Clinical Nutrition ESPEN 71 (2026) 102883

Contents lists available at ScienceDirect

Clinical Nutrition ESPEN

journal homepage: http://www.clinicalnutritionespen.com

Randomized Controlled Trial

The impact of olive oil polyphenol supplementation on metabolic syndrome parameters The OleoMetS study: A randomized, controlled clinical trial

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ARTICLE INFO

Article history:
Received 16 November 2025
Accepted 15 December 2025

Keywords:
Oleocanthal
Oleacein
Metabolic syndrome

SUMMARY

Background & aims: Olive oil aldehydic phenols (OOPs), including oleocanthal, oleacein, oleuropein aglycone, and ligstroside aglycone, are highly bioactive secoiridoids with unique health-protective properties. Preclinical investigations have demonstrated that OOPs possess anti-inflammatory and antioxidant attributes. The aim of the study was to assess the direct health properties of OOPs as a food supplement in humans diagnosed with metabolic syndrome. Fasting Blood Glucose (FBG) and Hemoglobin A1c (HbA1c) were chosen as primary endpoints because they are key indicators of glucose control, a core aspect of metabolic syndrome. Their combined assessment offers a comprehensive view of both short-term and long-term glucose regulation, making them highly relevant for evaluating interventions.

Methods: This double-blind, randomized, controlled clinical trial screened 110 individuals for eligibility between September 16, 2024, and January 27, 2025. Eight did not meet the inclusion criteria for metabolic syndrome, and 102 eligible participants were randomly assigned to the OOPs supplement or placebo group using a computer-generated randomization sequence to receive either the OOPs supplement or placebo. The 12-week intervention provided either a 10 mg/day polyphenol-rich olive oil extract food supplement (80 % oleocanthal/oleacein, 18 % oleuropein aglycon/ligstroside aglycon) or a placebo. No changes to lifestyle were recommended, and diet and physical activity were assessed at week 6. Fasting blood glucose (FBG) and hemoglobin A1C (HbA1c) were the primary outcomes. Secondary outcomes included lipid profile, C-reactive protein (CRP), body mass index (BMI), blood pressure, waist circumference, liver function tests (LFTs), estimated glomerular filtration rate (eGFR), uric acid, and fatigue scores. Repeated measures ANOVA/Linear Mixed Models were used to compare the mean changes (12 weeks-baseline) in fasting blood glucose levels between the intervention group and the placebo arm. Similar analysis (changes since baseline) was conducted for the continuous secondary outcomes. For categorical outcomes, changes since baseline were compared using chi-square methods. Bonferroni adjustments were made for multiple comparisons as appropriate.

Results: All 102 randomized participants completed the 12-week intervention and were included in the final analysis. The intervention group demonstrated significant improvements across various metabolic and physiological markers compared to the placebo group. Notable reductions were observed in FBG (mean difference: −7.06 mg/dL, $p < 0.0001$) and HbA1c (−0.29 %, $p < 0.0001$). Additionally, the intervention led to significant decreases in BMI (−1.15, $p < 0.0001$), systolic blood pressure (−7.66 mmHg, $p = 0.004$), triglycerides (−8.57, $p = 0.0003$), oxidized LDL (−5.01, $p < 0.0001$), uric acid (−1.04,

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https://doi.org/10.1016/j.clnesp.2025.102883

$p < 0.0001$), ALT (−4.92, $p = 0.0002$), and fatigue scores (−16.88, $p < 0.0001$). A significant increase in eGFR was also noted (+3.38, $p = 0.0002$).

Conclusion: Supplementation with OOPs shows promise in improving key metabolic markers in patients with MetS. The intervention was well-tolerated, with no serious adverse events reported. Further studies with longer intervention periods, follow-ups, and even higher OOPs dosages are needed to assess the long-term durability and magnitude of the effect.

Trial registration: ClinicalTrials.gov Identifier: NCT07144488.

1. Introduction and background

Metabolic syndrome (MetS) is a cluster of interrelated metabolic abnormalities—central obesity, dyslipidemia, hypertension, and insulin resistance—that significantly elevate the risk of cardiovascular disease (CVD), stroke, and type 2 diabetes mellitus (T2DM). The prevalence of MetS is rising globally due to increasing rates of obesity and sedentary lifestyles. Individuals with MetS are up to five times more likely to develop T2DM and have approximately double the risk of cardiovascular events compared to those without MetS.

Habitual olive oil consumption offers significant heart-protective benefits, as firstly evidenced by the Seven Countries Study in the 1960s [1]. This study revealed that the mortality rate from coronary heart diseases in the Mediterranean region was two to three times lower compared to North Europe and the USA. This finding was consistently observed in subsequent research [2,3]. Furthermore, a large U.S. cohort study linked higher olive oil consumption with fewer cardiovascular disease events, lower cardiovascular and all-cause mortality, and significantly reduced stroke risks [4]. The underlying pathophysiology of MetS includes chronic low-grade inflammation, endothelial dysfunction, and increased oxidative stress, all of which contribute to accelerated atherosclerosis. Dietary polyphenols have emerged as potential modulators of these processes. In particular, extra virgin olive oil-derived polyphenols such as oleocanthal and oleacein have demonstrated strong anti-inflammatory effects through cyclooxygenase (COX) inhibition [5], as well as several other beneficial properties [6], while oleacein, oleuropein aglycon and ligstroside aglycon have shown antioxidant and endothelial-protective properties as well as properties related to obesity regulation, cancer and neurodegeneration prevention, and reducing the risk of atherosclerosis in preclinical studies [7–9].

Since 2012, EFSA has officially recognized the protective role of olive oil's phenolic compounds against blood lipid oxidation [10], which has been accepted as a permitted health claim by the European Commission [11]. Clinical trials conducted by members of our group have demonstrated the benefits of olive oil with high OOPs content in conditions such as chronic lymphocytic leukemia [12], mild cognitive impairment [13,14], oxidative stress [15], and platelet aggregation [16,17]. Previous independent clinical studies have also shown that olive oils with high phenolic content exhibit significantly stronger activity compared to those with low phenolic content in relation to cholesterol, ox-LDL, blood glucose, blood pressure, and various other parameters associated with metabolic syndrome [18–22].

Until now, the attribution of the observed activities in humans to phenolic ingredients has been based indirectly on comparisons between the activity of high-phenolic and low-phenolic olive oils, because the large-scale isolation of OOPs presented technical difficulties that prevented their use in nutritional supplements. In this study, we describe a novel method for the isolation and formulation of OOPs and evaluate, for the first time in humans with metabolic syndrome, their direct health effects as a food supplement.

