL-1β (pg/mL)	0.98 ± 0.25	0.06 ± 0.23	−0.50 to 0.61	1.24 ± 0.29	−0.28 ± 0.14	−0.62 to 0.06	0.252
IL-6 (pg/mL)	1.74 ± 0.25	−0.03 ± 0.35	−0.80 to 0.73	1.76 ± 0.22	0.16 ± 0.26	−0.39 to 0.71	0.655
IL-10 (pg/mL)	0.86 ± 0.08	0.189 ± 0.08	0.01 to 0.37	1.11 ± 0.14	0.05 ± 0.09	−0.14 to 0.24	0.259
TNF- (pg/mL)	0.25 ± 0.10	0.00 (0.00)	−1.96 to 3.13	0.61 (0.45)	0.09 ± 0.29	−0.65 to 0.84	0.905
IL-12p70 (pg/mL)	2.08 ± 0.45	−0.14 ± 0.47	−1.17 to 0.88	2.15 ± 0.39	−0.10 ± 0.31	−0.77 to 0.56	0.942
IL-10/IL-6 (pg/mL)	2.15 ± 0.39	−0.39 ± 0.29	−1.01 to 0.25	1.63 ± 0.21	0.12 ± 0.28	−0.49 to 0.72	0.227

Values are mean ± SE or median (interquartile range)

HOMA-IR homeostasis model assessment of insulin resistance [36], *HDL-c* high-density lipoprotein cholesterol, *LDL-c* low-density lipoprotein cholesterol, *ALP* alkaline phosphatase, *AP* alkaline phosphatase, *Gamma GT* γ-glutamyltransferase, *AST* aspartate amino transferase, *ALT* alanine amino transferase, *IL-8* interleukin-8, *IL-1β* interleukin-1β, *IL-6* interleukin-6, *IL-10* interleukin-10, *TNF-α* tumor necrosis factor-α, *IL-12p70* interleukin-12p70, P_{Inter} between-group Δ values (9 week—baseline) are not significantly different (Student's *t* test or Mann–Whitney *U* signed-rank test, $P > 0.05$)

such increase [39]. Furthermore, our results show that while IL-10 increased only in the control group, HDL-c concentrations reduced in that same group. On the other hand, serum creatinine increased, alkaline phosphatase reduced, and there was a trend for IL-1β reduction in the EVOO group along the nine experimental weeks.

It has been widely suggested that the consumption of a Mediterranean diet rich in olive oil can prevent type 2 diabetes mellitus [40, 41] metabolic syndrome [40] and obesity [17, 40]. However, randomized clinical trials in which the effect of olive oil on body weight/fat was investigated are scarce and presented conflicting results [18–20, 42]. In a recent study [42] involving 7447 asymptomatic high-CVD risk individuals, daily consumption of 50 mL of EVOO for 4.8 years associated with an unrestricted-calorie, high-vegetable Mediterranean diet reduced body weight and promoted less central adiposity gain compared with the consumption of a low-fat diet. In our study, the daily consumption of energy-restricted normal-fat diet containing 25 mL of EVOO reduced total body fat compared to 25 mL/day of soybean oil. Additionally, to the aforementioned study, our findings support the prescription of EVOO not only for preventing weight gain, but also for promoting body weight/fat loss.

The current hypothesis that EVOO could improve body composition was mainly based in the effect of oleic acid (C18:1) on stearoyl-CoA desaturase 1 (SCD1) [11]. This enzyme catalyzes a key step in the endogenous biosynthesis of MUFA from saturated fatty acids. The preferential substrates for its action are palmitic acid and stearic acid, which are converted by SCD1 into palmitoleic acid and oleic acid, respectively [43]. The influence of increased SCD1 activity on obesity is supported by studies using mice with natural or SCD1-direct mutations. SCD1-deficient mice consume 25% more food but accumulate less fat and are considerably thinner than normal mice [44, 45]. In addition, SCD1-deficient animals consume more oxygen and have higher rates of β-oxidation in liver and fat tissue [46]. The lack of SCD1 also beneficially modulates the expression and activity of some genes related to adiposity [47]. According to this hypothesis, SCD1 activity is regulated by the amount of substrate and final product. Thus, while consumption of the saturated fatty acids palmitic and stearic acid acts as substrate stimulating SCD1 action and favoring obesity, oleic acid down regulates SCD1 activity favoring weight loss [11]. The effect of EVOO consumption on SCD1 expression and activity must be investigated in metagenomic studies.

