Reduction of LDL and total cholesterol levels with Kalamon table olive extract: A natural approach to dyslipidemia

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Abstract. The present study investigated the effects of a nutritional supplement derived from high-phenolic Kalamon variety table olives on lipid parameters in individuals with mild dyslipidemia. The supplement, which corresponds to the consumption of five table olives daily, was produced through a process involving the removal of olive oil, the extraction of tyrosol, hydroxytyrosol and lactic acid, and the removal of water and salt. In a 30-day clinical analysis, volunteers with mild dyslipidemia were enrolled and instructed to take two capsules daily, while maintaining a healthy lifestyle and diet. The results revealed significant reductions in total cholesterol (4.16%) and low-density lipoprotein (LDL) cholesterol (5.67%) levels following supplementation. Although the triglyceride levels exhibited a modest reduction, the difference in these levels did not reach statistical significance. High-density lipoprotein cholesterol levels were not affected throughout the study period. The individualized responses to supplementation were observed in all lipid parameters, with varying ranges in initial and final measurements among the participants. These findings suggest that the nutritional supplement may have beneficial effects on reducing total cholesterol and LDL cholesterol levels, highlighting the potential health benefits of the phenolic compounds found in table olives, particularly hydroxytyrosol and tyrosol, related to cardiovascular well-being and metabolic health. However, further research is required to confirm these results and investigate the underlying mechanisms.

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Introduction

Table olives have been known since antiquity to have beneficial effects on human health. Edible table olives contain substances which potentially can protect the body from oxidative stress and cardiovascular diseases. These substances are phenolic compounds, which are found in extra virgin olive oil, but not in any other edible fruit. While a number of studies have been conducted on the phenolic compounds contained in extra virgin olive oil (1-5), the number of studies conducting in-depth investigations concerning table olives and the phenols contained therein with regards to potential the biological effects on human health are limited (6-8).

In previous research, the health benefits associated with tyrosol and hydroxytyrosol, prominent bioactive compounds present in table olives, were extensively explored. Hydroxytyrosol, a compound found in olive oil and table olives, has shown promising results in mitigating cardiovascular risk factors. Notable studies include a randomized double-blind, placebo-controlled parallel study by Knaub *et al* (9), which investigated the low-density lipoprotein (LDL)-cholesterol lowering effect of hydroxytyrosol (HTEssence®). Additionally, others studies (10,11) have provided insight into the hypocholesterolemic effects of hydroxytyrosol, demonstrating its potential to lower cholesterol levels and to function as an antioxidant. The study by Tabernero *et al* (12) further contributed to the understanding of the metabolic effects of hydroxytyrosol, emphasizing its role in ameliorating hypercholesterolemia.

By directly targeting the mitochondria in inflamed endothelial cells, hydroxytyrosol reduces mitochondrial peroxide production, enhances superoxide dismutase activity and suppresses inflammatory angiogenesis, thereby mitigating oxidative stress and inflammation at the cellular level (13,14). Additionally, hydroxytyrosol prevents lipid peroxidation, a key process in protecting LDLs from oxidative damage, which is critical for reducing the cardiovascular risk (15,16). Experimental studies have also highlighted the ability of hydroxytyrosol to address liver-related metabolic dysregulation. By alleviating oxidative stress and liver inflammation, hydroxytyrosol prevents early-stage insulin resistance and

non-alcoholic fatty liver disease (NAFLD), restoring glucose homeostasis. This protective effect was exemplified in animal studies, where hydroxytyrosol administration led to marked reductions in cholesterol levels and improved insulin sensitivity and glucose tolerance (17,18). Taken together, these findings expand the understanding of the hypolipidemic and metabolic effects of hydroxytyrosol, aligning with its observed benefits in clinical trials.

