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Randomized Control Trials

Hydroxytyrosol supplementation improves antioxidant and anti-inflammatory status in individuals with overweight and prediabetes: A randomized, double-blind, placebo-controlled parallel trial

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SUMMARY

Background & aim: Hydroxytyrosol (HT), an olive-derived phenolic compound, possesses well-known antioxidant and anti-inflammatory properties. While its benefits in healthy individuals and as part of extra virgin olive oil are well studied, its preventive role as a dietary supplement in at-risk populations remains less explored. This study investigates the potential of HT supplementation in preventing aging-related diseases in overweight individuals with prediabetes.

Methods: A randomized, double-blind, placebo-controlled trial was conducted in adults with overweight and prediabetes (40–70 years). For 16 weeks, volunteers consumed either 15 mg of HT or a placebo daily. The primary outcome were oxidized LDL (oxLDL) levels, while secondary outcomes included biochemical and metabolic parameters, oxidative stress and inflammation biomarkers, and lifestyle assessments. Compliance was verified through urinary HT-3′-sulphate levels.

Results: A total of 52 participants were recruited and randomized, with 49 completing the study. They were then allocated to either the HT-treated group (n = 24) or the placebo group (n = 25). Compliance was confirmed, as the HT-supplemented group showed increased urinary HT-3′-sulphate levels, whereas the placebo group exhibited a significant decrease ($p = 0.039$). Compared with placebo, HT supplementation significantly reduced oxLDL levels ($p = 0.045$), protein carbonyls ($p = 0.031$), and 8-OHdG ($p < 0.01$). Additionally, it prevented a decline in total antioxidant status ($p < 0.01$) and GPx activity ($p < 0.01$). An anti-inflammatory effect was also observed, with reduced IL-6 levels ($p = 0.05$). No significant changes were found in lipid profile, anthropometric parameters, or lifestyle factors such as sleep, mental well-being, or physical capacity. No adverse events were observed throughout the intervention.

Conclusion: Chronic supplementation with 15 mg/day of HT for 16 weeks significantly improved antioxidant and anti-inflammatory status in individuals with overweight and prediabetes, suggesting a potential preventive role against aging-related diseases.

Registration number of Clinical Trial: NCT 06295913 (https://clinicaltrials.gov/study/NCT06295913?intr=Hydroxytyrosol&page=2&rank=1).

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1. Introduction

Over recent decades, the global prevalence of chronic non-communicable diseases (NCDs) has been rising, accounting for 41 million deaths annually [1]. Although human life expectancy has increased, achieving healthy aging remains an urgent priority to preserve both physical and cognitive function. In this context, lifestyle modifications have been shown to reduce the incidence of obesity, type 2 diabetes (T2D), and cardiovascular diseases and help to control associated risk factors such as overweight and prediabetes, [2,3]. In the context of aging combined with prediabetes and overweight, there exists a complex interplay of metabolic imbalance, chronic low-grade inflammation, and increased production of reactive oxygen species [4]. These processes are closely intertwined; oxidative stress, for instance, amplifies inflammatory signaling and further impairs insulin sensitivity. Together, they contribute to cumulative molecular and cellular damage that gradually leads to organ dysfunction and, ultimately, the development of NCDs [5]. Therefore, interventions aimed at mitigating these detrimental mechanisms hold viable promise in slowing the progression from metabolic risk to overt disease and in preventing adverse aging trajectories.

One of the most accessible and cost-effective strategies to address an optimal aging is the adherence to a healthy diet. Among dietary patterns, the Mediterranean diet (MedDiet) has been extensively studied due to its contrasted benefits in overall health [6], particularly in large-scale trials such as the PREDIMED-Plus study, which linked diet to improvements in health-related quality of life [7] and the original PREDIMED study, which demonstrated a significant reduction in several health outcomes including primary cardiovascular prevention [8]. Among the emblematic foods of the MedDiet, extra virgin olive oil (EVOO) stands out as the primary dietary fat in the Mediterranean area. Its composition is primarily based on monounsaturated fatty acids (MUFA), and it also contains a minor unsaponifiable fraction rich in tocopherols, squalene, and polyphenols, among others [9]. This overall composition is associated with its bioactive properties, including antioxidant, anti-inflammatory, and lipid-modulating effects, contributing to the prevention of NCDs [9,10].

Olive oil polyphenols have attracted significant scientific interest, particularly after the European Food Safety Authority (EFSA) approved the claim that “olive oil polyphenols contribute to the protection of low-density lipoproteins (LDL) from oxidative damage” [11], an effect linked to their antioxidant capacity. Meanwhile, the market for dietary supplements has significantly grown in recent years, offering a way to achieve higher concentrations of bioactive compounds than those naturally present in foods [12], particularly in regions where the consumption of certain foods is not a common dietary habit. However, although supplementation provides easier access and a controlled method for delivering bioactive compounds, it remains unclear whether olive polyphenols exert the same protective effect against LDL oxidation when consumed as supplements and not within the whole food matrix.

Hydroxytyrosol (HT), one of the most studied olive polyphenols, is widely recognized for its potent antioxidant and anti-inflammatory properties, demonstrated in both *in vitro* and *in vivo* studies [13], thus making it a promising candidate for the prevention of NCDs [14]. At the same time, since HT was approved as a novel food in the European Union after safety assessment [15], it may be used as a dietary supplement or ingredient in other foods. A few studies have examined the effects of HT in food matrices other than oil through clinical trials. Binou et al. (2023) assessed the impact of daily consumption of bread enriched with HT (32.5 mg/day) for 12 weeks, reporting positive effects on lipid profile, inflammation markers and body weight in individuals with overweight/obesity and T2D but no effect on oxidized LDL (oxLDL) levels [16]. And Mateos et al. (2016) observed that HT consumed in an HT-enriched biscuit was highly bioavailable and led to a reduction in oxLDL levels in healthy humans [17]. Regarding pure HT supplementation, a short trial (three weeks) in healthy individuals found no significant reduction in oxLDL after consuming 15 mg/day of pure HT [18], while another six-month study in individuals with overweight or obesity (5–15 mg/day) did not assess oxLDL or other oxidative markers [19].