In our study, EVOO significantly reduced (~5 mmHg) diastolic blood pressure compared to the control (soybean oil). Soybean oil could be considered a good control for assessing blood pressure due to its little effect on that variable [48]. Therefore, despite the differences in baseline values observed in diastolic blood pressure, our results suggest that EVOO contribute to hypertension control. The role of EVOO in reducing blood pressure is supported by a growing body of scientific evidence [46–49]. Despite the fact that minor components characteristic of olive oil could contribute to the cardioprotective activity of EVOO, such as a-tocopherol, polyphenols, and other phenolic compounds, Terés et al. [49] demonstrated that its high oleic acid content is responsible for the antihypertensive effects of olive oil consumption. This effect is likely to be attributed to the incorporation of oleic acid into cell membranes, which regulates membrane lipid structure in such a way as to control G protein-mediated signaling, causing a reduction in blood pressure [49].

There is still no consensus about the role of EVOO on dyslipidemia. While some studies reported beneficial increase in HDL-c [49, 50] and reduction in LDL-c [51], others showed no significant changes in lipid profile [47, 52–55]. In our study, EVOO presented cholesterol-neutral effect, since HDL-c reduced in the control group at the end of the experiment. Our results corroborated with those reported by [56], in which there was a decrease in HDL-c concentrations after the consumption of ~50 g of soybean oil and maintenance of HDL-c in response to the consumption of similar amount of olive oil. The authors attributed the reduction to soybean oil linoleic acid high content and the maintenance of HDL-c to the competition between olive oil chylomicron remnants and HDL for hepatic lipase [56]. Thus, olive oil could prevent HDL-c postprandial decrease, and maybe contribute for a more favorable lipid profile.

We observed a significant, but no clinically relevant increase in serum creatinine in the EVOO group. This was an unexpected result since creatinine was assessed as a biomarker of renal function, and we expected that EVOO could protect kidneys from obese lipotoxicity [57]. However, we believe that the increase in serum creatinine was a reflect of lean mass preservation during the study since creatinine is a lean mass content marker and EVOO was the only group in which lean mass percentage increased at the end of the experiment. On the other hand, there was a reduction in alkaline phosphatase in EVOO. Despite the fact that alkaline phosphatase is not specific from liver, data from animal studies provide some evidences that polyphenols from olive oil could improve liver function by reducing lipid peroxidation in this tissue [58, 59]. Thus, the slight reduction in that enzyme may reflect and improve in liver function. This result deserves to be confirmed in individuals with non-alcoholic fatty liver disease.

In our study, there was a significant increase in IL-10 in the control group. Soybean oil was provided to the control group to match fat consumption between groups, but was responsible for an increased consumption of α-linolenic acid (C18:3) in that group. Increased consumption of α-linolenic acid can down-regulate inflammatory pathways and reduce plasma levels of IL-10 [60]. In turn, EVOO

showed a trend for IL-1β reduction. A very similar effect of olive oil was observed in another study conducted by Kremer et al. [61]. In that study, the effect of fish oil vs. olive oil (placebo) on active human rheumatoid arthritis was investigated. Olive oil consumption led to unexpected beneficial effects on the improvement of clinical aspects of the disease. These benefits were associated with decreased macrophage IL-1 production although not to the same extent as the fish oil group [61]. As IL-1β has potent and vast pro-inflammatory effect over a number of cells, including macrophages, monocytes, and dendritic cells [62], the role of EVOO on IL-1β deserves to be further explored.

Our study has several strengths, including the rigorous subjects' eligibility criteria, the use of DXA for body composition assessments, use of double blind protocol, double digitation of data, controlled breakfasts consumption, and evaluation of diet compliance. However, the study also has limitations. This study showed a relatively high attrition rate due to secondary reasons not related to the study protocol. Despite the fact that we selected woman with very high body fat content (~48% at baseline), they were also young and it is possible that we were not able to detect the influence of dietary treatment in some metabolic biomarkers (e.g., some cytokines which were not detected). Furthermore, women are more prone to present changes in anthropometric parameters and body compositions due to menstrual cycle. Despite our efforts to reduce the influence of water retention, we cannot assure that our results were not affected by participant hormonal fluctuations. Finally, the interference of EVOO higher diastolic blood pressure at baseline in our results cannot be totally neglected.

Conclusion

Daily consumption of 25 mL of extra virgin oil (EVOO) associated with an energy-restricted Western-diet increased body fat loss and reduced blood pressure. The beneficial effects of EVOO were independent of an increase in caloric restriction, indicating a positive direct role of this oil on adiposity. EVOO also increased serum creatinine, reduced hepatic alkaline phosphatase, and tended to reduce IL-1β concentrations. The intriguing impact of EVOO on SCD1 expression and activity must be better explored in metagenomic studies.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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