Given the established role of oxidative stress and inflammation in promoting insulin resistance, central obesity, and dyslipidemia—the core components of metabolic syndrome as defined by the IDF—olive oil polyphenols may exert beneficial metabolic effects through their antioxidant and anti-inflammatory actions. Therefore, we aimed to evaluate the impact of olive oil aldehydic phenol (OOP) supplementation on metabolic and inflammatory parameters in individuals with MetS, hypothesizing that OOP supplementation would improve glycemic and lipid profiles compared with placebo [23].

2. Patients and methods

2.1. Study design, randomization and participants

This 12-week interventional trial was a randomized, single-center, double-blind, two-arm parallel group study performed between 16/09/2024 and 27/01/2025. This trial is registered with ClinicalTrials.gov, NCT07144488. Participants were recruited from Apostolos Loukas Medical Centre - the largest outpatient medical centre in Cyprus serving more than 30,000 patients, with academic oversight and protocol monitoring provided by the University of Nicosia Medical School. The Ethics Committee of Cyprus approved the clinical study protocol. It was designed to assess the effects of OOPs as a food supplement on various metabolic syndrome parameters. No changes to lifestyle were recommended, and diet and physical activity were assessed at week 6.

In this study we employed the Pyramid based Mediterranean Diet Score (PyrMDS) questionnaire [24] to assess participants' adherence before, during and after the end of the study to the main components of the Mediterranean diet and their physical activity levels. The PyrMDS is a structured, validated tool that quantifies the frequency of consumption of core dietary elements including vegetables, fruits, whole grains, fish or seafood and subsequently determines the primary source of dietary fat, with an emphasis on olive oil use. Furthermore, it incorporates a Physical Activity Questionnaire [25] that classifies individuals as active or inactive, contributing to an overall score that reflects the combined quality of diet and lifestyle. This integrated approach facilitates robust investigation of the effects of olive oil polyphenol supplementation on key parameters of metabolic syndrome. In addition, we utilized a structured Energy Level Questionnaire [26] to assess participants' perceived levels of fatigue and activity. This questionnaire was used to capture multiple aspects of fatigue including general, physical, and mental fatigue as well as reduced activity, through self-reported items rated on a graded scale. Participants scored the extent to which they experienced tiredness, exhaustion, lack of energy, difficulties with concentration and memory and limitations in initiating or completing daily activities. In doing so, a comprehensive profile of subjective energy levels was collected, which can be correlated with dietary interventions to evaluate potential effects of OOPs supplementation on fatigue and activity-related outcomes.

Eligible participants were consented, screened, and randomized to either the arm receiving a 12-week OOPs food supplement or the exact similar placebo capsule. Participants randomized to the intervention arm were prescribed a daily dose of 2 capsules, containing 5 mg each (10 mg total daily dose) of polyphenols, namely oleocanthal/oleacein (80 %) and oleuropein aglycon/ligstroside aglycon (18 %). Participants randomized to the control arm were prescribed a daily dose of 2 placebo capsules. All participants followed routine care at the medical center. At enrollment, all participants received the same guidance on dietary intervention, medication regimen and physical activity. At baseline, demographic characteristics (including ethnicity, sex, age, level of education) and family history of metabolic syndrome, were collected via an interview by a study physician. We recruited individuals (30–70 years old) with a current diagnosis of metabolic syndrome based on the International Diabetes Federation (IDF) criteria. According to the International Diabetes Federation (IDF) definition, metabolic syndrome was diagnosed by the presence of central obesity, defined by ethnicity-specific waist circumference thresholds ($\geq$94 cm in men and $\geq$80 cm in women for Europid populations), together with at least two of the following: elevated triglycerides ($\geq$150 mg/dL or treatment), reduced HDL cholesterol (<40 mg/dL in men or <50 mg/dL in women or treatment), elevated blood pressure ($\geq$130/85 mmHg or antihypertensive treatment), or elevated fasting plasma glucose ($\geq$100 mg/dL) or previously diagnosed type 2 diabetes [27]. The following outcomes were recorded at baseline and 12 weeks: Biochemical indicators via fasting blood tests: lipids, CRP, fasting glucose, HbA1c, lipid profile, Uric acid, LFTs, RFTs; smoking status; BMI; Systolic and Diastolic BP; waist-to-hip (WHR) ratio; nutritional habits (diet high in saturated fat and carbohydrates); physical activity diary. An electronic questionnaire on physical activity was sent monthly in order to capture any changes in exercise that could contribute to the changes in the participants' study outcomes. Telephone call reminders were used monthly. Serious adverse events were assessed throughout the trial by the principal investigator (GS). All data were entered on a secure, password-protected website by trained and approved clinicians. The study's data system, including screening and randomization processes, and data capture was developed by a contractor and validated in collaboration with the study biostatistician (TCK). Access to the accumulated data was restricted to the biostatistician (TCK) until the study was completed and all data were cleaned and the database closed for analysis. All statistical analyses were conducted using SAS v9.4. Data entry quality was ensured through real-time range checks and automated data validation algorithms. Any discrepancies were resolved through source data verification and review by the principal investigator. Trial integrity was safeguarded by routine monitoring of protocol adherence and data completeness.

All clinical and biochemical measurements were performed using standardized methods. Fasting blood glucose and HbA1c were analyzed using automated enzymatic assays. Lipid profile (TC, HDL, LDL, TG) and CRP were measured via enzymatic colorimetric assays using the same platform. Liver and renal function tests (ALT, AST, creatinine, eGFR) were conducted with validated spectrophotometric methods following manufacturer instructions in an accredited laboratory. Blood pressure was measured in triplicate using a calibrated Omron M6 Comfort digital sphygmomanometer after a 5-min rest in a seated position, and BMI was calculated as weight (kg)/height (m^2). All laboratory analyses were performed at the Apostolos Loukas Medical Centre Clinical Laboratory, which participates in external quality assurance programs to ensure accuracy and reproducibility.

The CONSORT flow diagram of the OleoMetS study is presented in Fig. 1. Participants and researchers were blinded to the assigned arm in this study, randomly assigned to the OOPs supplement or placebo group using a computer-generated randomization sequence. Both the intervention and placebo capsules were designed to have an identical appearance and taste.