Given that the diverse modes of HT administration, whether within a food matrix or as a dietary supplement, can influence its health effects, several important questions remain unresolved due to the limited number of clinical trials testing HT supplementation. Specifically, it remains unclear whether HT, when consumed as a supplement, effectively protects circulating LDL from oxidation, as attributed to its role in EVOO-based health claims. Moreover, the relevance of these effects in populations at risk for NCDs is still uncertain, since most supporting evidence has been derived from studies conducted in healthy individuals [11], where antioxidant mechanisms may primarily act as preventive measures.

With the aim of exploring the mentioned aspects, this study investigates, for the first time, the potential beneficial effects of chronic HT-rich extract supplementation (15 mg/day) in individuals at-risk with prediabetes and overweight—metabolic alterations commonly associated with aging.

2. Material and methods

2.1. Study design and intervention

This study was a parallel, randomized 1:1, double-blinded, placebo-controlled trial with a duration of 16 weeks. The enrollment and study were conducted by researchers between December 2023 and September 2024. All visits took place at the Human Nutrition Unit (HNU) of the Institute of Food Science, Technology and Nutrition (ICTAN-CSIC, Madrid, Spain). The study protocol was reviewed and approved by both the Ethics Committee of the Hospital Universitario Puerta de Hierro-Majadahonda, (Madrid, Spain) and the Ethics Committee of the CSIC. The study is registered at ClinicalTrials.gov (NCT: 06295913) https://clinicaltrials.gov/study/NCT06295913?intr=Hydroxytyrosol&page=2&rank=14. All volunteers provided written informed consent before recruitment, in accordance with the provisions of the Ethics Committee and the Helsinki Declaration of 1975, as revised in 1983 and Good Clinical Practice Guidelines of the International Council for Harmonization (CPMP/ICH/135/95) and the current Spanish directives (RD 1090/2015).

2.2. Sample size calculation

The sample size was determined using oxLDL levels as the primary variable. A 30 % reduction in this parameter was considered effective. The statistical power was set at 95 %, with a significance level of $\alpha = 0.05$. The variance estimates and the percentage reduction considered significant were based on a previous clinical trial of a HT-supplemented food product that evaluated this parameter [17]. Based on these criteria, the required number of volunteers was calculated to be 20 per study group, increased to 25 to account for an anticipated 20 % dropout rate. Consequently, the total sample size was established at 50 participants.

2.3. Treatment characterization

The HT-rich extract was obtained from the vegetation water of olives (*Olea europaea* L.) through physical and mechanical processes. This olive fruit extract was characterized by containing 10–12 % natural HT (high performance chromatography or HPLC assay) and 1.5–3 % natural tyrosol (HPLC assay), with moisture ≤5 % and ash ≤8 %. The HT-rich extract was formulated into capsules containing 15 mg of HT. Placebo capsules were prepared, composed of inactive excipients to ensure blinding of participants and researchers. HT-rich extract and placebo capsules were manufactured by IF3Lab S.L (Granada, Spain).

2.4. Participants and recruitment

Forty-nine adults with overweight (body mass index or BMI 25.0–30.0 kg/m^2) and prediabetes (HbA1c 5.7–6.4 % and/or fasting glucose 100–126 mg/dL) were randomized in a double-blind intervention design. The participants were aged 40–70 years old (mean age 55.9 years). Exclusion criteria included any prior diagnosis of diabetes, hypertension, hyperlipidemia, other cardiovascular disease, thyroid disease, asthma, arthritis or inflammatory conditions, cirrhosis, or other liver disease; use of any type of pharmacological treatment or dietary supplements containing antioxidants other than vitamins; smoking or regular alcohol consumption; human immunodeficiency virus-positive status; pregnancy or lactation. Recruitment was conducted by the research team, targeting individuals who met the inclusion criteria. Blood analysis was performed to confirm prediabetes status. The participants were allocated into groups HT treatment (Group HT) or placebo (Group P) through simple randomization via random number generator in Microsoft Excel 2019 MSO by research team. Each participant was assigned a unique random code, with group allocation revealed only after the intervention was completed. During the 16-week intervention period, subjects consumed one capsule/day with meals. Additionally, all participants were instructed to avoid antioxidant supplements, EVOO, virgin olive oil (VOO) or table olives during the study due to their HT content. To prevent EVOO consumption during the study, participants were provided with olive oil at a rate of 1 L per week for household consumption. A two-weeks washout period without these products was required before the intervention. At each visit, the dietary intake was assessed using 72 h recall, the level of physical activity was recorded, and compliance with capsule consumption was evaluated by checking the return of empty bottle and measuring HT-3′-sulphate in the first morning void on an empty stomach as biomarker of HT intake.

2.5. Primary and secondary outcomes

oxLDL is a key marker in the pathophysiology of atherogenesis and cardiovascular disease, and HT have been widely described as antioxidant and lipid oxidation protector. Hence, oxLDL was selected as primary endpoint and measured by ELISA (enzyme-linked immunosorbent assay) kit. As secondary endpoints we selected oxidative stress and inflammation biomarkers, markers of glucose homeostasis, cardiometabolic profile, anthropometric parameters, physical fitness, sleep quality and mental well-being, described in detail in Section 2.6. Assessments were done at two time points, before and after intervention.

2.6. Samples and data collection

At the beginning and end of the study, urine samples corresponding to the first morning void on an empty stomach were collected in sterile urine collection vessels and stored at −80 °C until subsequent analysis to assess adherence through HT-3'sulphate determination. Additionally, fasting blood samples (20 mL distributed into tubes containing different anticoagulants for the measurements) were collected before and after intervention. Blood samples were left to rest for 30 min before being centrifuged at 3000 rpm for 10 min at 4 °C. Plasma and serum aliquots were then extracted from the blood and stored at −80 °C until further analysis.

Blood pressure was measured and a complete anthropometric and body composition analysis was performed. Subsequently, mental well-being, sleep quality and fatigue were evaluated. Fig. 1 illustrates the study design and the assessments conducted at each stage.