Similarly, studies on tyrosol, another phenolic compound found in *Olea europaea*, have highlighted its antihyperlipidemic effects. Chandramohan and Pari (19) conducted research showcasing the antihyperlipidemic effects of tyrosol in rats with streptozotocin-induced diabetes. Furthermore, Boronat *et al* (20) explored the cardiovascular benefits of tyrosol, indicating its potential to contribute to cardiovascular health and its conversion into hydroxytyrosol in humans. Tyrosol exhibits notable cardiometabolic protective effects through diverse biochemical mechanisms. Its anti-inflammatory properties are attributed to its ability to modulate the upregulation of cluster of differentiation 14 (CD14) and suppress inflammatory processes, which play pivotal roles in hypertension, coronary artery disease and insulin resistance (21). Moreover, tyrosol enhances high-density lipoprotein (HDL) functionality by reducing oxidative modifications and promoting cholesterol efflux via the ATP-binding cassette transporter A1 (ABCA1) pathway, a critical step in maintaining cholesterol homeostasis (22). It also demonstrates anti-atherogenic activity by inhibiting leukotriene B4 production, thereby preserving endothelial function and reducing vascular inflammation (23). The hepatoprotective effects of tyrosol have been highlighted in studies demonstrating its capacity to reduce lipid synthesis in primary rat hepatocytes and mitigate NAFLD through the upregulation of cystathionine β-synthase and cystathionine γ-lyase expression, leading to increased hepatic hydrogen sulfide (H_2S) synthesis (24,25). Taken together, these findings underscore the potential health-promoting properties of both tyrosol and hydroxytyrosol, emphasizing their key role in the context of cardiovascular well-being and metabolic health.

In a previous study by the authors, biochemical tests were performed in a small group of normal control volunteers after consuming table olives of 'Kalamon' variety with a high phenolic content (unpublished data, derived from the Master's thesis of Ms. Maria Vlachakou, performed by the Research Group of Clinical Pharmacology and Pharmacogenomics, National and Kapodistrian University of Athens, 2017). The material of the study was selected due to the high content in tyrosol and hydroxytyrosol, measured using quantitative nuclear magnetic resonance (qNMR) protocols, established by Mousouri *et al* (26) in 2014. The results of the biochemical blood measurements following a period of a 30 days of the daily consumption of five olives suggested that there was an improvement in the levels of specific critical markers, characterized as risk factors of metabolic syndrome. Based on the initial results, herein, the 30-day study was repeated with volunteers with mild dyslipidemia consuming a nutritional supplement that was developed in a daily dosage corresponding to five table olives of high-phenolic Kalamon variety. In the initial study, volunteers consumed table olives that were standardized for their content in tyrosol and hydroxytyrosol. In the present follow-up study, these bioactive compounds were extracted from the olive matrix to confirm whether the extract itself is responsible for the observed effects. By using this approach, the present study also aimed to explore the potential of utilizing olive by-products to create a dietary supplement, thus providing access to the health benefits of olives to individuals who may not have regular access to table olives.

Subjects and methods

Supplement production. The selected Kalamon table olives, provided by Sakellaropoulos Organic Farms (Laconia, Greece), were initially processed into a paste using a mill, which served as the starting material for the extraction of bioactive compounds. The olive paste in this stage consisted of ~15% olive oil, solid materials of the olive flesh and an aqueous phase, containing the bioactive compounds implemented in the supplement. The first step of the extraction of bioactive compounds was the olive oil removal, using an OlioMio decanter (Pieralisi). Tyrosol, hydroxytyrosol and lactic acid were then extracted from the paste by the addition of purified water at a 1:1 w/w ratio and stirring continuously for 40 min. The aqueous phase was obtained by filtration and evaporated under vacuum using a rotary evaporator. This procedure yielded 85% of the bioactive compounds present in the original material. The dry material obtained from this procedure contained significant amounts of salt, that was removed using a 3:1 v/w dilution in ethanol, filtration and evaporation. Finally, the extracted bioactive compounds were formulated into capsules using microcrystalline cellulose as a carrier. To validate the concentration of bioactive compounds in the developed supplement, a re-extraction of the compounds from the carrier was performed. This process involved the addition of syringaldehyde (MilliporeSigma), as an internal standard to the re-extracted solution, followed by qNMR analysis (Fig. 1). The proton NMR signals were integrated and quantified relative to the internal standard, allowing for the accurate determination of the concentrations of tyrosol, hydroxytyrosol and lactic acid. This method was adapted from a previously published study by the authors (27), which has been successfully applied to quantify bioactive compounds in thousands of table olive and olive oil samples annually. Using this approach, it was determined that two capsules of the supplement, hereby referred to as CHOLESTOLIVE, contained 28 mg hydroxytyrosol, 12 mg tyrosol and 81 mg lactic acid, an amount corresponding to five Kalamon table olives of high-phenolic content. Following the described procedure, 180 kg Kalamon table olives yielded a total of 3,000 daily doses of the supplement, with an extraction efficiancy of 85% achieved during the upscale process.

