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The effect of minimal dietary changes with raisins in NAFLD patients with non-significant fibrosis: a randomized controlled intervention†‡

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Aiming at investigating the potential effect of minimal dietary changes in NAFLD patients with non-significant fibrosis, 55 patients with NAFLD were enrolled in a randomized controlled clinical trial. Patients were assigned into two isocaloric dietary treatment groups for 24 weeks: (a) nutritional counseling (Control arm, N = 27), (b) nutritional counseling with currants included (two fruit servings, 36 g per day), substituting snacks of similar caloric content (Currant arm, N = 28). Clinical tests, anthropometrics, inflammatory and oxidative stress markers were conducted pre- and post-intervention. A total of 50 patients completed the trial. Significant differences between the two arms post-intervention were observed in fasting glucose and in IL-6 levels, these being significantly decreased only in Currant patients. Body weight, BMI, HbA_{1c}, CRP and EUS values decreased in both arms, differences being insignificant between the two arms post-intervention. Participants in the Currant arm had significantly reduced total body fat, WC and trunk fat. Ultrasound scanning improved significantly in patients snacking currants daily. Also, volunteers enrolled in the Currant arm showed a reduced intake of saturated fatty acids. Because BW regulation has been officially recognised as a treatment approach in NAFLD an additional analysis was repeated in patients adhering to this. Post-intervention, the decrease in IL-6 and in fasting glucose was significantly higher in Currant patients who lost BW compared to their counterparts in the Control arm. Conclusively, minimal modifications in snacking choices, such as the inclusion of dried grapes in diet, are beneficial in NAFLD patients with non-significant fibrosis.

1 Introduction

Non-alcoholic fatty liver disease and steatohepatitis (NAFLD/NASH) is one of the most common liver disorders and a major public health problem worldwide.[1] It includes a spectrum of liver injury beginning from simple steatosis to nonalcoholic steatohepatitis (NASH) that leads to advanced fibrosis and cirrhosis.[2] The rising prevalence of NAFLD is closely related to the convergent epidemics of obesity, insulin resistance and type 2 diabetes, while it is considered the hepatic complication of obesity. The estimated worldwide prevalence of NAFLD is approximately 30% and this doubles within the type 2 diabetic population.[3] The prevalence of NASH is approximately 7%, and this too is estimated to be at least two-fold higher among people with type 2 diabetes. Since obesity is a growing worldwide epidemic, the prevalence of NAFLD/NASH tends to increase ranging from 2.8% to 46%, depending on the study population and on the diagnostic tool.[4,5] Pathogenesis is unclear with the most widely supported theory implicating

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insulin resistance (IR), which impairs lipid metabolism leading to fat accumulation within the liver, as the key mechanism. Additionally, oxidative injury is required to manifest the necroinflammatory component of steatohepatitis resulting from the combination of oxidative stress, lipid peroxidation, mitochondrial dysfunction, hormonal abnormalities and cellular toxicity from free fatty acids. Mitochondrial dysfunction is crucial in the pathogenesis of NAFLD/NASH, leading to the overproduction of reactive oxygen species that promote hepatocyte injury.[6,7] Oxidative stress triggers cell membrane peroxidation, cell degeneration and apoptosis, and the expression of pro-inflammatory and pro-fibrogenic cytokines leading to progressive liver damage. NAFLD is considered a chronic inflammatory disease[8] and even mild inflammation has been associated with the risk of disease progression.[9] In this context, the changes in adipokine production play a crucial role in the pathogenesis.[10] For example, leptin has been shown to exhibit pro-inflammatory effects in the liver.[11] To this end, clinical research on the potential management of NAFLD is mounting. Data derived from clinical trials have indicated that managing the body weight may be beneficial in patients with NAFLD or with obesity,[12] lowering triglycerides (TG),[13] reducing liver fat and inhibiting *de novo* lipogenesis[14] or improving insulin sensitivity.[15] The restriction of excessive carbohydrate intake has also been proven to improve several important factors related to cardiovascular diseases[16] or to reduce the hepatic triglyceride content.[17] Not only should NASH be treated, but so should benign fatty liver.[18] The last guidelines on NAFLD clearly report that diet is the first-line treatment for NAFLD.[19,20] Adherence to the Mediterranean diet has been reported to reduce liver fat on 1H-MRS, when compared with a low fat/high carbohydrate diet in a cross-over comparison.[21] Recent studies in food science have focused on identifying bioactive ingredients that can suppress hepatic lipid accumulation and although weight management is the main intervention, only a few studies on the effect of bioactive microconstituents on NAFLD have been performed. Polyunsaturated n-3 fatty acids from seal oils were efficacious for patients with NAFLD associated with hyperlipidemia and improved their total symptom scores, alanine aminotransferase (ALT), serum lipid levels and normalized ultrasonographic evidence.[22] Synbiotic supplementation attenuated inflammatory markers in a randomized, double-blind, placebo-controlled pilot study in NAFLD patients.[23] It has been proposed that since NAFLD/NASH pathogenesis involves the increased production of reactive oxygen species and oxidative stress, bioactive phytochemicals may be useful in its management.[24] For example, vitamin E has been associated with fibrosis reversal,[25] improvement in insulin sensitivity and several of its associated parameters, including ALT levels in overweight otherwise healthy subjects[26] or improvement in ALT levels and insulin resistance in children with NAFLD in addition to lifestyle intervention.[27] Focusing on improving the clinical features and markers of oxidative stress and inflammation in NAFLD patients, we designed a pilot randomized controlled clinical trial to investigate the effects of two isocaloric diets of different "snacking" choices in between meals in addition to nutritional counseling for minimal body weight decrease. To this end, naturally sun-dried black grapes (currants), previously characterized for their phenolic content (217.4–354.2 mg GAE g^{-1}) and composition,[28] were examined *vs.* other isocaloric snacks.