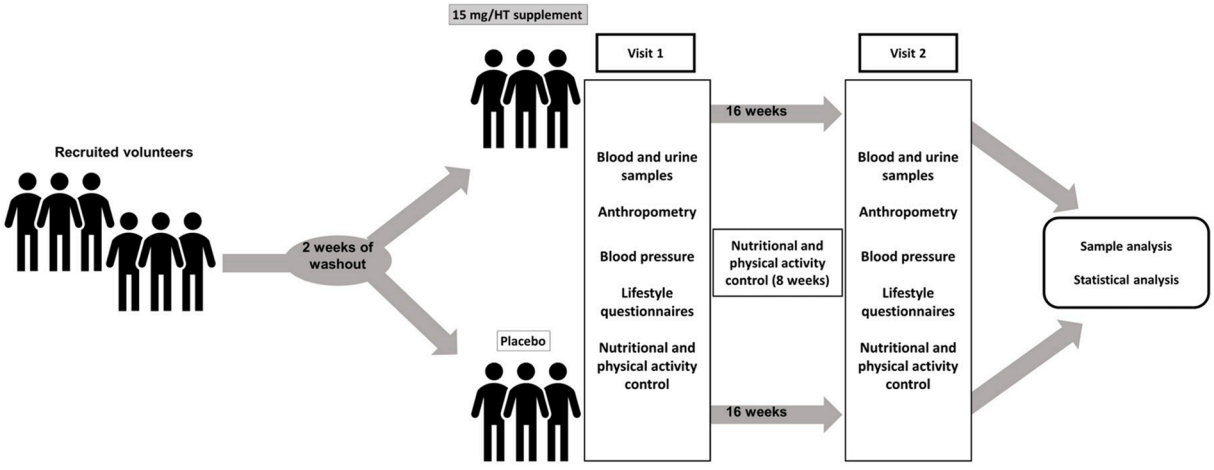

Fig. 1. Schematic representation of the intervention study. Participants were recruited and, after a two-week washout period, were randomized to placebo or supplementation with 15 mg/day of hydroxytyrosol (HT). During the first visit (baseline point), blood and urine samples were collected, and anthropometric measurements, blood pressure assessments, lifestyle-related questionnaires, as well as nutritional and physical activity evaluations, were conducted. A follow-up assessment of nutrition and physical activity was performed at week 8. After 16 weeks of intervention, the same procedures as in the first visit were repeated. Biological samples were analyzed, and all collected data were subjected to statistical analysis.

2.6.1. Oxidative stress and inflammation biomarkers

Oxidative stress biomarkers, including protein carbonyls, NADPH oxidase (NOX), total antioxidant status (TAS), total oxidant stress (TOS) and thiobarbituric acid reactive substances (TBARS) were measured using an ELISA kit from MyBioSource (San Diego, USA). Superoxide dismutase (SOD) and glutathione peroxidase (GPx) were measured using an ELISA kit from Sigma Aldrich (Madrid, Spain), 8-hydroxy-2′-deoxyguanosine (8-OHdG) with an ELISA kit from Elabscience (USA), glutathione (GSH) with an ELISA kit from Invitrogen ThermoFisher (Madrid, Spain) and oxLDL with an ELISA kit from Cloud-Clone Corp (USA). Inflammation biomarkers, including and C-reactive protein (CRP), interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α) were measured by an autoanalyzer.

2.6.2. Biochemical parameters

Biochemical parameters related to glucose and lipid homeostasis, and liver function including HbA1c, glucose, insulin, cholesterol, triglycerides, high-density lipoproteins (HDL), LDL, apolipoprotein B (ApoB), apolipoprotein A1 (ApoA1), uric acid, alanine transaminase (ALT) and aspartate transaminase (AST) were measured by an autoanalyser. Glucagon-like peptide 1 (GLP-1) was measured using an ELISA kit from Merck (Madrid, Spain). The Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) and Beta-cell Function (HOMA-B) were calculated using fasting insulin and glucose data.

2.6.3. Blood pressure

Blood pressure measurements were performed with the automatic blood pressure monitor M6 Comfort (Omron, Madrid, Spain). Beats per minute, systolic and diastolic blood pressure (SBP/DBP) were assessed three times in a seated position after a resting period.

2.6.4. Anthropometric parameters

Anthropometric parameters were measured at baseline and after the 16-weeks of follow-up, using InBody 707 body composition analyzer (Microcaya, Bilbao, Spain). Weight, BMI and fat-related measures, including fat percentage, waist-to-hip ratio, fat mass index, visceral fat level and visceral fat area, were determined with minimal clothing and without shoes. Height was measured in a standing position without shoes by a stadiometer with the nearest 0.5 cm.

2.6.5. Lifestyle assessment

Mental well-being was evaluated using the Warwick–Edinburgh Mental Well-being Scale (WEMWBS) [20]. This 14-item scale evaluates subjective well-being and psychological functioning. Participants responded to each item on a 1-to-5 Likert scale, with total scores ranging from 14 (lowest well-being) to 70 (highest well-being).

Sleep quality was assessed using the Pittsburgh Sleep Quality Index (PSQI) [21]. This is a self-rated questionnaire assessing sleep quality and disturbance over the past month. Seven items were scored from 0 to 3: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleep medication, and daytime dysfunction. The total score ranges from 0 to 21, with scores of $\leq$5 indicating good sleep quality and scores >5 indicating poor sleep quality (worsening with higher values of the scale).

Physical fitness was measured using the Senior Fitness Test [22], a comprehensive battery assessing a range of physical abilities, including flexibility, strength, balance, and endurance, including a 6-minute walking test. After completing the physical tasks, participants rated their perceived physical exertion using the Borg Scale, ranging from 0 (resting) to 10 (extreme fatigue).

2.7. Nutritional and physical activity control throughout the study

Participants were asked to complete a 72-h dietary recall for each period of the study, including a weekend day, a weekday, and the day preceding each study visit. The data from each questionnaire were analyzed using the EvalFinut software (Iberoamerican Nutrition Foundation, Granada, Spain) to determine the macronutrient composition.

The Global Physical Activity Questionnaire (GPAQ) was completed by each participant to ensure that there were no significant changes in their levels of physical activity throughout the study. The physical activity value was calculated by adding the number of minutes spent on vigorous physical activities (e.g., strength training, intense exercise) multiplied by 8, to the number of minutes spent on moderate physical activities (e.g., walking, stair climbing) multiplied by 4. Values greater than 3000 were categorized as a high level of physical activity, values between 600 and 3000 as a moderate level of physical activity, and values below 600 as a low level of physical activity.

2.8. Assessment of intervention adherence

HT-3′-sulphate was determined in urine samples by HPLC-ESI-QTOF (High-Performance Liquid Chromatography-Electrospray Ionization-Quadrupole Time-of-Flight) analysis to assess adherence of participants to the corresponding intervention.