2.2. Primary and secondary endpoints

We assessed key health indicators including FBG and HbA1c as primary outcomes, and HDL, LDL, TC, TG, CRP, BMI, BP, abdominal obesity, LFTs, RFTs, uric acid, and fatigue scores as secondary outcomes. Assessments were done at three points: at baseline/randomization, at 6 weeks (diet and physical activity), and at 12 weeks post intervention.

2.2.1. Characterization of OOPs supplement, extraction of olive oil and formulation

The target compounds that were selectively extracted from the olive oil are depicted in Fig. 2. They include mainly the olive oil aldehydic phenols (OOPs) such as oleocanthal, oleacein, oleuropein aglycone, and ligstroside aglycone which are highly bioactive secoiridoids with unique health-protective properties and minor simple phenolics like hydroxytyrosol and tyrosol.

The identity of the compounds extracted from olive oil was confirmed using NMR spectroscopy with a Bruker Avance 400 MHz. The 1H NMR spectrum of the OOPs in CDCl3 is presented in Fig. 3.

The 1H NMR spectra were processed using MNova 6.0.2 (Mestrelab Research). The quantitation of the OOPs in the olive oil that was used as starting material as well as in the obtained extracts and final formulated products was performed using qNMR as previously described [28].

Olive oil produced at OMPHAX SA from a mix of olives of Kalamon and Olympia variety, specifically selected to contain oleocanthal, oleacein, oleuropein aglycon and ligstroside aglycon at high concentration (>1 g/kg), was vigorously mixed with PEG400 and subsequently the mixture was left to stand for 10 days in a conical tank. The two layers were separated by gravity and the heavier layer of PEG400 was collected, filtered to remove any insoluble particles and used for formulation [29].

The active capsules (Thousand Olives ®) were formulated by mixing the olive oil extract in PEG400 with cellulose, silicon dioxide and magnesium stearate. The placebo capsules were prepared using only PEG400, cellulose, silicon dioxide and magnesium stearate and had the same weight and appearance

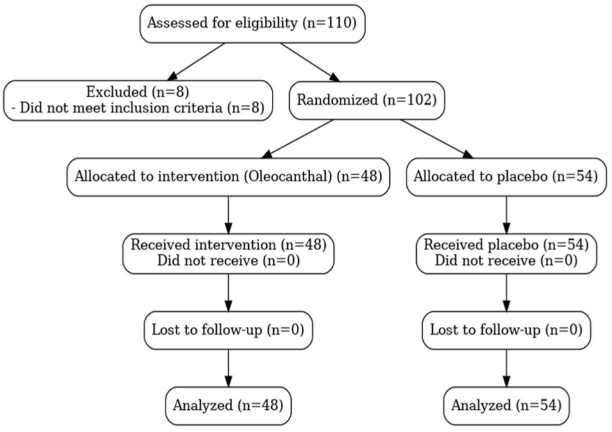

Fig. 1. Flow diagram of the OleoMetS study.

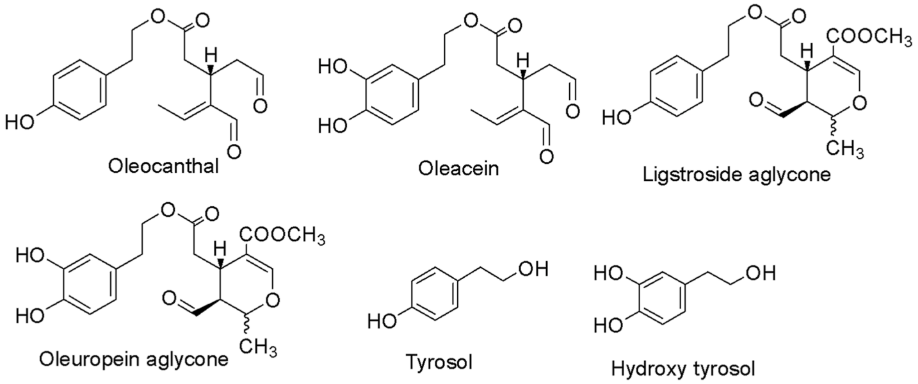

Fig. 2. Structures of secoiridoids/phenolics selectively extracted from olive oil.

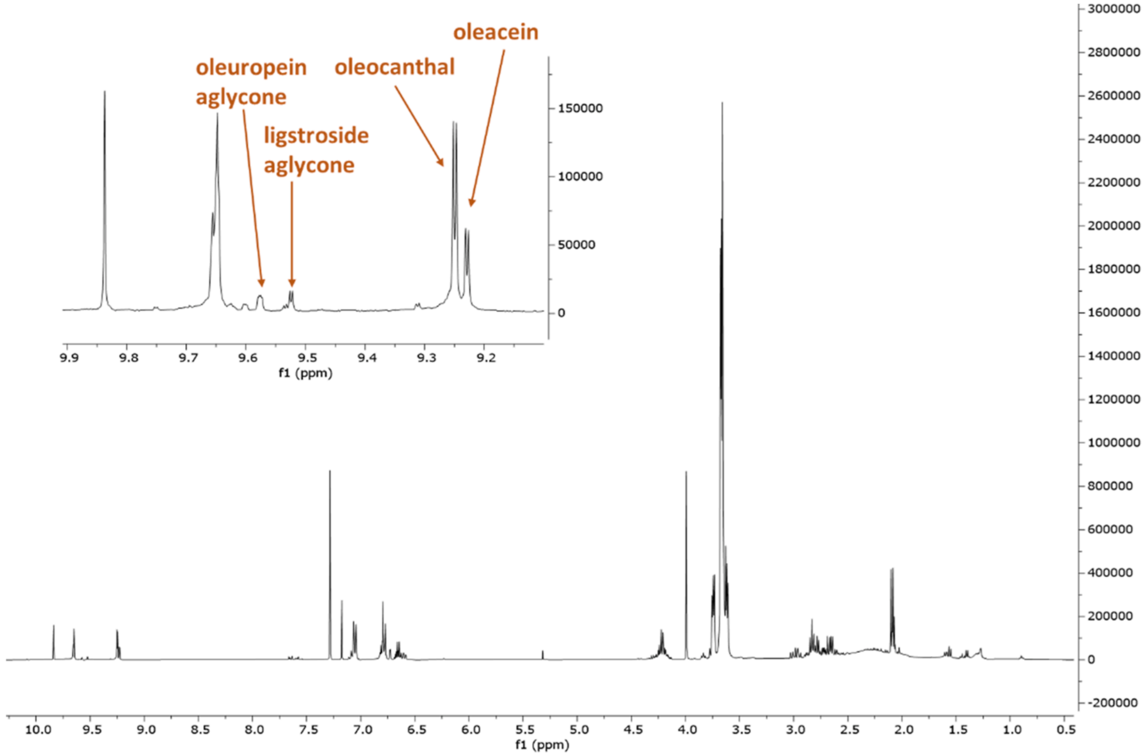

Fig. 3. NMR profile of the Oleoprotect extract diluted in $CDCl_3$ showing that it contains only the secoiridoid phenolics.

with the active ones. The exact content of each OOPs in the capsule is described in Fig. 4 and was measured by qNMR.