2. Materials and methods

2.1 Patient recruitment and eligibility criteria

Patients were identified and recruited at Laiko University Hospital. The diagnosis of NAFLD was made by ultrasound examination. Included in the trial were patients with no significant fibrosis (evaluated by liver stiffness <7.5 kPa in ShearWave Elastography/Fibroscan). Men and women >18 years of age, without any change in body weight (BW) during the last 6 months prior to the trial, with a body mass index (BMI) >25 kg m^{-2} and with adherence to the "westernized" diet indicated by MedDietScore values lower than 35 were recruited. Exclusion criteria encompassed the presence of chronic viral hepatitis, as well as the presence of congenital or acquired liver disease, history of prior exposure to hepatotoxic drugs, evidence of hepatic cirrhosis, ultrasonography values less than 1 Hz, bariatric surgery, daily consumption of ethanol more than 20 g for women and more than 30 g for men for over 6 months during the last 5 years, any medication effective on fatty liver disease, the co-presence of reduced life expectancy disease, psychiatric disorders impairing the patient's ability to provide written informed consent, age >65 years, pregnant or lactating women or subjects supplemented with n-3 fatty acids, probiotics/synbiotics, antioxidant vitamins and/or phytochemicals. Additionally, the exclusion criterion was any planned, structured, and repetitive physical activity. Trial investigators excluded from final analyses patients who changed their medication during the trial.

2.2 Study design

All eligible patients with NAFLD signed an informed consent form after a full review of the inclusion and exclusion criteria and an explanation of the risks and benefits of the study, which were approved by the Ethics Committees of both Harokopio University and Laiko University Hospital, based on the Helsinki Declaration. In a two-armed, single center, randomized, controlled, 24-week prospective intervention trial, NAFLD patients were randomly assigned either to the Control arm or to the Currant arm. The allocation of patients in the two groups was randomised. In this study simple randomization was chosen and the randomisation sequence was computer generated. An independent statistician used a computer randomisation software. After randomisation, the statistician sent the randomisation list to the trial principal investigator who completed a participant form for each subject, including the treatment and the patient trial number and put it in a sealed envelope. Blinding of the allocated treatment was maintained to data analysts and was exposed only after the assessment of outcomes. Daily energy needs were determined

according to the basic metabolic rate equation of Harris–Benedict and sedentary lifestyle. The aim of nutritional counseling was a weight loss of approximately 5% of the initial BW within 6 months. Subjects attended appointments with experienced dieticians to receive guidance regarding calorie restriction. Nutritional counseling was centered on the distribution of nutrients in relation to the total caloric value as follows: 30% of the total energy as fat (<10% as SFAs, ~10% as MUFAs, and ~10% as PUFAs), 20% as protein, 50% as carbohydrate, 300 mg d^{-1} as dietary cholesterol, and 20–30 g fiber per d. Participants in the Control arm received the above dietary counseling. Participants in the Currant arm received dietary counseling and incorporated in their daily diet the consumption of 36 g of Corinthian currants equal to two fruit servings replacing snacks of alike nutritional value (low fat yogurt, mini crackers, or bread with low fat cheese). Currants were provided in packages of 36 g each, kindly donated by the Agricultural Cooperatives Union, Aegion, Greece.

2.3 Anthropometrics, blood pressure and clinical analyses

Anthropometric indices such as BW, height, waist and hip circumferences (WC, HC), waist to hip ratio (WHR) and BMI were recorded both at baseline and after the end of the trial. BW was measured early in the morning in the fasting state with subjects in light clothing without shoes using a flat scale (Tanita WB-110MA, Japan) recorded to the nearest 0.1 kg and height was measured on a stadiometer (Seca Model 220, Germany) recorded to the nearest 0.1 cm. Body composition evaluation using bioimpedance analysis was also conducted pre- and post-intervention on the Tanita WB. BMI was calculated as weight (in kg) divided by height^2 (in m^2). The WC was measured at the midpoint between the lower margin of the last palpable rib and the top of the iliac crest, using a stretch-resistant tape. HC was measured around the widest portion of the buttocks, with the tape parallel to the floor. All anthropometric measurements and body compositions were recorded after a ≥12-hour fast. Diastolic and systolic blood pressure (DBP and SBP in mmHg, respectively) and heart frequency (HF) were evaluated at baseline and after the study completion using an electronic sphygmomanometer (OMRON HEM-907 XL, OMRON, Kyoto, Japan). Participants were asked to lie down and relax for a few minutes, after which, two consecutive blood pressure measurements were recorded at an interval of 1–2 minutes. The recorded value was the mean of the two measurements. Blood samples were drawn at baseline and at the end of the study (week 24) through a catheter in an antecubital vein after a 12 h overnight fast. Freshly drawn blood samples were used for the determination of liver enzymes, lipid profile, glucose, insulin, glycated hemoglobin (HbA_{1c}), albumin, urea, uric acid, ferritin, Fe, transferrin and complete blood count using an automatic analyzer. Low density lipoprotein (LDL) cholesterol was calculated using the Friedewald formula.[29] HbA_{1c} was measured using high performance liquid chromatography. For assays to determine inflammation and oxidative stress biomarkers, serum samples were collected, separated by centrifugation at 1800*g* for 10 min at 4 °C, and stored at −80 °C for subsequent analyses.