Urine samples were diluted with an equivalent volume (1:1, v/v) of Milli-Q water and centrifuged at 14,000 rpm (20 min, 4 °C). Supernatants were filtered (0.45 μm cellulose acetate membrane filters) and a 5 μL aliquot was directly injected into the LC-MS-QTOF equipment

(Agilent 1200 series LC system coupled to an Agilent 6530 A Accurate-Mass Quadrupole Time-Of-Flight with ESI-Jet Stream Technology from Agilent Technologies). Compounds were separated on a reverse-phase Luna C18(2) 100 A (50 × 2 mm, 5 μm) column (Phenomenex, Spain) preceded by a Phenomenex 613477-5 precolumn at 30 °C. Then, 5 μL of urine was injected and separated using a mobile phase consisting of Milli-Q water (phase A) and acetonitrile (phase B), both containing 0.1 % formic acid, at a flow rate of 0.4 mL min^{-1}. The mobile phase was initially programmed with 95 % of solvent A and 5 % of B. The elution program increased to 30 % of solvent B in 15 min, 50 % solvent B in 5 min and 90 % solvent B in 5 min, maintaining 90 % solvent B for 4 min. Then, the initial conditions (5 % solvent B) were recovered in 1 min and maintained for 5 min. The QTOF acquisition conditions were as follows: drying gas flow (nitrogen, purity >99.9 %) and temperature were 10 L min^{-1} and 325 °C, respectively; sheath gas flow and temperature were 6 L min^{-1} and 250 °C, respectively; nebulizer pressure was 25 psi; cap voltage was 3500 V, and nozzle voltage was 500 V. The mass range selected was from 100 up to 970 m/z in negative and positive mode and the fragmentor voltage was 150 V. Data were processed in Mass Hunter Workstation Software.

For quantification, a standard curve of commercial HT-3′-sulphate was prepared from 5 to 5000 nM. Linear response was checked by linear regression analysis.

2.9. Statistical analysis

All statistical analyses were conducted using SPSS 22.0 (SPSS Inc., Chicago, USA), Microsoft Excel 2019 MSO, and RStudio 4.4.2. Categorical data, such as sex, are presented as percentages, while quantitative data are expressed as mean ± standard deviation (SD)

or 95 % confidence interval. Outliers and extreme values were identified and excluded using the interquartile range method. Normality (based on kurtosis) and homogeneity of variances were assessed to ensure the use of appropriate statistical tests. Variables with skewed distributions were logarithmically transformed prior to analysis.

Baseline data were analyzed to identify any significant inter-group differences in the inclusion parameters and other variables at the start of the study. For quantitative variables, unpaired *t*-tests were employed, while the Chi-squared (χ^2) test was used for categorical variables such as sex.

Rates of change in primary and secondary outcomes were analyzed using the Mann–Whitney U test for nonparametric distributions and unpaired *t*-tests for parametric distributions. To compare changes from baseline to end between Group HT and Group P, a one-way analysis of covariance (ANCOVA) was conducted for nutritional intake and physical activity. Age and sex were included as covariates in the ANCOVA model. A significance threshold of $p < 0.05$ was applied to all statistical tests.

3. Results

3.1. Recruitment and baseline characteristics

This study was a parallel, randomized, double-blinded, placebo-controlled trial with a duration of 16 weeks. Recruitment was conducted via email and flyers, reaching a total of 202 potential participants. They were all asked for eligibility, resulting in the exclusion of 150 individuals due to lack of interest or failure to meet inclusion criteria. Fifty-two participants were successfully recruited from December 2023 to March 2024 and were randomly assigned to either the intervention (Group HT) or placebo (Group P) groups. During the course of the study between March 2024 and September 2024, three participants (two from Group HT and one from Group P) withdrew due to personal reasons. Finally, the remaining 49 participants who completed the study were included in the final analysis. The CONSORT diagram of the study is shown in Fig. 2. Additionally, no adverse effects related to the treatments was recorded and liver function was normal since transaminases (ALT and AST) were unaltered following chronic administration in both groups, as Supplementary Table 1 shows.

As shown in Table 1, participant baseline characteristics were comparable, with no significant differences observed between the groups prior to the start of the intervention. Notably, the eligibility parameters (BMI, glucose and HbA1c) did not differ significantly between the groups.

3.2. Adherence assessment

HT is primarily metabolized in the body into HT-3′-sulphate, which is subsequently excreted in urine. Treatment adherence was evaluated by monitoring changes in urinary HT-3′-sulphate levels, as previously described. Statistical analysis revealed a significant increase in HT-3′-sulphate concentration in Group HT, while Group P showed a slight but significant decrease compared to Group HT (Table 2). These findings confirm that Group HT adhered to the treatment, while Group P effectively excluded EVOO, VOO and table olives from their diet, avoiding dietary sources of HT.

3.3. Oxidative stress and inflammation biomarkers

Oxidative stress was assessed using a range of parameters, including TOS, TAS and, GSH, as well as enzymes related to the redox status such as SOD, GPx, and NOX. Indicators of oxidative