Pilot observational study. In collaboration with three major hospitals of Greece (Hippokration General Hospital of Athens, Elpis General Hospital of Athens, both in Athens Greece, and Achilopoulio, Volos General Hospital, Volos, Greece), 48 volunteers (19 males and 29 females; mean age, 54 years; range, 26-76 years) exhibiting mild dyslipidemia were enrolled in a 30-day pilot study. All participants were extensively briefed, before signing an informed consent form. All collected samples were anonymized before

Table I. Inclusion and elimination criteria for the study participants.

Inclusion criteria	Elimination criteria
Age between 18 and 70 years	Currently treated for hyperlipidemia
No pharmaceutical treatment	Known olive allergies
Low or medium heart condition risk (HEARTSCORE <5%)	High/very high cardiovascular disease risk
Cognitive competence	Non-compliant to the study

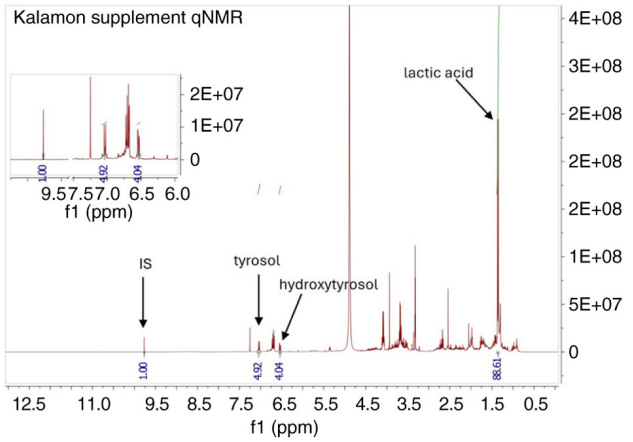

Figure 1. Quantitative NMR spectrum of Kalamon olive extract supplement, highlighting the key bioactive compounds: tyrosol, hydroxytyrosol, and lactic acid. The internal standard (IS) used for quantification was syringaldehyde (1 mg/ml). The inset displays a magnified region (6.0-9.5 ppm) for improved visualization of the aromatic signals corresponding to tyrosol and hydroxytyrosol.

further processing. The research protocol was reviewed and approved by the 55th Scientific Council of Hippokration General Hospital during its meeting on March 22, 2021 (Reference no. ΕΣ. 55Ο/22-6-2021) and expanded without any modifications, in accordance with institutional guidelines. The inclusion and exclusion criteria, referring to total cholesterol levels <290 mg/dl, and a low-to-medium cardiovascular risk, as assessed using HeartScore (<5%), are summarized in Table I. HeartScore is a risk assessment tool developed by the European Society of Cardiology to estimate the 10-year risk of an individual of fatal cardiovascular disease based on factors such as age, sex, blood pressure, cholesterol levels and smoking status. Volunteers were instructed not to alter anything regarding their lifestyle, to consume the daily dosage of CHOLESTOLIVE and to record their diet for 30 days. The first volunteer was enrolled on January 31, 2022, and the final lipid profile examination of the last enrolled participant was conducted on July 20, 2023. No control group was included, as this was a pilot observational study for the daily consumption of a naturally derived supplement. The design did not involve blinding or randomization, as the primary aim was to assess feasibility and collect preliminary data for future studies. Additionally, this pilot study was specifically designed to focus on lipid parameters, and therefore did not assess other parameters, such as blood pressure or kidney and liver function.