2.4 Liver imaging

Abdominal ultrasound (US) was applied for the diagnosis of NAFLD. ShearWave elastography/Fibroscan was applied for liver stiffness evaluation and for the identification of the fibrosis stage. The NAFLD Fibrosis Score (NFS) was also calculated.[30]

2.5 Dietary history and analysis

Food data were collected. Each participant was asked to keep a 3-day food record (non-consecutive days, including one weekend day). Dietitians trained participants and reviewed unclear descriptions, errors, omissions, or doubtful entries in records and asked the participants to clarify them. The research dietitian supervisor checked all completed records for accuracy. The MedDietScore questionnaire was applied to estimate adherence to the Mediterranean dietary pattern.[31] Furthermore, during the trial, dietary counseling was monitored by non-scheduled phone calls receiving 24-hour dietary recalls. To calculate energy intake and macronutrient breakdown (fat, protein, and carbohydrate) nutritional data were analyzed by Nutritionist Pro nutrient analysis software version 5.2.0 (Axxya Systems, Nutritionist Pro, Stafford, TX).

2.6 Inflammatory biomarkers

Sera were separated from blood samples after centrifugation at 1800*g* for 10 min at 4 °C and were stored at −80 °C for further analyses. Sandwich Enzyme Linked-Immunosorbent Assay (ELISA) kits were used to measure CRP (ng ml^{-1}) (Invitrogen Corporation, Camarillo, USA), hsIL-6 (pg ml^{-1}) (R&D Systems Inc., Minneapolis, USA), tumor necrosis factor alpha (hsTNF-α) (pg ml^{-1}) (Invitrogen Corporation, Camarillo, USA), visfatin (ng ml^{-1}) (BioVendor-Laboratorni medicina a.s., Brno, Czech Republic) and leptin (ng ml^{-1}) (Invitrogen Corporation, Camarillo, USA).

2.7 Oxidative stress biomarkers

A sandwich ELISA kit (Mercodia AB, Uppsala, Sweden) was used to measure oxidized LDL (oxLDL). The total serum oxidizability was estimated photometrically applying the method described by Esterbauer and Jurgens[32] using a Biotek PowerWave XS2 ELISA reader.

2.8 Statistical analysis

All analyses were conducted applying the Statistical Package for the Social Sciences (SPSS 21.0 for Windows, Chicago, IL, USA). Descriptive statistics were calculated for all parameters and the Kolmogorov–Smirnov test was applied to investigate if all measures were characterized by normal distribution. Parametric data are expressed as mean values (±SD), while non-parametric data are expressed as medians and interquartile ranges. For variables with a normal distribution, the independent samples' *t*-test was applied to compare the differences between the two arms pre- and post-intervention, while

for variables without a normal distribution, the Mann–Whitney test was applied. Before the intervention this test served to ensure that the study population was characterized by homogeneity. For investigating possible intra-group differences, a paired sample *t*-test was applied for parametric variables and the Wilcoxon test for non-parametric ones. After NAFLD patients were classified with the criterion of weight loss (Control arm N = 17, Currant arm N = 18), the above mentioned analyses were repeated. For all statistical analyses significance was set at $p < 0.05$.

3 Results

Of the 285 patients invited for screening to this study, 230 were found ineligible to participate. All 55 patients found eligible to participate consented to trial and enrolled in the protocol. No differences were found between the two arms in biochemical and anthropometric indices pre-intervention, except for insulin levels, which were lower in the Control arm compared to the Currant arm (Table 1). Also, energy and nutrient intake did not differ at baseline (Table 1). By the end of the trial, 4 out of 27 patients in the Control and 1 out of 23 in the Currant arm gave personal reasons for dropping out. In addition, 2 patients in the Control and 4 patients in the Currant arm were ineligible, as 5 modified the lipid lowering treatment and one started antimetabolite treatment during the trial. By the end of the study, 21 patients in the Control arm and 23 in the Currant arm were eligible for analysis (Fig. 1).

Significant differences between the two arms post-intervention were observed in fasting glucose and IL-6 levels (p = 0.023 and p = 0.032, respectively) (Fig. 2). These were significantly decreased only in Currant patients (variation in baseline glucose 13.6% and in endpoint 16%, p = 0.004; variation in baseline IL-6 87.5% and in endpoint 55.6%, p = 0.009) (Table 3).

At the end of the trial anthropometric measures were improved in both groups compared to the baseline, BW (Control arm: p = 0.001, Currant arm: p = 0.002), BMI (Control arm: p = 0.001, Currant arm: p = 0.001) and HC (Control arm: p = 0.010, Currant arm: $p < 0.001$), as well as HbA_{1c} (Control arm: p = 0.028, Currant arm: p = 0.001), CRP (Control arm: p = 0.027, Currant arm: p = 0.001) and Elastography Ultrasound Scanning (EUS) values (Control arm: p = 0.043, Currant arm: p = 0.008) (Table 2). However, differences were insignificant between the two arms post-intervention (Table 3). Furthermore, adherence to the Mediterranean dietary pattern, illustrated by MedDietScore, was increased both in the Control arm (p = 0.005), and in the Currant arm ($p < 0.001$) (Table 4). However, at the end of the trial, the adherence difference was significantly higher in the Currant arm (p = 0.044) (Table 4). Patients in the Control arm had significantly decreased SBP and DBP (p = 0.001 and p = 0.003, respectively), LDL and HDL (p = 0.016 in both), NFS (p = 0.018), as well as the consumption of total fat (p = 0.047) (Tables 2–4). Participants in the Currant arm had significantly reduced