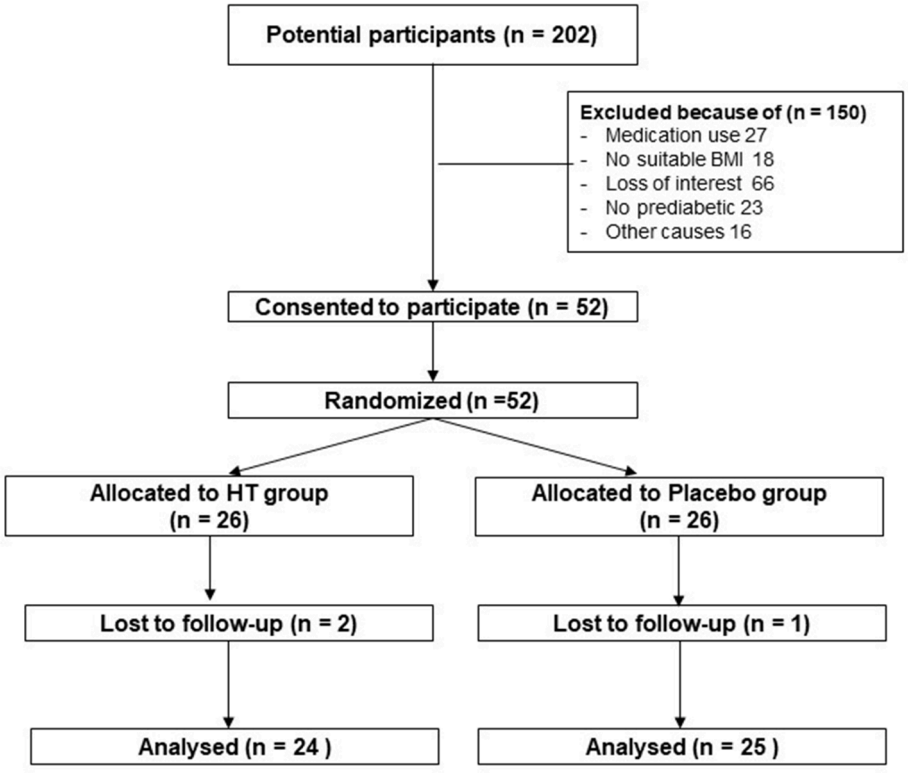

Fig. 2. CONSORT Diagram. Flow-chart showing enrollment, recruitment, randomization and allocation into hydroxytyrosol (HT) or placebo (P) group and analysis throughout the study.

Table 1
Baseline characteristics of HT and P groups. Values are presented as means ± standard deviations. Groups were compared with $\chi 2$ statistics for categorical measures (e.g. sex) and t-tests for continuous variable[a].

	HT (n = 24)	P (n = 25)	*p*-value
Age (years)	54.50 ± 8.54	57.40 ± 7.90	0.112
Sex, female (%)	45.83 %	44.00 %	0.897
BMI (kg/m^2)	29.17 ± 2.86	27.94 ± 2.71	0.067
HbA1c (%)	5.80 ± 0.31	5.78 ± 0.29	0.427
Glucose (mg/dL)	97.50 ± 10.11	93.08 ± 6.94	0.083

[a] BMI (body mass index); HbA1c (glycosylated hemoglobin).

Table 2
Rates of change of hydroxytyrosol-3′-sulphate in HT and P groups. Values are means with their respective confidence intervals 95 %. Groups were compared with unpaired t-student (*$p < 0.05$).

	HT (n = 24)	P (n = 25)	*p*-value
HT-3′-sulphate Rate of change (μM)	0.26 (−0.10; 0.63)	−0.12 (−0,34; 0,10)	0.039*

macromolecular damage, including oxLDL, protein carbonyls, TBARS, and 8-OHdG, were also analyzed. Additionally, inflammation was evaluated by measuring CRP, TNF-α and IL-6 levels.

The differences in oxidative stress and inflammation biomarkers between Groups HT and P are summarized in Table 3, which shows the comparison between rates of change observed after 16 weeks (post-intervention minus pre-intervention) in both groups. HT supplementation exhibited antioxidant properties, as evidenced by a significant reduction in oxLDL ($p = 0.045$), protein carbonyls ($p = 0.031$) and 8-OHdG ($p < 0.01$) compared to placebo. Additionally, HT prevented a decline in GPx levels ($p < 0.01$) and TAS ($p < 0.01$), whereas placebo treatment failed to preserve these parameters. Regarding inflammation, IL-6 levels decreased significantly ($p = 0.05$) in Group HT compared to Group P, while no significant differences were observed in the remaining inflammatory markers. Overall, HT supplementation significantly improved oxidative and inflammatory status in individuals with overweight and prediabetes.

3.4. Glucose homeostasis

In order to assess the effects of treatments HT and P on glucose homeostasis, HbA1c, glucose, insulin, HOMAB, HOMAIR and GLP-1 were analyzed and the results are summarized in Table 4. As shown, the increase in GLP-1 levels was significantly greater in Group P compared to Group HT ($p = 0.014$). Additionally, although HOMAB did not show a statistically significant difference between groups, Group P exhibited an evident increasing trend that was not observed in Group HT.

3.5. Cardiometabolic profile

The cardiometabolic profile combines vascular pressure and lipid homeostasis. The parameters analyzed include triglycerides, cholesterol, LDL, HDL, ApoB, ApoA1 and ApoB/ApoA1. Table 5 shows the rates of change in these cardiometabolic parameters in Groups HT and P. No significant differences were observed between the two groups in any of the measured parameters.

3.6. Anthropometric parameters

Several anthropometric variables were measured, including weight and BMI, along with specific body fat-related parameters such as body fat percentage, waist-to-hip ratio, fat mass index, and visceral fat level and area. Regarding the rates of change in anthropometric parameters, no statistically differences were observed between Groups HT and P (Table 6).

3.7. Healthy lifestyle assessment

Physical fitness and aerobic capacity are key factors in the prevention of age-related diseases. The participants performed several tasks, including assessments of flexibility, strength, and walking. Following these tasks, subjective fatigue was evaluated using the Borg Scale. The Supplementary Table 2 shows the rates of change in physical fitness in groups HT and P. No significant difference was observed between the rates of change of each group.

To evaluate parameters related to sleep quality and mental health, participants fulfilled the PSQI and the WEMWBS, respectively. The results presented in Supplementary Table 3 indicate that there were no significant changes between the Groups HT and P.

Table 3
Rates of changes in oxidative stress and inflammation biomarkers in Groups HT and P. Values are means ± standard deviation or means with their respective confidence intervals 95 % for rates of change. Groups were compared with unpaired t-student (*$p < 0.05$)[a].