Biomarker evaluation. Blood samples were collected from the participants at baseline and after the 30-day supplementation period. Triglyceride, HDL and LDL cholesterol levels were measured using enzymatic colorimetric assays at the clinical laboratories of the collaborating hospitals. These biomarkers were evaluated against established clinical guidelines for normal ranges: LDL cholesterol <130 mg/dl, triglycerides <150 mg/dl, and HDL cholesterol ≥40 mg/dl for males and ≥45 mg/dl for females These parameters were selected as they are clinically significant and widely recognized biomarkers for evaluating dyslipidemia and cardiovascular risk (28).

Statistical analysis. Statistical analyses were performed using R Studio. The Kolmogorov-Smirnov test was used to assess the normality of the data. For normally distributed variables, the paired sample t-test was applied to compare measurements before and after the intervention. For non-normally distributed variables, the Wilcoxon signed-rank test was used as a non-parametric alternative. A two-sided P-value <0.05 was considered to indicate a statistically significant difference. A power analysis was conducted prior to the study, targeting 80% power with an alpha level of 0.05. This analysis suggested the required sample size to be 80 participants. Despite the final sample size being 48 participants, the data generated statistically significant findings that align with the objectives of this pilot observational study.

Results

Total cholesterol. In the initial examination, the mean total cholesterol levels were recorded at 232.54±26.88 (SD) mg/dl. Following a 30-day daily intake of CHOLESTOLIVE, the final examination revealed a mean total cholesterol level of 222.85±36.68 (SD) mg/dl. This represents a notable mean reduction of 4.16% (Table II). Statistical analysis, employing the Wilcoxon test, demonstrated a significant difference between the mean total cholesterol levels before and after CHOLESTOLIVE supplementation (p.s.=5%, Z=-2.518, P=0.012). The reduction observed in the total cholesterol levels was statistically significant. It is noteworthy that the initial measurement of total cholesterol levels ranged from 178 to 291 mg/dl, while the final measurement exhibited a range of 167 to 341 mg/dl (Fig. 2).

HDL. At the commencement of the study, the mean HDL cholesterol level was 56.37±14.53 (SD) mg/dl; and following the 30-day CHOLESTOLIVE supplementation, the final examination reported a marginal decrease to 56.06±13.20 (SD) mg/dl. On average, there was a minor change of -0.54%

Table II. Overall changes in lipid parameters following intervention with the supplement.

Before/after supplementation	Cholesterol	HDL	LDL	TG
Prior to supplementation	232.54±26.88	56.37±14.53	150.95±22.33	125.93±64.50
After supplementation	222.85±36.68	56.06±13.20	142.39±36.13	118.37±46.26
Difference	-4.16%	-0.54%	-5.67%	-6.01%
P-value.	0.012	0.783	0.048	0.430

Percent changes and corresponding P-values for each lipid parameter following the intervention. Statistically significant changes (P<0.05) are indicated for cholesterol and LDL, reflecting reductions of -4.16 and -5.67%, respectively. HDL, high-density lipoprotein; LDL, low-density lipoprotein; TG, triglycerides.