Table 1 Baseline characteristics of NAFLD patients enrolled in the study protocol

Parameters	Control arm (N = 27)	Currant arm (N = 28)	P value
Age (y)	51.6 ± 9.4	50.7 ± 10.9	0.747
Sex (n)			0.480
Males	10	13	
Females	17	15	
Anthropometrics			
Height (m)	1.7 ± 0.0	1.70 ± 0.0	0.390
BW (kg)	82.0 ± 3.0	85.7 ± 14.3	0.357
BMR (kcal per 24 h)	1633.3 ± 349.4	1712.2 ± 344.4	0.403
BMI (kg m^{-2})	29.1 ± 21.8	29.7 ± 22.2	0.595
Fat (%)	33.3 ± 9.4	33.3 ± 8.5	0.993
Trunk fat (%)	32.0 ± 8.3	32.5 ± 6.9	0.801
WC (cm)	99.1 ± 12.5	102.2 ± 11.6	0.336
HC (cm)	110.0 ± 10.9	111.8 ± 8.9	0.522
WHR	1.0 ± 0.0	0.9 ± 0.0	0.678
Clinical data			
SBP (mmHg)	130.2 ± 13.0	130.5 ± 13.2	0.929
DBP (mmHg)	81.5 ± 8.3	81.5 ± 8.9	0.988
HF (bpm)	70.3 ± 12.0	70.2 ± 45.0	0.974
US (Hz)	1.7 ± 5.2	2.0 ± 0.5	0.108$^{\neq}$
EUS (kPa)	5.6 ± 1.6	5.4 ± 1.0	0.655
NFS	−2.4 ± 1.0	−2.3 ± 1.0	0.692
Glucose (mg dl^{-1})	90.9 ± 15.6	95.7 ± 12.2	0.201
TC (mg dl^{-1})	203.7 ± 31.2	209.1 ± 31.2	0.523
HDL-C (mg dl^{-1})	57.2 ± 16.6	54.4 ± 12.7	0.483
LDL-C (mg dl^{-1})	122.3 ± 28.6	126.8 ± 33.3	0.590
TG (mg dl^{-1})	112.2 ± 46.8	137.2 ± 90.4	0.259$^{\neq}$
SGOT (U L^{-1})	22.3 ± 6.2	22.7 ± 5.3	0.809
SGPT (U L^{-1})	28.0 ± 12.5	29.6 ± 12.7	0.624
SGOT/SGPT	0.9 ± 0.52	0.8 ± 0.0	0.560
γGT (U L^{-1})	101.2 ± 69.7	128.5 ± 338.6	0.021$^{\neq}$
ALP (U L^{-1})	64.6 ± 16.6	69.4 ± 23.8	0.396
TBIL (mg dl^{-1})	0.6 ± 0.0	0.7 ± 0.5	0.752
DBIL (mg dl^{-1})	0.2 ± 0.0	0.2 ± 0.0	0.653
TPR (g dl^{-1})	7.3 ± 0.5	7.3 ± 0.5	0.968
Fe (μg dl^{-1})	107.8 ± 44.2	94.3 ± 24.3	0.164
Fer (ng dl^{-1})	94.8 ± 102.4	124.4 ± 97.3	0.275
HbA_{1C} (%)	5.5 ± 0.5	5.8 ± 0.5	0.058
Insulin (μIU ml^{-1})	12.5 ± 3.6	16.1 ± 6.9	0.016
WBC (×10^3 μl^{-1})	6.3 ± 1.6	6.8 ± 2.1	0.490$^{\neq}$
RBC (×10^6 μl^{-1})	5.0 ± 0.5	5.1 ± 0.5	0.606
Plt (×10^3 mm^{-3})	256.4 ± 58.2	240.7 ± 40.7	0.249
MCV (fl)	83.9 ± 9.4	83.0 ± 7.9	0.350$^{\neq}$
MCH (pg)	28.6 ± 3.6	28.1 ± 3.2	0.448$^{\neq}$
Hb (g dL^{-1})	14.2 ± 1.6	14.1 ± 1.1	0.877
Hct (%)	41.6 ± 4.2	41.7 ± 2.6	0.912
Na (mmol L^{-1})	141.0 ± 2.1	141.5 ± 1.6	0.267
K (mmol L^{-1})	4.5 ± 0.5	4.6 ± 0.5	0.656
Urea (mg dl^{-1})	27.9 ± 8.8	29.0 ± 5.7	0.593
Creatinine (mg dL^{-1})	0.8 ± 0.0	0.8 ± 0.0	0.448
Uric acid (mg dL^{-1})	5.1 ± 1.0	5.3 ± 1.1	0.394
CRP (ng ml^{-1})	2661.6 ± 2831.4	2780.8 ± 2703.7	0.528$^{\neq}$
Visfatin (ng ml^{-1})	8.4 ± 2.1	9.4 ± 5.3	0.381
Leptin (ng ml^{-1})	75.7 ± 53.6	91.8 ± 76.7	0.368
IL-6 (pg ml^{-1})	1.6 ± 2.6	2.4 ± 4.2	0.057$^{\neq}$
oxLDL (U L^{-1})	125.7 ± 29.6	137.5 ± 32.8	0.171
Serum oxidizability (s)	6033.6 ± 1188.2	5775.0 ± 1185.0	0.424
TNF-α (pg ml^{-1})	1.3 ± 1.0	0.9 ± 1.1	0.120
Nutritional data			
MedDietScore	32.1 ± 4.7	30.0 ± 4.2	0.095
Energy (kcal)	1996.3 ± 617.2	1927.2 ± 645.4	0.688
Carbohydrates (g)	197.6 ± 68.1	203.0 ± 73.5	0.776
Sugars (g)	59.3 ± 22.9	63.1 ± 27.5	0.586
Fat total (g)	103.1 ± 48.4	87.2 ± 40.7	0.192
SFA (g)	28.3 ± 17.2	30.6 ± 13.2	0.572
PUFA (g)	15.5 ± 9.9	12.2 ± 9.0	0.209
MUFA (g)	49.2 ± 25.0	35.4 ± 21.2	0.051