Oxidative stress biomarkers	HT (n = 24)		P (n = 25)		*p*-value
	Post-int	Rate of change	Post-int	Rate of change	
oxLDL (μM)	969.48 ± 45.89	−5.11 (−34.46; 24.24)	969.86 ± 53.20	29.69 (0.10; 59.27)	0.045*
TOS (μmol/L)	7.36 ± 1.89	2.98 (2.03; 3.92)	7.85 ± 2.63	3.22 (2.08; 4.35)	0.367
TAS (μmol/L)	1.04 ± 0.09	0.06 (0.01; 0.11)	1.06 ± 0.08	−0.04 (−0.07; −0.00)	<0.001*
SOD (U/mL)	18.63 ± 2.52	0.35 (−0.66; 1.37)	18.81 ± 2.58	0.14 (−0.95; 1.24)	0.273
GPx (U/mL)	348.55 ± 44.10	−3.85 (−20.66; 12.95)	319.01 ± 41.75	−60.84 (−79.98; −41.69)	<0.001*
TBARS (μM)	2.67 ± 0.58	1.20 (1.02; 1.38)	2.71 ± 0.89	1.29 (0.96; 1.63)	0.307
Protein carbonyls (μM)	19.14 ± 4.69	−0.45 (−1.78; 0.87)	25.02 ± 4.10	2.21 (−0.32; 4.74)	0.031*
GSH (μM)	5.88 ± 0.85	−1.12 (−1.91; −0.34)	5.69 ± 1.00	−0.68 (−1.46; 0.09)	0.204
NOX (ng/mL)	7.15 ± 1.04	1.12 (0.73; 1.51)	9.03 ± 1.62	1.89 (−0.96; 4.75)	0.065
8-OHdG (ng/mL)	61.46 ± 40.43	−36.69 (−46.44; −26.94)	83.51 ± 48.26	−8.13 (−22.83; 6.57)	<0.001*
Inflammation biomarkers					
CRP (μU/ml)	0.18 ± 0.15	−0.59 (−1.66; 0.47)	0.15 ± 0.19	−0.01 (−0.50; 0.02)	0.349
TNF-α (pg/mL)	8.55 ± 3.07	2.15 (0.96; 3.34)	9.45 ± 4.24	2.15 (0.70; 3.60)	0.39
IL-6 (pg/mL) (n = 28, HT = 14)	2.84 ± 2.33	−2.31 (−3.97; −0.65)	1.75 ± 1.43	−0.69 (−2.04; 0.67)	0.05*

[a] Post-int (post-intervention); oxLDL (oxidized low-density lipoproteins); TOS (total oxidative stress), TAS (total antioxidant status); SOD (superoxide dismutase); GPx (glutathione peroxidase); TBARS (thiobarbituric acid reactive substances); GSH (glutathione); NOX (NADPH oxidase); 8-OHdG (8-hydoxy-2′-deoxyguanosine); CRP (C reactive protein); TNF- α (tumor necrosis factor alpha); IL-6 (interleukin 6).

Table 4
Rate of change in glucose homeostasis parameters in Groups HT and P. Values are means ± standard deviation or means with their respective confidence intervals 95 % for rates of change. Groups were compared with unpaired t-student (*$p < 0.05$)[a].

Variable	HT (n = 24)		P (n = 25)		*p*-value
	Post-int	Rate of change	Post-int	Rate of change	
HbA1c (%)	5.87 ± 0.31	0.12 (0.05; 0.19)	5.85 ± 0.25	0.07 (0.02; 0.11)	0.093
Glucose (mg/dL)	99.52 ± 10.54	4.91 (0.53; 9.30)	98.92 ± 8.70	5.84 (3.38; 8.30)	0.191
Insulin (μIU/mL)	8.73 ± 3.34	1.52 (−0.13; 3.16)	10.06 ± 3.22	2.40 (0.74; 4.06)	0.219
HOMAB	94.10 ± 37.85	8.30 (−5.59; 22.19)	113.19 ± 42.85	20.24 (6.42; 34.05)	0.106
HOMAIR	2.42 ± 1.12	0.45 (−0.00; 0.91)	2.45 ± 0.84	0.77 (0.26; 1.28)	0.177
GLP-1 (pM)	25.15 ± 7.97	−0.62 (−3.65; 2.41)	34.12 ± 8.34	5.33 (0.85; 9.80)	0.014*

[a] Post-int (post-intervention); HbA1c (glycosylated hemoglobin); HOMAB (Homeostatic Model Assessment for Beta cell Function); HOMAIR (Homeostatic Model Assessment for Insulin Resistance); GLP-1 (glucagon-like peptide 1).

Table 5
Rates of change in cardiometabolic profile in Groups HT and P. Values are means ± standard deviation or means with their respective confidence intervals 95 % for rates of change. Groups were compared with unpaired t-student(*$p < 0.05$)[a].

Lipid homeostasis	HT (n = 24)		P (n = 25)		*p*-value
	Post-int	Rate of change	Post-int	Rate of change	
Cholesterol (mg/dL)	214.87 ± 27.70	4.96 (−7.67; 17.58)	221.33 ± 40.45	10.92 (−0.19; 22.03)	0.233
Triglycerides (mg/dL)	115.42 ± 34.99	17.21 (0.54; 33.88)	118.87 ± 50.52	16.60 (−2.94; 36.14)	0.481
HDL (mg/dL)	54.20 ± 11.09	4.64 (1.87; 7.41)	56.30 ± 12.83	4.17 (1.03; 7.30)	0.409
LDL (mg/dL)	132.72 ± 27.25	−0.72 (−8.99; 7.54)	141.56 ± 29.50	4.46 (−4.65; 13.56)	0.196
ApoA1 (mg/dL)	143.58 ± 16.43	3.56 (−0.52; 7.65)	147.96 ± 21.34	2.8 (−1.87; 7.47)	0.401
ApoB (mg/dL)	103.95 ± 21.49	4.55 (0.15; 8.94)	104.83 ± 20.17	6.29 (0.36; 12.22)	0.316
ApoB/ApoA1	0.74 ± 0.21	0.01 (−0.03; 0.05)	0.74 ± 0.18	0.04 (−0.01; 0.08)	0.178
Cardiovascular parameters					
SBP (mmHg)	121.44 ± 12.32	−3.50 (−7.12; 0.11)	122.39 ± 11.69	−0.72 (−4.59; 3.14)	0.142
DBP (mmHg)	82.57 ± 7.25	−0.94 (−3.54; 1.7)	79.89 ± 6.478	0.48 (−1.98; 2.95)	0.206
Beats per minute	69.68 ± 8.54	−1.88 (−5.87; 2.11)	67.60 ± 7.55	0.17 (−3.04; 3.38)	0.205

[a] Post-int (post-intervention); HDL (high-density lipoproteins); LDL (low-density lipoproteins); ApoA1 (apolipoprotein A1); ApoB (apolipoprotein B); SBP (systolic blood pressure); DBP (diastolic blood pressure).

Table 6
Rates of change on Anthropometric parameters in Groups HT and P. Values are means ± standard deviation or means with their respective confidence intervals 95 % for rates of change. Groups were compared with unpaired t-student (*$p < 0.05$)[a].