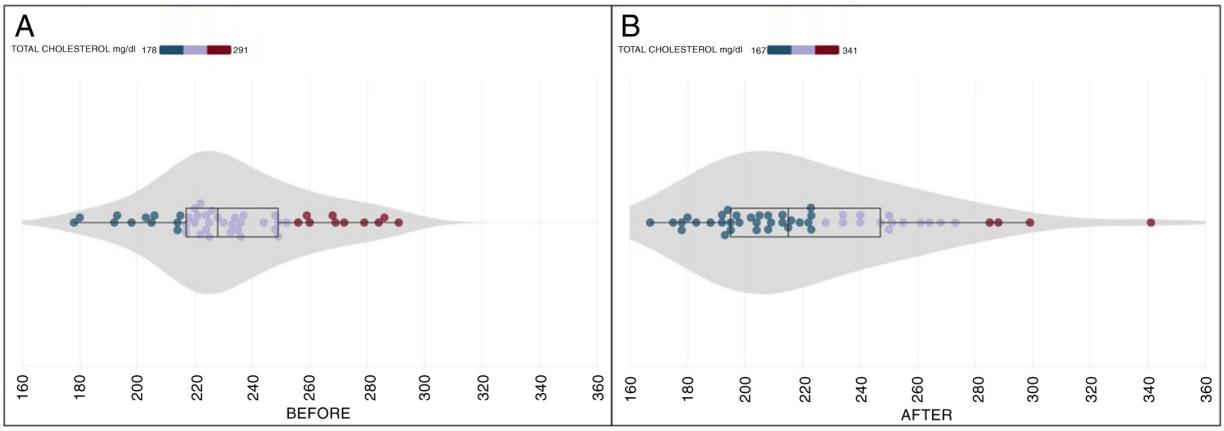

Figure 2. Violin plots representing total cholesterol measurements (A) before and (B) after the supplementation period.

in the HDL cholesterol levels (Table II), which was not substantial (p.s.=5%, t=0.277, P=0.783). This suggested that CHOLESTOLIVE intake did not lead to any modification in HDL cholesterol. The initial measurement of HDL cholesterol levels spanned from 28 to 96 mg/dl, while the final measurements showcased a broader range, fluctuating between 33 and 101 mg/dl (Fig. 3). This variance emphasizes the individualized response observed in HDL cholesterol levels among the participants.

LDL. At the study onset, the mean LDL cholesterol level was recorded at 150.95±22.33 (SD) mg/dl, with a noticeable reduction in the final examination after 30 days of CHOLESTOLIVE supplementation, registering at 142.39±36.13 (SD) mg/dl. The calculated mean reduction in LDL cholesterol amounted to a noteworthy 5.67% (Table II). Utilizing the paired t-test for statistical analysis, a significant difference emerged between the mean LDL cholesterol levels before and after CHOLESTOLIVE intake (p.s.=5%, t=2.033, P=0.048). This emphasizes the statistically significant impact of CHOLESTOLIVE on reducing LDL cholesterol levels. The initial measurement range for LDL cholesterol spanned from 105 to 196 mg/dl, showcasing variability among participants. Notably, the final measurements exhibited an even wider range, fluctuating between 56 and 251 mg/dl (Fig. 4). This variance in responses highlights the complex interplay of factors influencing LDL cholesterol levels post-CHOLESTOLIVE supplementation.

Triglycerides. In the initial examination, the participants exhibited a mean triglyceride level of 125.93±64.50 (SD) mg/dl. Following a 30-day daily intake of the dietary supplement, the final examination reported a marginal decrease to 118.37±46.26 (SD) mg/dl. The calculated mean reduction in triglyceride levels reached 6.01% (Table II). Contrary to the observed trends in other lipid parameters, the Wilcoxon test employed for statistical analysis indicated that mean triglyceride levels before and after CHOLESTOLIVE intake did not exhibit a significant difference (p.s.=5%, Z=-0.790, P=0.430). This suggests that the reduction in the triglyceride levels, while notable, did not reach statistical significance. The range of initial triglyceride values was diverse, spanning from 40 to 358 mg/dl, reflecting the heterogeneity among participants. Of note, the final measurements showed a reduction in variability, ranging from 26 to 228 mg/dl (Fig. 5). This nuanced pattern underscores the individualized response to CHOLESTOLIVE supplementation in modulating triglyceride levels.

Discussion

The health-promoting properties of olives have been recognized since antiquity, with olives and olive oil playing a central role in the Mediterranean diet, which is widely regarded as one of the healthiest diets globally (29,30). Historical references to the cardioprotective benefits of olives align with modern scientific findings that attribute these effects to compounds, such as tyrosol and hydroxytyrosol (31). The use of Kalamon