Table 1 *(Contd.)*

Parameters	Control arm (N = 27)	Currant arm (N = 28)	P value
Protein (g)	82.5 ± 35.9	82.4 ± 32.8	0.986
Insoluble fibre (g)	1.7 ± 2.6	0.9 ± 5.8	0.241$^{\neq}$
Crude fibre (g)	2.6 ± 1.6	3.2 ± 4.2	0.462

BW, body weight; BMR, basic metabolic rate; BMI, body mass index; WC, waist circumference; HC, hip circumference; WHR, waist to hip ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; HF, heart frequency; US, ultrasound; EUS, elastography ultrasound stiffness; NFS, NAFLD fibrosis score; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides; SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamic pyruvic transaminase; SGOT/SGPT, SGOT to SGPT ratio; γGT, γ-glutamyl transpeptidase; ALP, alkaline phosphatase; TBIL, total bilirubin; DBIL, direct bilirubin; TPR, total protein; Fe, Iron; Fer, ferritin; HbA_{1C}, glycated hemoglobin; WBC, white blood cells; RBC, red blood cells; Plt, platelets; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; Hb, hemoglobin; Hct, hematocrite; Na, sodium; K, potassium; CRP, C-reactive protein; IL-6, interleukin-6; oxLDL, oxidized LDL; TNF-α, tumor necrosis factor α; SFA, saturated fatty acid; PUFA, polyunsaturated fatty acid; MUFA, monounsaturated fatty acid. Data are mean values ± deviation (SD). CV, coefficient variation. Nutritional data were derived from food records, while the MedDiet score is given as well. P stands for the difference between the Control and the Currant arm at baseline parameters analyzed by independent sample t test, ≠ indicates the difference between the Control and the Currant arm parameters analyzed by the Mann–Whitney test. Difference was considered significant at $P < 0.05$.

total body fat (p = 0.010), WC (p = 0.007) and trunk fat (p = 0.045). Ultrasound scanning improved significantly in patients snacking currants daily (p = 0.029). Volunteers enrolled in the Currant arm showed a reduced intake of saturated fatty acids (SFAs) (p = 0.003). All participants in both arms received a common treatment, meaning nutritional counseling that aimed at a weight loss of approximately 5% of the initial BW within six months. However not all participants adhered to counseling; seventeen patients in the Control arm and eighteen in the arm that used currants as snacks showed a reduced body weight. Since weight loss has been officially recognised as a treatment approach in NAFLD[19] a secondary analysis was repeated in patients adhering to this lifestyle change. Overall, NAFLD patients with a decreased BW had a significantly improved total body fat, WC, and HC. Post-intervention, the decrease in fasting glucose and in IL-6 was significantly higher in Currant patients who lost BW compared to their counterparts in the Control arm (p = 0.005 and p = 0.035, respectively) (Table 2S‡). Variation in baseline glucose in the Currant arm was 9.8% and in the endpoint 10.8% while variation in baseline IL-6 in the Currant arm was 69.2% and in the endpoint 50.0%. Also EUS values, CRP, and leptin decreased significantly in these patients after 6 months (Table 2S‡). The assessment of nutritional intake showed a