The OOP extract, as shown in the NMR spectrum in Fig. 3, contains only the compounds described (oleocanthal, oleacein, oleuropein aglycon, ligstroside aglycon, and tyrosol). According to qNMR measurements, the total OOP content in each capsule is 5 mg, corresponding to the minimum daily dose proposed by EFSA for 20 g of olive oil. Administration of two capsules per day provides 10 mg of OOPs, an amount equivalent to that found in approximately three tablespoons of olive oil. Considering the long-established safety of olive oil consumption, the EFSA-recommended daily intake, and the equivalence of OOPs in the capsules to that obtainable through a typical diet of high-quality olive oil, the product is considered safe for human use.

The extraction methodology was developed by the authors (Panagiotis Diamantakos, Eleni Melliou, and Prokopios Magiatis). The OOPs extract was prepared by OMPHAX SA, and the final encapsulated product was manufactured by OnePharma SA, as registered with the Greek National Organization for Medicines (EOF). Quality control was performed on both the extract and the final formulated product using quantitative NMR (qNMR) for the measurement of olive oil polyphenols (OOPs). The quality control process also included microbial contamination testing and heavy metal residue analysis, all of which confirmed the product's safety and conformity with specifications. The stability testing of the supplement was performed as described in the Methods section, demonstrating stability throughout the study period. No stability testing was conducted for the placebo, as it contained only excipients (PEG400, cellulose, silicon dioxide, and magnesium stearate), which are chemically stable under identical storage conditions.

To ensure consistency and product integrity, the formulated OOPs supplement underwent stability testing under various storage conditions. Samples were stored for 3 months at 40 °C and 75 %

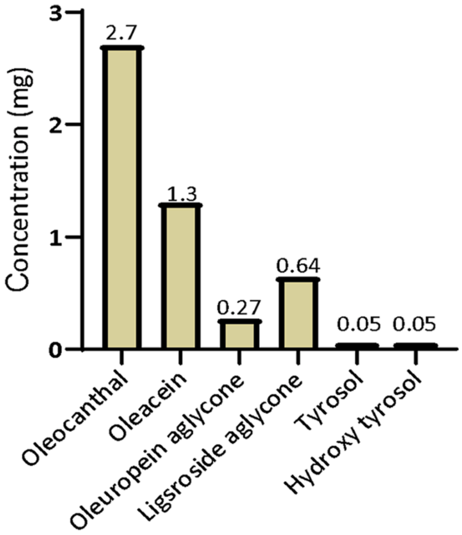

Fig. 4. Secoiridoid phenolics in Thousand Olives ®: mg per capsule.

relative humidity (RH), 30 °C and 65 % RH, and 25 °C and 60 % RH. In all cases, the formulation retained >90 % of the initially quantified OOPs content, indicating excellent short-term stability even under accelerated conditions.

Long-term monitoring was also performed at room temperature for 30 months, during which the capsules consistently retained >90 % of their OOPs content, confirming the durability of the formulation.

Furthermore, analyses of multiple independently manufactured batches demonstrated <5 % variability in total OOPs concentration across batches, confirming the reproducibility and high integrity of the product throughout the production process.

2.2.2. Ethics

The protocol was approved by the National Bioethics Committee of Cyprus file number ΕΕΒΚ/ΕΠ/2024/39. All participants signed the informed consent upon explanation of all the objectives and methodology of the trial. The OleoMetS study was conducted according to the recommendations of the Helsinki Declaration and Good Clinical Practice Guidelines of the International Council for Harmonization (CPMP/ICH/135/95).

2.3. Statistical analysis

The sample size was determined a priori based on the primary endpoint, fasting plasma glucose (FBG). The study was designed to detect a mean between-group difference (Δ) of 10 mg/dL in the change in FBG from baseline to week 12. This magnitude of change was selected because it is considered clinically meaningful and relevant in the context of metabolic syndrome and impaired glucose regulation. An estimated standard deviation (σ) of 15 mg/dL for the change in FBG was assumed based on prior clinical studies and pilot data from high-phenolic olive oil interventions.

Using these parameters, a two-sided significance level (Type I error $\alpha = 0.05$) and a desired statistical power of 90 %, the minimum required sample size was calculated to be 47 participants per group. To compensate for an anticipated 10 % rate of attrition or loss to follow-up, the final recruitment target was increased to 53 participants per arm, yielding a planned total enrollment of 106 participants. A total of 102 participants were ultimately randomized (control $n = 54$; intervention $n = 48$). Even with this slightly lower enrollment, the achieved sample size remained sufficient to preserve statistical power exceeding 90 % for detecting the predefined effect size in FBG change. Post-hoc power verification confirmed that the observed effect size was well within the detectable range for the final sample.

All analyses followed the intention-to-treat principle. For the primary outcomes (FBG and HbA1c), Repeated Measures ANCOVA/Linear Mixed Models were employed to compare mean changes from baseline between groups across study visits. Models were first run unadjusted, and subsequently adjusted for prespecified covariates (age, sex, and baseline BMI) to account for potential confounding effects. Between-subjects comparisons from these models demonstrated statistically significant treatment effects for both primary outcomes, and significance was maintained after covariate adjustment (Table 1).

For the primary outcome of FBG, a comparison of the mean changes since baseline (week 12 minus baseline) was conducted using repeated measures methods. For each participant, the difference between the 12 week and baseline fasting blood glucose levels was calculated. The mean difference in the primary outcome between the intervention groups was compared to those of the placebo arm using repeated measures ANOVA/Linear Mixed Models. Similar analysis (12 week change since baseline) was conducted for HbA1c and the continuous secondary outcomes. For categorical outcomes, changes from baseline to 12 weeks were compared using chi-square methods (Table 2).