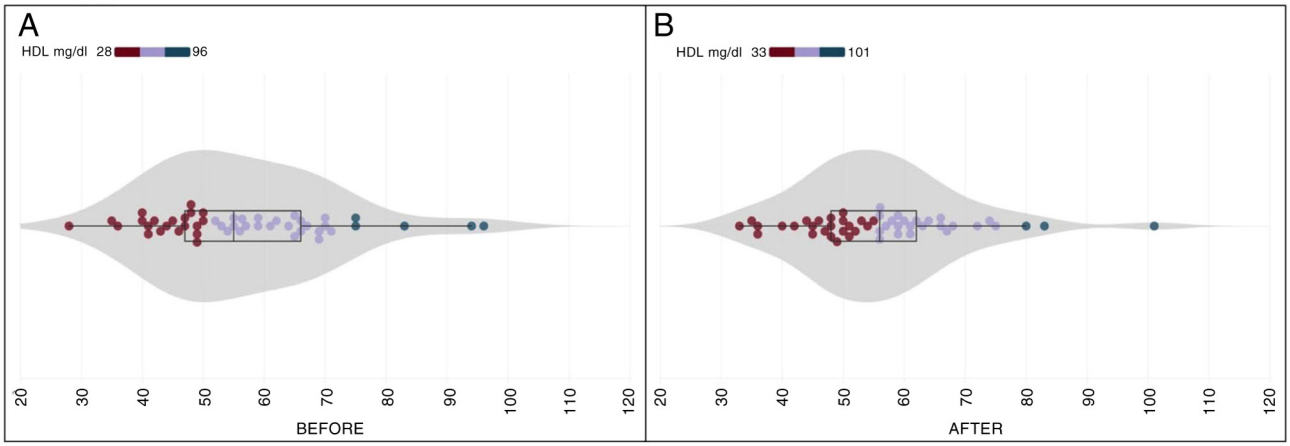

Figure 3. Violin plots illustrating HDL measurements (A) before and (B) after the supplementation period. HDL, high-density lipoprotein.

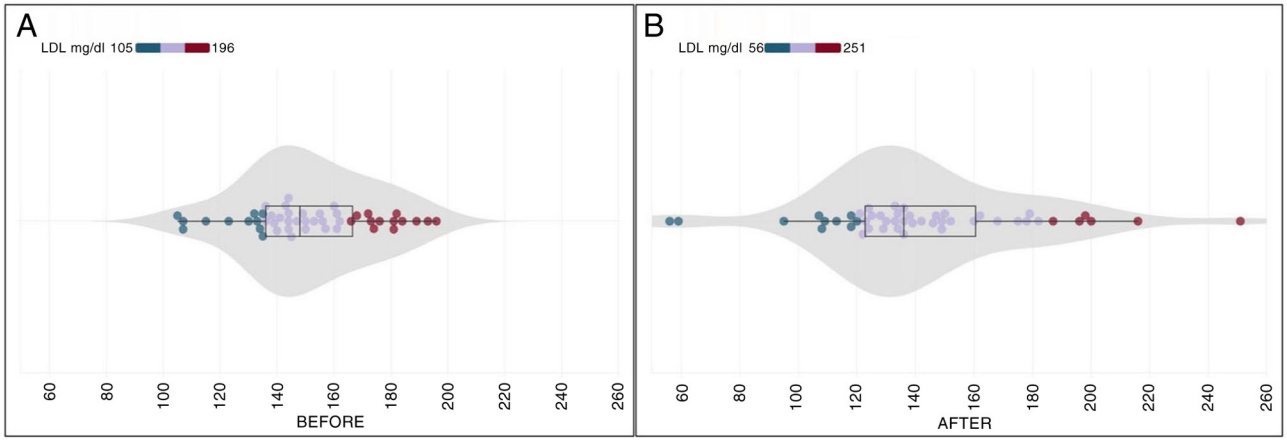

Figure 4. Violin plots representing LDL measurements (A) before and (B) after the supplementation period. LDL, low-density lipoprotein.