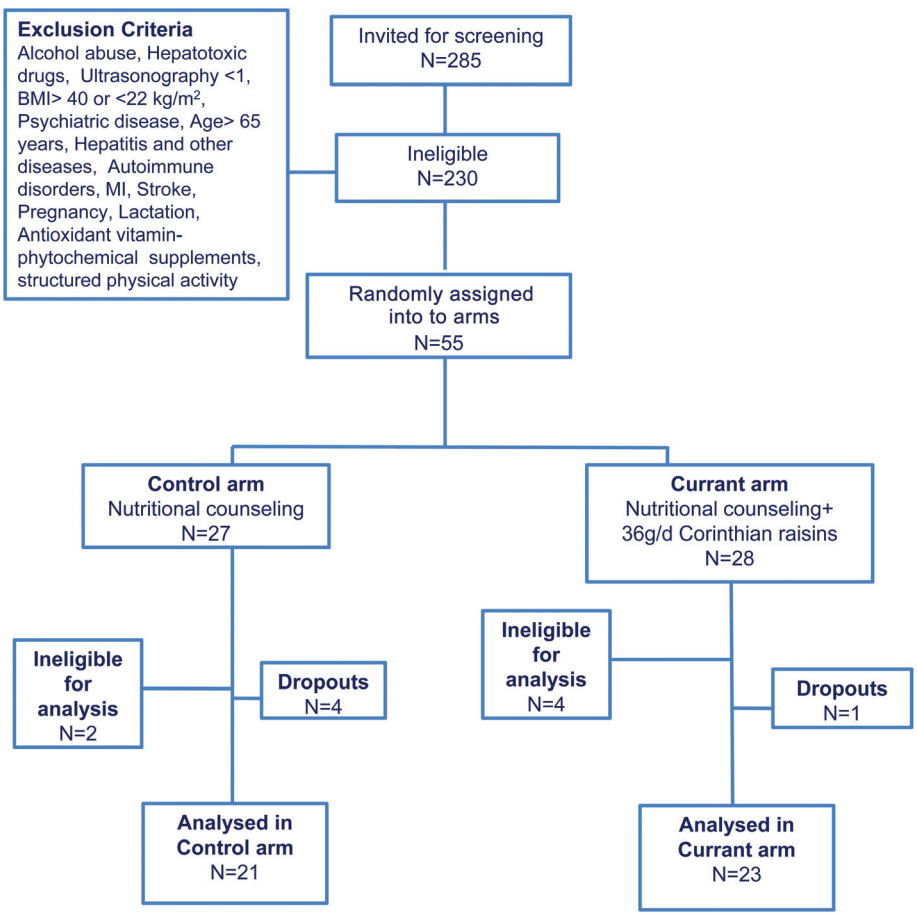

Fig. 1 Flowchart of the study.

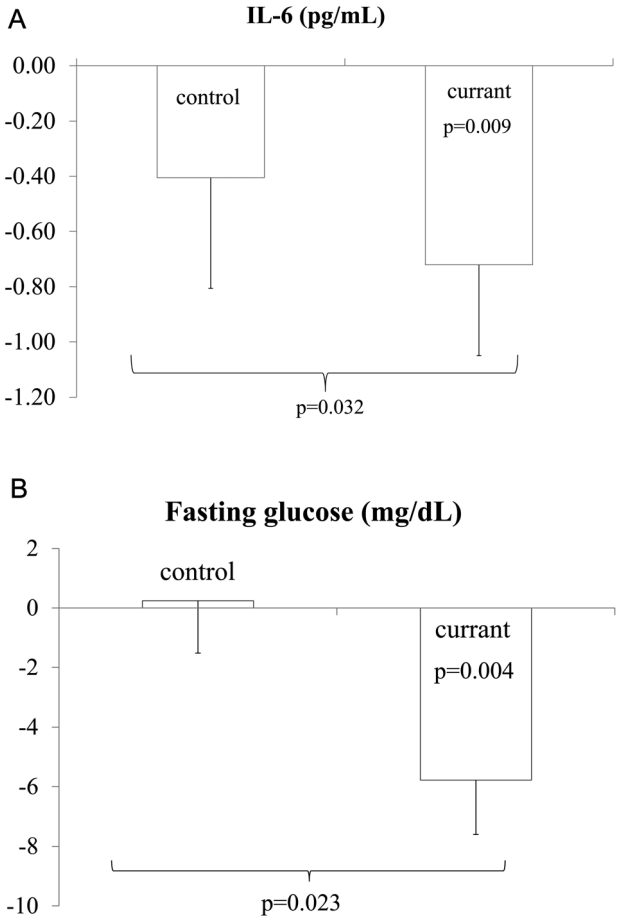

Fig. 2 Differences between patients in the control arm and in the raisin arm who completed the trial in serum IL-6 (A) and in fasting glucose (B). *P* values: comparison with the baseline values within groups, **P* values: significant differences in changes at the endpoint between the two groups, difference was considered significant at $P < 0.05$.

significant increase in MedDietScore values, and a significant decrease in SFA intake in patients who lost weight in both arms (Table 3S‡).

4 Discussion

To the best of our knowledge, this is the first randomized clinical trial that evaluated the effect of snacking along with standard nutritional counseling for a minimal body weight decrease in NAFLD patients of non-significant fibrosis. More specifically, in addition to nutritional counseling, the effect of incorporating a dried fruit in diet as a snack was evaluated. The main outcome of this study was that minimal dietary modifications in patients with NAFLD of non-significant fibrosis result in the reduction of serum IL-6 and in the reduction of fasting glucose.