3. Results

3.1. Recruitment, baseline characteristics and compliance

Between September 2024 and December 2024, 110 individuals were assessed for eligibility (see Table 2). Eight did not meet the inclusion criteria for metabolic syndrome and were excluded. The remaining 102 eligible participants were randomly assigned to the OOPs supplement or placebo group using a computer-generated randomization sequence. All randomized participants completed the 12-week intervention and were included in the final analysis. The key demographic and baseline characteristics of the randomized participants are summarized in Table 3. A key strength of the trial was the absence of any participant dropouts throughout its duration, ensuring the integrity of the collected data. Throughout the 12-week intervention, no adverse effects or safety concerns were reported in either study arm. The OOPs supplement was well tolerated, and no participant discontinued the intervention due to side effects.

Upon enrollment, a comprehensive assessment of baseline characteristics was performed for all participants. As presented in detail in Table 3, these characteristics were found to be consistent across both study arms, indicating successful randomization and minimizing the potential for confounding variables. Furthermore, a thorough analysis revealed no significant differences in the baseline characteristics of participants when considering the sequence of intervention, further reinforcing the robustness of the

Table 1
Adjusted linear mixed-model results for primary outcomes (FBG and HbA1c).

Outcome	Variable	Unadjusted	Adjusted
FBG	Treatment Group	**0.007**	**0.0065**
	Age	N/A	0.0235
	Sex	N/A	0.4990
	Baseline BMI	N/A	0.0024
HbA1C	Treatment Group	**0.0273**	**0.0318**
	Age	N/A	0.0440
	Sex	N/A	0.5590
	Baseline BMI	N/A	<0.0001

Note: Models adjusted for age, sex, and baseline BMI.

Table 2
Between-group differences in fasting blood glucose change (week 12 – baseline), with effect size and post-hoc power.

Group	n	Mean FBG Change (12wk-baseline)	SD	Cohen's d	Effect size	Power
Control	54	0.5463	4.08			
Oleocanthal	48	−6.52	7.60			
				−1.16	−0.5	>90 %*

Note: Power estimated with a two-sample t-test (unequal variances) using PASS 2022.

Table 3
Baseline demographic and clinical characteristics of randomized participants (n = 102).

	Control (n = 54)*	Intervention (n = 48)*	Total (n = 102)*
Gender			
Female	21 (39 %)	15 (31 %)	36 (35 %)
Male	33 (61 %)	33 (69 %)	66 (65 %)
Education Level			
Middle School	5 (9 %)	6 (13 %)	11 (11 %)
High School	22 (41 %)	18 (38 %)	40 (39 %)
University/College	15 (28 %)	17 (35 %)	32 (31 %)
Graduate School (Masters)	11 (20 %)	5 (10 %)	16 (16 %)
Graduate School (PhD)	1 (2 %)	2 (4 %)	3 (3 %)
Ethnicity			
Greek Cypriot	51 (94 %)	48 (100 %)	99 (97 %)
Turkish Cypriot	1 (2 %)	0 (0 %)	1 (1 %)
Other European	2 (4 %)	0 (0 %)	2 (2 %)
Family History of MetS			
No	26 (48 %)	23 (48 %)	49 (48 %)
Yes	28 (52 %)	25 (52 %)	53 (52 %)
Currently Smoking			
No	33 (61 %)	34 (71 %)	67 (66 %)
Yes	21 (39 %)	14 (29 %)	35 (34 %)
Current Diagnosis of Fatty Liver			
No	35 (65 %)	23 (48 %)	58 (57 %)
Yes	19 (35 %)	25 (52 %)	44 (43 %)
Age (in years)	58 (11.5)	55.1 (11.4)	56.6 (11.5)
Baseline BMI	31.3 (4.3)	32.3 (5.8)	31.8 (5.1)
Blood Pressure in mm Hg			
Diastolic	89.9 (11.5)	90.5 (11.2)	90.2 (11.3)
Systolic	140.1 (13.7)	139.6 (14.0)	139.9 (13.8)
Waist-Hip-Ratio	1.1 (0.3)	1.0 (0.2)	1.09 (0.29)
ELQ Scores			11.3 (3.8)
Overall Score	79.5 (17.8)	78.4 (15.6)	79.0 (16.7)
General Fatigue Score	11.4 (4.0)	11.3 (3.7)	11.3 (3.8)
Physical Fatigue Score	12.2 (4.9)	12.0 (4.5)	12.1 (4.7)
Mental Fatigue Score	10.9 (4.7)	10.6 (4.6)	10.8 (4.6)
Reduced Activity Score	10.8 (4.3)	10.7 (4.1)	10.7 (4.2)
Reduced Motivation Score	10.5 (4.6)	9.8 (4.1)	10.2 (4.4)
Well-Being Score	12.2 (3.9)	12.2 (4.1)	12.2 (4.0)
Sleep Quality Score	11.6 (2.0)	11.7 (2.3)	11.6 (2.2)
Adherence to Mediterranean Diet	3.7 (1.4)	3.3 (1.3)	3.5 (1.4)

Note: Total n = 102 reflects all randomized participants (110 screened, 8 excluded).

study design. Although fatty liver was more frequent in the intervention group (52 %) than in the control group (35 %), this difference was not statistically significant (χ^2 test, $p = 0.11$). Baseline comparisons further demonstrated no significant differences between groups for BMI, systolic or diastolic blood pressure, whereas fasting blood glucose and HbA1c levels were significantly higher in the intervention group at baseline ($p = 0.001$ and $p = 0.002$, respectively).

3.2. Primary and secondary outcomes

There was a statistically significant difference in the primary outcome (Fasting Blood Glucose; FBG) between the two study arms. In the intervention arm there was a mean reduction (12 weeks -baseline) in FBG of 6.5 mg/dL (95 % CI mean −4.3, −8.7); in the control arm the change of 0.5 mg/dL (12 weeks-baseline) was not statistically significant (95 % CI mean: −0.6, 1.7). The difference in the mean change between the two study arms, 7.06 mg/dL, was statistically significant (95 % CI mean: 4.6, 9.5; $p < 0.0001$) at the 0.05 Type I error, as well as an adjusted 0.025 Type I error (to allow for testing of HbA1c as a secondary primary outcome). Likewise, the difference in the mean HbA1c change (12 weeks-baseline) between the two study arms was 0.29 and was statistically significant at the 0.025 alpha level (adjusted for multiple comparisons when testing for HbA1c as a second primary outcome). (95 % CI; 0.19,0.39; $p < 0.0001$).