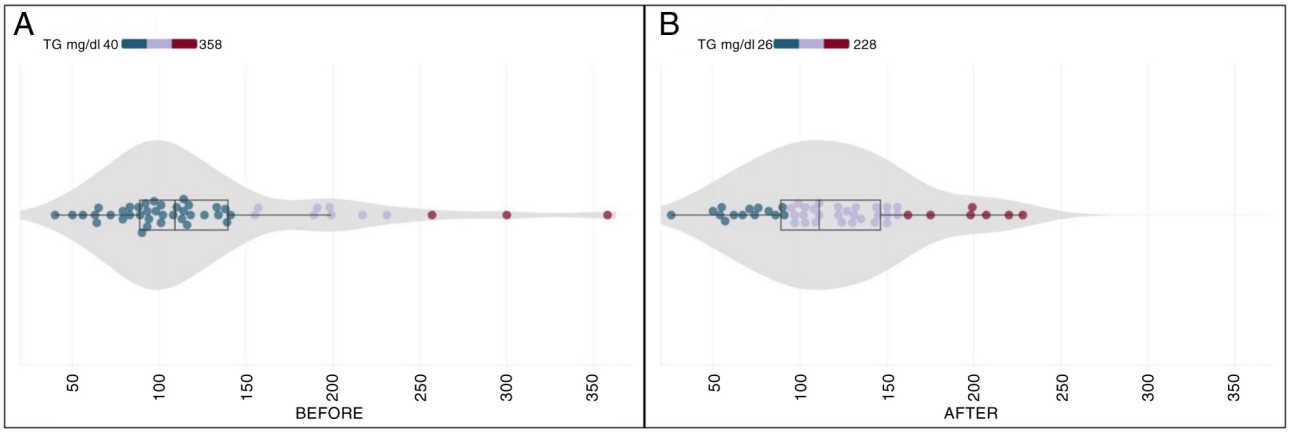

Figure 5. Violin plots depicting TG measurements (A) before and (B) after the supplementation period. TG, triglycerides.

olives in CHOLESTOLIVE connects these traditional uses with contemporary science, demonstrating that the bioactive compounds in olives can effectively reduce LDL cholesterol and total cholesterol, providing natural protection against cardiovascular diseases. While the present study was designed to evaluate CHOLESTOLIVE supplementation independently, further research is required to further explore how its bioactive compounds compare to direct Kalamon olive consumption in dietary applications.

Before commencing the production of CHOLESTOLIVE, multiple samples of different varieties of table olives were screened using qNMR to identify those with the highest content

of tyrosol and hydroxytyrosol. This screening process ensured that the selected Kalamon olives contained the optimal levels of bioactive compounds necessary for the formulation of a supplement that follows a patented production method (32). The innovative method involves a clean, efficient separation process that maintains the integrity of these phenolic compounds. To the best of our knowledge, the present study is the first to evaluate the lipid-lowering and cardioprotective effects of a dietary supplement produced using this method, offering a novel approach to utilizing table olives for health purposes.

In contrast to pharmaceutical interventions such as statins, which are often prescribed to reduce LDL cholesterol and prevent cardiovascular diseases, CHOLESTOLIVE provides a natural alternative derived from table olives. Statins are well-known for their effectiveness; however, they also carry a risk of side-effects, including muscle pain, liver damage and an increased risk of developing type 2 diabetes (33,34). The use of natural supplements such as CHOLESTOLIVE, rich in tyrosol, hydroxytyrosol and lactic acid, offers a safer approach with the possibility of fewer side-effects, rendering it a promising alternative for those with mild dyslipidemia who wish to avoid statins. The absence of reported side-effects or adverse events, as stated by the participants during the 30-day supplementation period further supports the safety of CHOLESTOLIVE, which is derived from natural, widely consumed table olives. Moreover, the extraction process used for CHOLESTOLIVE preserves the bioactive compounds found in Kalamon olives unaltered through an environmentally responsible method that avoids harsh chemical processing. The process achieves a high extraction yield of 85% using purified water and ethanol, which are recyclable, non-toxic and commonly employed in food and dietary supplement production. This ensures that the beneficial phenolic compounds are retained, maintaining their antioxidant properties, which are crucial for protecting cardiovascular health and combating oxidative stress.