Dietary counseling was effective in all NAFLD patients, both in the Control and Currant arms, as it significantly reduced BW, BMI and HC, and significantly improved HbA_{1c}, CRP, EUS and MedDietScore. Overall, the findings of our study are consistent with the findings of other clinical trials that have shown the beneficial effects of nutritional counseling and diet-induced weight loss in NAFLD patients[31,33–36]. Achieving and maintaining a 5% weight reduction has been suggested to improve several components of the metabolic syndrome and liver function.[37] A 9% weight loss in obese older adults resulted in significant decreases in the intrahepatic fat content accompanied by considerable improvements in insulin sensitivity.[15] Similarly, 6–8% weight loss reduced hepatic fat and improved glucose metabolism in younger NAFLD patients.[38,39] Corinthian currants are the dried products of their fresh homologues, grapes (*Vitis vinifera* L.). MedDietScore values were significantly different between the two groups at the end of the trial, a rather expected result as the snack was a dried fruit, however, its importance should be stressed. Adherence to the Mediterranean diet has been found to be a significant predictor of changes in the fat content of the liver in overweight patients with NAFLD.[40] It has been shown that dried fruit consumption in quantities more than 20 g on a daily basis was associated with improved nutrient intakes, a higher overall diet quality score, and lower body weight/adiposity measures, compared to those who did not consume such a quantity.[41] In our study, NAFLD patients in the Currant arm showed an additional decrease in the intake of SFAs and a higher adherence to the Mediterranean dietary pattern. In general, *Vitis vinifera* derived phenols, have known antioxidant properties and have been described to have beneficial effects also in NAFLD.[42,43] The beneficial effect of snacking in NAFLD, according to the results reported, could have also been influenced by the higher adherence to diet and by the intrinsic antioxidant effects of phenols.[44] Currants have been indicated as dried fruits of a medium glycemic index that regulate insulin and glucose response in both diabetic and non-diabetic subjects referenced to glucose.[45] In general, foods with a low glycemic index have been shown to exert anti-inflammatory effects,[46,47] as well as beneficial effects in glycemic control and blood pressure.[48] Furthermore, currants contain considerable amounts of phenolic compounds such as vanillic acid, protocatechuic acid, syringic acid, *p*-coumaric acid, gallic acid, ferulic acid, caffeic acid, quercetin and the flavonoid chrysin.[47] Seventeen out of 25 phytochemicals present in Corinthian currants have been found to increase in plasma after consumption, providing evidence for their absorption and bioavailability.[49] Phenolic compounds have been attributed with several health benefits, mainly with effects on vascular function, on cognitive performance and neurogenesis, on the inhibition of DNA oxidation and tumor development, and on the inhibition of adhesion molecules and inflammatory mediators.[50] The regulation of fasting glucose appearing herein might be attributed to the phenolic content of currants, as phenolic compounds, *i.e.* gallic acid, have been shown to ameliorate impaired glucose in experimental NAFLD/NASH.[51] In NAFLD, pro-inflammatory

Table 2 Differences in anthropometrics, BP, US, EUS and NFS within the Currant and Control arms of NAFLD patients who completed the 24-week trial

Variable	Arm	Week 0	Week 24	*P* value	**P* value
BW (kg)	Control	79.8 ± 61.8	76.9 ± 12.4	0.001	0.343
	Currant	84.9 ± 14.4	82.9 ± 14.4	0.002	
BMR (kcal per 24 h)	Control	1631.6 ± 318.8	1592.4 ± 292.6	0.003	0.151
	Currant	1689.5 ± 348.5	1674.7 ± 351.8	0.221	
BMI (kg m^{-2})	Control	28.2 ± 3.7	27.1 ± 3.7	0.001	0.241
	Currant	29.5 ± 4.3	28.8 ± 4.3	0.001	
Fat (%)	Control	31.2 ± 8.7	30.0 ± 8.2	0.133	0.965
	Currant	33.6 ± 8.2	32.4 ± 8.7	0.010	
Trunk fat (%)	Control	30.8 ± 7.3	29.5 ± 7.8	0.205	0.866
	Currant	32.6 ± 7.2	31.5 ± 7.7	0.045	
WC (cm)	Control	96.7 ± 11.5	94.5 ± 13.0	0.094	0.969
	Currant	101.0 ± 11.5	98.9 ± 11.5	0.007	
HC (cm)	Control	107.0 ± 10.1	105.3 ± 9.2	0.010	0.207
	Currant	112.5 ± 9.1	109.6 ± 9.1	<0.001	
WHR	Control	0.9 ± 0.0	0.9 ± 0.0.09	0.565	0.588
	Currant	0.9 ± 0.0	0.9 ± 0.0	0.972	
SBP (mmHg)	Control	130.3 ± 13.2	121.8 ± 11.0	0.001	0.179
	Currant	129.1 ± 13.4	124.8 ± 13.0	0.057	
DBP (mmHg)	Control	81.4 ± 8.7	76.5 ± 8.7	0.003	0.030
	Currant	80.3 ± 9.1	79.8 ± 10.1	0.737	
HF (bpm)	Control	68.4 ± 18.8	68.5 ± 13.3	0.967	0.255
	Currant	71.4 ± 8.6	69.0 ± 8.2	0.078	
US (Hz)	Control	1.6 ± 0.5	1.5 ± 0.5	0.058#	0.833#
	Currant	1.9 ± 1.0	1.8 ± 0.5	0.033#	
EUS (kPa)	Control	5.5 ± 1.8	5.0 ± 0.9	0.043	0.684
	Currant	5.3 ± 1.0	4.9 ± 1.0	0.008	
NFS	Control	−2.3 ± 1.4	−2.1 ± 1.4	0.018	0.279
	Currant	−2.3 ± 1.0	−2.2 ± 1.0	0.102	

Data are mean values ± standard deviation (SD). BW, body weight; BMR, basic metabolic rate; BMI, body mass index; WC, waist circumference; HC, hip circumference; WHR, waist to hip ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; HF, heart frequency; US, ultrasound; EUS, elastography ultrasound stiffness; NFS, NAFLD fibrosis score. *P* values: comparison with the baseline values by the paired samples *t* test or # the Wilcoxon test, difference was considered significant at $P < 0.05$. **P* values indicate significant differences in the change of risk factors in the raisin group as compared with the change in the Control group applying the independent samples *t* test # or the Mann–Whitney test difference was considered significant at $P < 0.05$.