No significant inter- or intra-treatment changes in adherence to the Mediterranean diet and physical activity were observed among participants in both arms, suggesting, though not definitively proving, the absence of contamination in our samples. Therefore, detailed 6-week assessment data for diet and physical activity were not included in the main results presentation.

Comparison of changes in primary and secondary outcomes, both continuous and categorical, are reported in Table 4.

In addition to improvements in primary glycemic parameters, the intervention group showed clinically and statistically significant

Table 4
Comparison of changes in Primary and Secondary Outcomes.

Outcome	Control mean change	Intervention mean change	Intervention mean change-Control mean change	95 % CI	p[a]
Primary					
FBG	0.55	−6.52	7.07	4.61,9.53	<.0001[b]
HbA1c	−0.14	−0.30	0.29	0.19,0.39	<.0001[c]
Secondary					
BMI	0.066	−1.09	1.15	0.81, 1.50	*<.0001*
WHR	−0.02	0.02	0.00	−0.05, 0.06	0.93
BP					
Diastolic	1.20	−4.85	6.06	1.18, 10.93	*0.015*
Systolic	0.43	−7.23	7.66	2.49, 12.81	*0.004*
Cholesterol	−1.67	−12.0	10.33	4.44, 16.22	*0.0007*
HDL	−0.07	−0.26	0.19	−0.75, 1.12	0.7
LDL	−0.78	−6.05	5.27	0.94,9.60	*0.018*
Oxidized LDL	1.06	−3.96	5.01	3.21,6.81	*<.0001*
Triglycerides	−1.22	−9.79	8.57	4.02, 13.11	*0.0003*
CRP	0.04	0.02	0.02	−1.25, 1.30	0.98
Uric Acid	0.70	−0.34	1.04	0.67, 1.41	*<.0001*
Creatinine	0.01	−2.07	2.08	−2.00, 6.20	0.31
ALT	1.05	−3.90	4.92	2.45, 7.39	*0.0002*
AST	0.85	−0.64	1.49	−0.39, 3.37	0.12
eGFR	−0.28	3.10	−3.38	−5.11,-1.65	*0.0002*
PyrMD Score[d]	0.07	0.46	−0.38	−1.04, 0.27	0.25
PyrMD Score[e]	0.12	0.54	−0.42	−1.10,0.24	0.21
ELQ Overall Score	6.94	−9.94	16.88	10.86,22.91	*<.0001*

Note: For secondary outcomes, the adjusted Type I error to account for multiple testing is 0.05/18 = 0.0028. p-values presented in italics are statistically significant at the adjusted Type I error.

[a] From paired t-test (3 month-baseline).

[b] From Repeated Measures ANOVA GLM model: p = 0.007 for between subcts effects.

[c] From Repeated Measures ANOVA GLM model: p = 0.0273 for between suects effects.

[d] PyrMD (6 week-baseline).

[e] PyrMD (12 week-baseline).

improvements in several secondary outcomes compared to the control group. Notably, participants receiving the OOPs supplement experienced a greater reduction in body mass index (mean difference: −1.15, $p < 0.0001$) and systolic blood pressure (−7.66 mmHg, $p = 0.004$). Significant improvements were also observed in oxidized LDL (−5.01, $p < 0.0001$), triglycerides (−8.57, $p = 0.0003$), and uric acid (−1.04, $p < 0.0001$), indicating favorable changes in lipid metabolism and inflammation. Liver function, particularly ALT, also improved (−4.92, $p = 0.0002$), while eGFR increased significantly (+3.38, $p = 0.0002$), suggesting possible renal benefits. Importantly, the intervention group reported significantly improved fatigue scores using the ELQ (−16.88, $p < 0.0001$), highlighting perceived improvements in quality of life.

Potential differences in medication use or adherence among participants could have influenced the metabolic or glycemic outcomes. However, all participants were instructed to maintain their existing medication regimens, and no changes were reported during the trial. Medication adherence was reinforced through monthly telephone reminders and review at follow-up visits. While residual confounding from differential adherence cannot be completely excluded, the balanced baseline characteristics and similar follow-up contact frequency between groups make a systematic medication-related bias unlikely.

3.3. *Sex-related differences in treatment response*

Exploratory analyses were performed to assess whether treatment effects differed between male and female participants. Independent-sample t-tests comparing changes in fasting blood glucose (FBG) and HbA1c between the intervention and control groups within each sex indicated statistically significant differences favoring the Oleocanthal group in both males and females.

Among female participants, mean changes in FBG and HbA1c differed significantly between treatment arms (FBG $p = 0.013$; HbA1c $p = 0.0004$). Among male participants, these differences were also significant (FBG $p < 0.0001$; HbA1c $p < 0.0001$).

However, in repeated-measures models adjusted for baseline BMI, sex, and baseline values of each outcome, the effect of sex was not statistically significant for either FBG ($p = 0.499$) or HbA1c ($p = 0.56$). These results suggest that both male and female participants experienced similar relative benefits from Oleocanthal supplementation.

4. Discussion

Oleocanthal, oleacein, oleuropein aglycone, and ligstroside aglycone are potent secoiridoids found in olive oil. These aldehydic phenols (OOPs) are celebrated for their distinct health-protective qualities. The OleoMetS randomized controlled trial offers compelling evidence for the efficacy of OOPs in enhancing glycemic control and a range of metabolic parameters in patients with MetS. Notably, the lipid profile showed significant benefits, including a reduction in both oxidized LDL and total LDL cholesterol, alongside a decrease in triglycerides. The outcomes of the OleoMetS trial strongly suggest that the OOPs are the main active ingredients of olive oil against specific diseases and that the lipids potentially play a secondary role. To our knowledge, this is the first clinical study to investigate the metabolic effects of a high-phenolic olive oil naturally enriched in oleocanthal in individuals with metabolic syndrome. While previous studies have examined olive oil phenolic content more broadly, data specifically isolating the role of oleocanthal in this population remain limited, underscoring the novelty and clinical relevance of the present findings.

We hypothesized that OOPs are the primary active ingredients in olive oil, with lipids potentially playing a secondary role in its disease-fighting properties. To our knowledge, no pure OOPs supplement has been tested globally. This significant gap in research stemmed from the lack of cost-effective methods for large-scale extraction of OOPs from olive oil [30], hindering their commercialization as supplements or pharmaceuticals. To overcome this, we developed a rapid and economical method for selectively extracting OOPs from olive oil. This led to the encapsulation of the commercially available extract (Oleoprotect®) in a food supplement (Thousand Olives®), enabling the first clinical trial with pure OOPs, separated from the olive oil's lipid matrix. This trial offers new insights into their bioactivity and provides strong data on their clinical efficacy against metabolic syndrome. It is a limitation of the study that data on the specific type of antidiabetic medication used were not collected; however, all participants were required to be on a stable treatment regimen for at least three months prior to enrollment, and no changes were permitted throughout the trial, minimizing potential confounding.