Variable	HT (n = 24)		P (n = 25)		*p* value
	Post-int	Rate of change	Post-int	Rate of change	
Weight (kg)	80.62 ± 9.83	0.34 (−0.46; 1.15)	79.25 ± 9.90	0.92 (0.14; 1.70)	0.149
BMI (kg/m^2)	28.77 ± 2.69	0.14 (−0.15; 0.44)	28.18 ± 2.66	0.14 (−0.14; 0.43)	0.499
Body fat (%)	35.29 ± 9.04	−0.14 (−0.75; 0.48)	32.68 ± 7.25	0.27 (−0.41; 0.95)	0.180
Waist-to-hip ratio	0.97 ± 0.07	0.00 (−0.01; 0.01)	0.96 ± 0.07	0.01 (−0.00; 0.02)	0.053
Fat Mass Index (kg/m^2)	10.28 ± 3.35	0.03 (−0.26; 0.31)	9.30 ± 2.69	−0.02 (−0.32; 0.29)	0.415
Visceral Fat Level	13.69 ± 4.94	0.00 (−0.64; 0.64)	12.12 ± 4.07	0.16 (−0.37; 0.69)	0.765
Visceral Fat Area (cm^2)	143.10 ± 51.47	1.07 (−2.37; 4.51)	126.44 ± 40.12	2.01 (−3.03; 7.05)	0.380

[a] Post-int (post-intervention); BMI (body mass index).

3.8. Nutrient intake and physical activity control

Supplementary Table 4 shows the results regarding nutrient intake and physical activity throughout the study, adjusting the statistical analysis by sex and age. Regarding nutritional intake, significant differences were observed between Group HT and Group P in total energy intake, as well as for saturated fatty acids (SFA) and polyunsaturated fatty acids (PUFA) intakes. However, the percentage intake of each macronutrient remained unchanged. For physical activity, no significant differences were observed between or within groups before and after the intervention. Both groups maintained a stable moderate-to-high level of physical activity throughout the study.

4. Discussion

The growing market for dietary supplements [12] has made key phytochemicals such as HT accessible to populations that do not traditionally consume EVOO or related products like table olives. While this expands accessibility, the health effects of HT supplementation in isolation remain poorly understood, particularly since EFSA health claim applies only when these phenols are consumed within EVOO. In this regard, it is important to note that research on HT has been conducted in healthy individuals [17] or those with risk factors such as overweight [19]. Also, some studies have examined its effects in combination with oleuropein (OLE) [23,24], which is metabolized in the body to release free HT [25].

However, there is a lack of studies assessing HT supplementation in individuals with prediabetes, a condition that significantly increases the risk of multiple aging-related diseases and involves distinct metabolic and oxidative alterations. Considering all the aforementioned aspects, our study takes a novel approach by investigating the effects of daily supplementation with 15 mg of HT (three times EFSA recommended intake) for 16 weeks in individuals with both overweight and prediabetes, aiming to enhance our understanding of its potential role in aging-related disease prevention.

Oxidative stress associated with prediabetes and overweight contributes to macromolecular damage and the onset of pathological processes. Therefore, a major focus of our study was to evaluate potential improvements in oxidative status. We demonstrated that HT supplementation led to a reduction in oxLDL levels, a well-established biomarker of atherogenesis risk [26]. This finding aligns with the EFSA health claim (2011), which recognizes the benefits of olive oil phenolic compounds within EVOO in reducing LDL oxidation [11]. However, evidence on the effects of HT in isolation remains inconsistent, likely due to differences in study design, duration, and formulation. For instance, studies administering HT alone at 15 mg/day for three weeks in healthy volunteers [18] or in combination with OLE (HT 9.7 mg/day and OLE 51.1 mg/day) for 12 weeks [23] in middle-aged overweight men did not observe changes in oxLDL levels. In contrast, acute postprandial studies have reported significant reductions: a study where 12 healthy volunteers received a single dose (25 mL) of a liquid olive phenolic supplement (30.6 or 61.5 mg HT) showed a reduction in oxLDL levels 1 h after ingestion [27], while a fortified biscuit portion containing 5.25 mg HT led to a reduction of oxLDL at 0.5- and 4-h post-ingestion [17]. In this complex landscape, our study provides novel insights by demonstrating that HT alone, administered as a dietary supplement, can reach a similar effect against lipoprotein oxidation as that achieved by olive phenols within EVOO.

Regarding other oxidative stress biomarkers, in the present study, HT supplementation maintained TAS and GPx levels in the Group HT, whereas both parameters decreased in the Group P. This preservation of GPx activity could explain, at least in part, the observed reduction in oxidative damage markers for macromolecules such as DNA (8-OHdG) and proteins (carbonyls), although no significant effects were observed for lipid peroxidation (TBARS). Similarly, Colica et al. (2017), using the same dose of HT as in our study, found that three weeks of supplementation in healthy individuals led to a reduction in lipid peroxidation (MDA), nitrates, and nitrites, while increasing TAS and SOD [18] Overall, our findings suggest that HT may reduce oxidative stress in individuals with overweight and prediabetes by its ability to modulate antioxidant enzymes.

Along with oxidative stress, low-grade chronic inflammation is a common underlying physiopathological process strongly linked to age-related chronic diseases. Since HT's anti-inflammatory activity is well-established *in vitro* and *in vivo* [28], it is pertinent to assess its effects in at-risk populations. In our study, we found a mild effect of HT supplementation on inflammation with a significant reduction in IL-6 levels, a key pro-inflammatory marker, while no significant changes were detected in CRP or TNF-α levels. Similarly, in prehypertensive individuals, daily supplementation with OLE (136.2 mg) and HT (6.4 mg) for six weeks reduced IL-8 levels [24]. Likewise, the levels of IL-6, IL-8, and TNF-α decreased significantly in patients with hypertension supplemented with an olive leaf extract for 12 weeks [29]. And Binou et al. (2023) reported that the consumption of HT-enriched whole wheat bread consumption (32.4 mg HT) for 12 weeks by subjects with overweight/obesity and T2D led to attenuation of TNF-α levels while no effect regarding CRP was reported [16]. Accordingly, previous studies on the effects of dietary polyphenols in different steps of the nucleotide-binding oligomerization domain-like receptors protein 3 (NLRP3) inflammation signaling cascade have shown discrepancies in the specific cytokines that were modified, but with a clear overall anti-inflammatory effect [30]. Taken together, our results indicate the potential of HT supplementation to improve inflammatory status in people with overweight and prediabetes.

Although this study did not assess specific markers of cardiovascular outcomes, previous studies reported that reduction in oxidative stress and inflammation biomarkers, was accompanied by improvements in cardiac and vascular performance in subjects with chronic coronary artery syndrome supplemented with isolated HT [31] or combined with other olive polyphenols [32]. Together, these findings reinforce the potential of HT as a preventive strategy against NCDs, particularly cardiovascular diseases.