The lipid-lowering effects observed with CHOLESTOLIVE are comparable to those reported for other natural supplements, such as those derived from plant sterols or fiber supplements, such as psyllium husk (35,36). Plant sterols, which are structurally similar to cholesterol, reduce intestinal cholesterol absorption and are widely recognized for their LDL-lowering efficacy, with studies reporting reductions of 10-15%. Psyllium husk, a soluble fiber, also demonstrates LDL-lowering effects by binding bile acids in the gut, with reported reductions ranging from 5-10%. While both interventions are effective at lowering LDL cholesterol, their effects on total cholesterol or triglycerides are less pronounced. However, the unique combination of phenolic compounds in Kalamon olives, particularly hydroxytyrosol, provides additional antioxidant and anti-inflammatory benefits, rendering CHOLESTOLIVE more than just a cholesterol-lowering agent. Unlike plant sterols and psyllium husk, which primarily function through intestinal mechanisms, CHOLESTOLIVE offers a broader cardioprotective profile. The reductions in total cholesterol (4.16%) and LDL cholesterol (5.67%) observed in the present study are noteworthy, particularly in comparison to other over-the-counter natural supplements (9). However, further studies are required to explore the synergistic effects of combining CHOLESTOLIVE with other lifestyle interventions or supplements to maximize its potential.

The results of the present study demonstrate significant reductions in total cholesterol and LDL cholesterol following CHOLESTOLIVE supplementation, with some variability in individual responses. Several factors could account for this variability. Genetic differences among participants may influence the metabolism of tyrosol and hydroxytyrosol, resulting in varying degrees of lipid reduction. Additionally, the baseline lipid levels of participants were diverse, which likely contributed to differences in their response to the supplement. Lifestyle factors, such as diet and physical activity, which were not controlled during the study, may also have affected the outcomes. Furthermore, individual differences in the absorption and bioavailability of the bioactive compounds from the supplement could explain the range of responses observed.

The present study has certain limitations, which should be mentioned. The present observational pilot study was designed as an initial exploration to assess the feasibility and potential benefits of the first nutritional supplement derived only from Kalamon table olives. As such, certain aspects, such as the short follow-up period and the absence of randomization or a control group, reflect the exploratory nature of the study. These characteristics were necessary to focus on generating preliminary data and evaluating the practicality of this approach. The authors are confident that future studies with extended durations, larger sample sizes, and more comprehensive designs will build upon these findings and provide deeper insight into the long-term effects and broader applications of the supplement.

In conclusion, the present study revealed that the use of the CHOLESTOLIVE supplement resulted in a significant reduction of total cholesterol (-4.16%, p.s.=5%, Z=-2.518, P=0.012) and LDL cholesterol (-5.67%) levels. The triglyceride levels exhibited a modest reduction, although this difference did not reach statistical significance. HDL cholesterol was not affected throughout the study (Table II). These findings suggest that the nutritional supplement may have beneficial effects on reducing total cholesterol and LDL cholesterol levels, highlighting the potential health benefits of the bioactive compounds found in table olives, particularly hydroxytyrosol, tyrosol and lactic acid, related to cardiovascular well-being and metabolic health. However, further larger-scale research is required to confirm these results and investigate the underlying mechanisms.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

ND, EM and PM were involved in the conception of the study. DK and CPT performed the medical examinations of the participants. NP and CP were involved in the acquisition of data and functioned as clinical research associates. AT, PM and ND were involved in the design of the study and in the writing of the manuscript. VE performed the statistical analysis. PD and PM provided scientific input in the design and development of the product. All authors have read and approved the final manuscript. NP, AT and ND confirm the authenticity of all the raw data.

Ethics approval and consent to participate

All participants were extensively briefed, before signing an informed consent form. All collected samples were anonymized before further processing. The research protocol was reviewed and approved by the 55th Scientific Council of Hippokration General Hospital during its meeting on March 22, 2021 (Reference no. ΕΣ. 55Ο/22-6-2021) and expanded without any modifications, in accordance with institutional guidelines.

Patient consent for publication

Not applicable.

Competing interests

Although the present study was supported by funding from Mediakos GmbH, it should be noted that the funding body had no role in the study design, data collection, analysis, or interpretation of the results. It should also be noted that, although some of the authors are listed as inventors on the patent for the nutritional supplement, they were not compensated for their contributions to this study (32).

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