The bioavailability of olive oil polyphenol (OOP) compounds, both in the specific formulation used and in olive oil itself, presents a complex area with limited published data in humans. Preclinical animal studies have shown that after intravenous administration in mice, compounds such as oleocanthal or oleacein are not directly measurable in plasma. Therefore, future research may include the analysis of biomarkers in blood or plasma—specifically phenolic compounds and their metabolites—to better characterize the systemic exposure and mechanism of action of OOPs. Furthermore, the significant interindividual variability in the presence of phenolics and their metabolites in urine should also be considered and discussed in future pharmacokinetic studies, as it presents a methodological challenge for assessing absorption and metabolism. Our previous research Darakjian L et al. demonstrated that oleocanthal spontaneously reacts with plasma amino acids, forming new metabolites like oleoglycine, which has been utilized as a pharmacokinetic marker for oleocanthal administration [30]. However, data concerning oral administration in humans are currently unpublished and remain under investigation. Preliminary findings from our group indicate that in humans, metabolites resulting from the reaction of oleocanthal or oleacein with certain plasma amino acids (e.g., glycine or cysteine) reach peak concentrations at 30 min and are detectable for up to 60 min, while oleocanthal or oleacein themselves are not detectable at any time point.

After screening more than 10,000 olive oil samples [31], we have discovered varieties that under specific conditions of malaxation can produce olive oil containing oleocanthal and oleacein as the main phenolic ingredients. Among them, the Kalamon variety presents the highest capability for producing oleocanthal. Another Greek variety able to produce olive oil very rich in oleuropein and ligstroside aglycons is the Olympia variety. Using a mix of these two types of olive oil as starting material and following a newly developed selective extraction procedure we managed to obtain an extract containing oleocanthal, oleacein, oleuropein aglycon and ligstroside aglycon without lipids or any other ingredients of olive oil. The selective extraction procedure had been previously reported using water as extraction medium [32,33] and was based on the property of the aldehydic secoiridoids to react with water and be transformed into water-soluble 1,1-diols. However, the solubility of the hydrated aldehydes in water is relatively low (e.g. for oleocanthadiol is 0.2 %) requiring big amounts of water for extraction and increasing the risk for hydrolysis and microbial spoilage when applied on an industrial scale. To overcome these problems, we developed an alternative way for selective extraction using polyethyleneglycol PEG400. With PEG, oleocanthal and all the other secoiridoid aldehydes are transformed into pegylated hemiacetals (Fig. 5) which present solubility in PEG up to 30 % w/w and in addition their solution remains stable for at least 2 years at room temperature and without microbial spoil risk. For example, 1000 kg of olive oil can be selectively and efficiently extracted with only 10 kg of PEG (in contrast with water which requires more than 1000 kg). The PEG solution can be directly used for formulation after mixing with appropriate solid excipients (e.g. cellulose) or alternatively can be further enriched using adsorption resin technology. The pegylated forms of the secoiridoids are readily soluble in water. They react quantitatively and spontaneously with water affording the corresponding 1,1-diols which are exactly the same compounds that would be formed in the gastric fluids after ingestion of the aldehydic OOPs when consumed in the form of olive oil.

The innovative method for the large-scale selective extraction of olive oil aldehydic phenols (OOPs) using polyethylene glycol 400 (PEG 400) marks a significant advancement in the practical utilization of these bioactive compounds. PEG 400 forms water-soluble hemiacetals with OOPs, addressing economic production challenges and enabling the creation of the OLEOPROTECT® extract.

Oleocanthal, which is the major OOPs contained in the studied food supplement, is a well-known transient receptor potential TRPA1 ligand responsible for the pungent taste of the olive oil coming from unripe olives. TRPA1 is an ion channel found on sensory neurons, pancreatic beta cells, and various metabolic tissues. It is activated by pungent compounds like allyl isothiocyanate (mustard oil), cinnamaldehyde (cinnamon), allicin (garlic), and environmental irritants and it plays roles in pain, inflammation, oxidative stress, and metabolic regulation. More specifically, the role of TRPA1 in blood glucose regulation has been recently reviewed [34]. Interestingly, TRPA1 is expressed in pancreatic beta cells, where its activation can enhance insulin secretion [35] and in addition several natural TRPA1 agonists like cinnamon, garlic, and mustard oil have been reported to lower blood glucose in animal models. Specifically, cinnamaldehyde (derived from cinnamon), a TRPA1 agonist, has demonstrated an improvement in insulin sensitivity and a reduction in fasting blood glucose levels in diabetic mice [36]. TRPA1 is also present in enteric neurons and might regulate glucagon-like peptide-1 (GLP-1) release [37], which affects insulin secretion and satiety. Furthermore, it may indirectly influence hepatic glucose production via neural pathways.

Specifically, in OleoMetS study the OOPs supplementation (intervention arm) resulted in statistically significant reductions in fasting blood glucose and HbA1c, crucial indicators of glucose homeostasis and critical predictors for the progression to type 2 diabetes mellitus (T2DM). These outcomes align with existing preclinical and mechanistic research that highlights the anti-inflammatory and insulin-sensitizing properties of OOPs. Beyond the primary glycemic outcomes, the intervention also elicited favorable changes in secondary parameters, including BMI, systolic blood pressure, triglycerides, oxidized LDL, and uric acid, thereby underscoring its broad metabolic benefits. Further systemic advantages of the supplement were suggested by improvements in liver function (ALT) and kidney function (eGFR), alongside a significant reduction in self-reported fatigue. These multifaceted effects are likely attributable to the synergistic antioxidant and anti-inflammatory mechanisms of the secoiridoids present in the formulation. Moreover, adherence to the Mediterranean Diet remained consistent between the two study arms throughout the study's duration, thereby removing potential confounding factors from the reported results.