Notably, we observed increased GLP-1 levels and a non-significant rise in HOMA-B (β-cell function) in Group P. The increase in HOMA-B is consistent with a prediabetic state, in which pancreatic β-cells increase insulin synthesis and secretion as an adaptive response to reduced tissue sensitivity to insulin [33]. Likewise, the elevation in GLP-1—a key incretin hormone that stimulates insulin secretion—may reflect a pathophysiological compensation, likely driven by hyperglycemia and insulin resistance, which are hallmarks of prediabetes. This interpretation is supported by previous studies that have reported that individuals with isolated impaired fasting glucose may exhibit elevated GLP-1 secretion during oral glucose tolerance tests. This is potentially a compensatory mechanism to preserve near-normal insulin output in the early stages of prediabetes [34]. However, GLP-1 levels tend to decline throughout T2D progression, making GLP-1 agonists an indicated pharmacological treatment [35]. Interestingly, these compensatory responses were not present in Group HT, suggesting that participants in this group required less physiological adaptation to maintain glucose homeostasis. Overall, this may indicate a protective effect of HT in preserving insulin sensitivity and reducing metabolic stress on β-cells, although further investigation is needed to substantiate this hypothesis due to the absence of consensus among studies [36].

As regards lipid profile, in the present study no significant changes were observed in the participants following HT supplementation, possibly because both groups initially exhibited a normolipidemic profile. Similarly, a study by Colica et al. (2017), which administered 15 mg/day of HT to healthy individuals over a shorter period (three weeks), also reported no changes in lipid parameters, including cholesterol and triglycerides [18]. However, other studies using olive leaf infusions with a known concentration of HT (11.9 mg) and OLE (320.8 mg) per day demonstrated a reduction in LDL and triglycerides levels after 12 weeks of supplementation in a prediabetic population [37], possibly due to the higher total polyphenol content.

Importantly, the maintenance of body weight throughout the study ensures that the observed changes in oxidative and inflammatory profiles were not confounded by weight loss. Similarly, dietary intake and physical activity remained stable, further reinforcing the reliability of our findings. These results align with a previous study reporting no effect of HT on these parameters, including a 12-week trial in overweight individuals receiving 9.7 mg/day of HT combined with 51.1 mg/day of OLE [23]. However, significant reductions in body weight and fat mass were observed when HT was administered as a pure compound at 15 mg/day for six months in overweight/obese women [19]. Another study, which used whole-grain bread enriched with 32.5 mg/day of HT in overweight/obese individuals with T2D for 12 weeks [16], reported

significant reductions in body weight, body fat and waist circumference. However, the participants in that study had a higher baseline BMI than those in our study (33.3 *vs* 28.8 kg/m^2), which may have contributed to the observed weight loss, as greater initial excess weight often facilitates more pronounced reductions. This suggests that HT's effects on body composition may depend on factors such as population characteristics, sex or prolonged exposure. Additionally, we explored potential effects on mental well-being and sleep quality, but no substantial changes were detected. Nonetheless, evidence suggests that polyphenols positively modulate these variables [38,39]. Future studies should investigate these effects in populations with existing sleep or mood disorders, where more pronounced improvements may be observed.

The strengths of the present study include the high adherence of participants to the intervention, a sample size exceeding the required 20 participants per group, ensuring adequate statistical power and a sufficiently long intervention period (16 weeks). Additionally, the intervention's simple dosing regimen (a single daily capsule) contributed to compliance, which was validated by a biological biomarker (HT 3′-sulphate). The main limitations of the study are related to design aspects. A crossover study design could have provided better control over individual variability, leading to more robust data and more precise insights into the intervention's effects. Similarly, the lack of postprandial analyses limited our ability to assess potential improvements in glucose modulation following oral intake. At the same time, future studies should integrate omics approaches, including microbial profiling, metabolite analysis, and gene expression modifications, to provide a comprehensive understanding of the effects of olive polyphenols on health and the underlying mechanisms of their benefits.

Regarding external validity, it should be mentioned that this study was conducted in a Mediterranean population, which is habitually exposed to HT through dietary sources. It would be valuable to explore whether similar improvements occur in populations with lower baseline exposure to this phytochemical, where the impact of supplementation might be even more pronounced. Finally, it is worth emphasizing that this trial was conducted under free-living conditions, which increases the translational relevance of the findings to everyday clinical and public health contexts. The beneficial effects observed with HT supplementation could be particularly relevant when envisioned as part of a comprehensive lifestyle approach that includes balanced nutrition and regular physical activity. Future research should explore this integrative framework, as the combination of targeted supplementation with healthy lifestyle practices may produce synergistic effects—further enhancing the regulation of oxidative stress and inflammation, and ultimately contributing to the control of cardiometabolic risk.

5. Conclusion

A randomized, double-blind, placebo-controlled parallel study was conducted in individuals with overweight and prediabetes, who were supplemented with 15 mg/day of HT for 16 weeks. The results demonstrated a significant effect on oxidative status markers, including improvements in oxLDL levels, protein carbonyls and 8-OHdG, as well as the prevention of a decline in TAS and GPx activity. Additionally, a beneficial effect was observed on inflammation, specifically in IL-6 levels.

These findings highlight the potential of HT, when consumed as a dietary supplement, to protect lipids from oxidative damage, thus expanding previously reported beneficial effects for this compound when found outside of EVOO matrix. Moreover, the study demonstrates that these effects are also relevant in populations at increased risk for NCDs, thereby underscoring its promise in early preventive strategies.

Further studies should assess the effects of HT supplementation on other processes closely linked to age-related diseases, such as miRNA expression or dysbiosis.

Author contribution

Conceptualization: R.M, M.A.M; Data curation: I.M-R, S.R; Formal analysis: I.M-R; Funding acquisition: R.M, M.A.M; Investigation: R.M, M.A.M, J.P-J, S.R; I.M-R; Methodology: R.M, M.AM, J.P-J, S.R, I.M-R; Project administration: R.M, M.A.M; Supervision: R.M; M.A.M, J.P-J, S.R, M.P.P; Validation: R.M; M.A.M; Writing original draft; I.M-R; Writing-review and editing: R.M; M.A.M; J.P-J; S.R; M.P.P.

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Conflict of Interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnu.2025.07.006.

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