The study design incorporated robust methodologies, including rigorous randomization, masking, and meticulous compliance monitoring, which collectively enhanced the internal validity of our findings. Furthermore, adherence to the intervention was high, and it was well tolerated, with no serious adverse events reported. However, we acknowledge the modest sample size of this single-center trial and recommend validation through larger, multi-center studies. Exploratory sex-specific analyses showed significant improvements in both male and female participants;

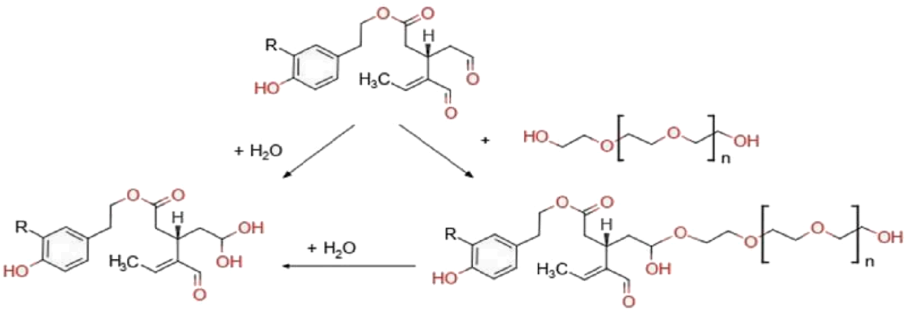

Fig. 5. Reaction of aldehydic secoiridoids with PEG400 or water used for the selective extraction from olive oil.

however, after adjusting for baseline BMI and other covariates, sex did not significantly influence treatment response for either fasting glucose or HbA1c, indicating a broadly consistent effect across sexes. However, several limitations warrant consideration. The relatively short trial duration of 12 weeks necessitates longer-term studies to ascertain sustained efficacy and safety. Additionally, while the study was adequately powered for its primary endpoints, subgroup analyses and an investigation into long-term outcomes such as cardiovascular events were beyond the scope of this particular investigation.

A recent double-blind crossover trial in adults with obesity and prediabetes, though short in duration, showed that replacing common dietary oils with high-oleocanthal EVOO for one month significantly reduced inflammatory markers (e.g., interferon-γ), improved oxidative stress, and led to modest decreases in body weight, BMI, and fasting glucose. However, this study did not assess long-term metabolic endpoints. In contrast, the OleoMetS trial, a 12-week parallel-group study, utilized a standardized olive polyphenol supplement and demonstrated more comprehensive metabolic benefits. These included significant improvements in glycemic control (FBG and HbA1c), lipid profile, blood pressure, and renal and liver function in individuals with diagnosed metabolic syndrome.

Collectively, these studies reinforce the role of olive oil–derived polyphenols in modulating key pathophysiological processes associated with inflammation, oxidative stress, and metabolic dysfunction. The OleoMetS trial expands upon this body of evidence by demonstrating sustained efficacy over a longer intervention period in a higher-risk and clinically heterogeneous population.

The findings provide a promising indication of the potential utility of dietary polyphenol supplementation as an adjunctive approach in the management of metabolic syndrome (MetS). In view of the increasing global prevalence of MetS, the development of safe, cost-effective, and naturally derived interventions warrants continued rigorous clinical evaluation. Future studies should also investigate higher dosages of oleocanthal-based formulations, as preliminary evidence suggests a dose-dependent effect.

5. Conclusions

Our innovative method for the large-scale selective extraction of olive oil aldehydic phenols (OOPs) using polyethylene glycol 400 (PEG 400) has demonstrated significant potential for commercial and therapeutic applications. This biocompatible solvent effectively transforms OOPs into water-soluble hemiacetals, facilitating their extraction and enhancing their stability in various formulations. The resulting extract, OLEOPROTECT®, has been successfully scaled to multi-ton production and formulated into a stable food supplement (Thousand Olives ®). The OleoMetS clinical trial validated, for the first time, the benefits of olive oil biophenols in humans, paving the way for the broader use of OOPs in health and disease prevention. Across all participants, the supplement demonstrated excellent safety and tolerability, with no adverse events reported during the 12-week intervention. In summary, the OleoMetS trial provides the first clinical evidence that supplementation with a standardized olive oil polyphenol extract rich in oleocanthal, oleacein, oleuropein aglycone, and ligstroside aglycone leads to significant and clinically meaningful improvements in fasting blood glucose and HbA1c—our primary outcomes—alongside beneficial effects on BMI, blood pressure, lipid profile, liver and renal function, and fatigue scores. These findings directly support the hypothesis that olive oil phenolic secoiridoids (OOPs) are key bioactive components responsible for the health benefits attributed to extra virgin olive oil [38,39].

Despite the strong and consistent effects observed, this study has certain limitations. The sample size, while adequate for detecting the primary outcomes, was modest and may not fully capture population variability. The study duration of 12 weeks limits conclusions about long-term efficacy and safety. Additionally, most participants were habitual consumers of olive oil, which may have influenced baseline sensitivity to OOPs.

Future studies should therefore aim to recruit larger, more diverse populations—including individuals with minimal previous olive oil consumption—and extend the intervention period beyond three months to evaluate whether the observed metabolic improvements are sustainable over time. Such studies would further elucidate the dose–response relationship, durability, and mechanistic underpinnings of OOP bioactivity in metabolic health.

Author contributions

G. Samoutis conceptualized, coordinated the RCT and oversaw ethical approvals. TC. Kyriakides was involved in study design, oversaw data management, and conducted statistical analysis. N. Demetriou, E. Poulianiti, G. Samouti and S. Samouti were responsible for participant assessments and data collection. Panagiotis Diamantakos, Eleni Melliou, and Prokopios Magiatis significantly contributed to the extraction and formulation of olive oil phenols. All authors contributed to and approved the final manuscript.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT in order to improve readability and language of the work. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Funding

This study was funded by *World Olive Center for Health* and the *A.G. Leventis Foundation* for their generous support and providing a scholarship, and the *Botanic Art*, by donating the study OOPs nutritional supplement capsules as well as the placebo capsules.

Acknowledgements

This study was funded by the World Olive Center for Health and the A.G. Leventis Foundation, which also awarded a scholarship to Mr. Nicholas Demetriou. We also warmly thank Botanic Art for kindly providing the OOPs nutritional supplement and the placebo capsules used in this study. Their contributions were instrumental in facilitating the research presented in this paper.

The sponsors had no role in the study's design, data collection, analysis, interpretation, or writing of the report.

The authors declare no conflicts of interest. All co-authors have reviewed and approved the manuscript contents and report no financial interests. Botanic Art provided the supplement free of charge but was not involved in the data analysis, interpretation, or the decision to publish the findings.

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