cytokines such as TNF-α and IL-6 are secreted by adipocytes. TNF-α and IL-6 have been proven pro-inflammatory in the liver.[11] Herein, TNF-α did not differ between the two arms post-intervention. Leptin was significantly decreased in patients with reduced BW concurrently with currants but not in those with reduced BW in the Control arm, however the decrease was not significantly different between these groups. NAFLD patients snacking polyphenol-rich currants daily experienced reduced IL-6 concentrations, the reduction being significant in all analyzed patients in the Currant arm irrespective of BW loss. Pro-inflammatory IL-6 is expressed in several tissues and is related to insulin resistance and inflammation.[52] Plasma IL-6 has been associated with its expression in the liver, which in turn has been positively associated with the stage of inflammation, as well as with fibrosis severity.[53] IL-6 is highly specific in confirming the absence of NASH at normal values and normal values are strongly associated with a fatty liver.[54] In our study, after including only patients who had lost weight, EUS values remained significantly decreased only in the Currant arm. Although the patients enrolled in the study did not have significant fibrosis at baseline, the decrease in liver stiffness could be attributed to the decrease in inflammation.[55] In obesity related NAFLD, IL-6 is largely produced by macrophages, but not only of adipose tissue derivation. In fact the main role is played by spleen and it has been demonstrated that spleen cells produce high levels of inflammatory cytokines and that NASH patients demonstrate high IL-6 blood levels and spleen longitudinal diameter values.[56] However, it has also been demonstrated that spleen also mediates a protective role, as it supports a pool of innate-like B cells in white adipose tissue that protect against obesity-associated insulin resistance.[57]

Surprisingly, we did not observe any change in the markers of oxidative stress in serum. In a previous study in patients with type 2 diabetes,[58] the daily consumption of 36 g of Corinthian currants for a total of 6 months resulted in an increase of the plasma antioxidant capacity, the increase being significantly different to that in the Control group post-intervention. As in the case of leptin, also in markers of oxidative stress, the relatively small study size may have limited our power to detect the differences among the Currant and Control groups for respective improvements in leptin or oxidative stress.

While this study reports interesting findings regarding the effect of lifestyle modifications on the health of patients with

Table 3 Differences in biochemical, inflammatory and oxidative stress markers within the Currant and Control arms of NAFLD patients who completed the 24-week trial

Variable	Arm	Week 0	Week 24	*P* value	**P* value
Glucose (mg dL^{-1})	Control	92.1 ± 16.9	92.3 ± 16.9	0.894	0.023
	Currant	95.7 ± 13.0	89.9 ± 14.4	0.004	
TC (mg dL^{-1})	Control	202.8 ± 32.0	209.2 ± 35.7	0.309	0.479
	Currant	209.6 ± 33.6	211.4 ± 32.2	0.548	
HDL-C (mg dL^{-1})	Control	56.7 ± 15.1	52.5 ± 13.7	0.016	0.853
	Currant	56.1 ± 14.4	52.4 ± 11.0	0.065	
LDL-C (mg dL^{-1})	Control	120.8 ± 29.8	134.9 ± 32.5	0.016	0.310
	Currant	126.7 ± 37.4	133.1 ± 34.1	0.225	
TG (mg dL^{-1})	Control	114.1 ± 51.8	109.1 ± 47.6	0.602#	1.000#
	Currant	137.3 ± 97.9	132.3 ± 79.7	0.399#	
SGOT (U L^{-1})	Control	23.1 ± 6.4	22.1 ± 6.4	0.511	0.827
	Currant	22.8 ± 5.8	22.2 ± 6.7	0.634	
SGPT (U L^{-1})	Control	29.5 ± 13.7	27.2 ± 15.6	0.394#	0.991#
	Currant	30.0 ± 14.0	27.7 ± 14.0	0.402#	
SGOT/SGPT	Control	0.9 ± 0.5	0.9 ± 0.5	0.352	0.991
	Currant	0.9 ± 0.5	0.9 ± 0.5	0.071	
γGT (U L^{-1})	Control	32.7 ± 29.3	36.6 ± 49.5	0.504#	0.465#
	Currant	32.1 ± 30.2	35.0 ± 37.4	0.475#	
ALP (U L^{-1})	Control	59.9 ± 14.2	61.2 ± 16.5	0.492	0.118
	Currant	70.1 ± 26.9	67.6 ± 28.3	0.108	
TBIL (mg dL^{-1})	Control	0.6 ± 0.5	0.7 ± 0.5	0.793	0.317
	Currant	0.7 ± 0.5	0.6 ± 0.5	0.219	
DBIL (mg dL^{-1})	Control	0.2 ± 0.0	0.2 ± 0.0	0.135	0.185
	Currant	0.2 ± 0.0	0.2 ± 0.0	0.905	
HbA_{1C} (%)	Control	5.5 ± 0.5	5.4 ± 0.5	0.028#	0.090#
	Currant	5.8 ± 0.5	5.4 ± 1.5	0.001#	
Insulin (μIU mL^{-1})	Control	11.7 ± 3.7	11.5 ± 6.0	0.839	0.288
	Currant	15.8 ± 7.2	17.5 ± 9.6	0.210	
Urea (mg dL^{-1})	Control	28.5 ± 8.2	32.3 ± 9.6	0.052	0.191
	Currant	29.4 ± 6.2	30.5 ± 7.2	0.239	
Uric acid (mg dL^{-1})	Control	5.0 ± 0.9	5.1 ± 1.4	0.585	0.556
	Currant	4.9 ± 1.4	4.84 ± 1.6	0.791	
CRP (ng mL^{-1})	Control	2399.2 ± 3048.9	841.6 ± 1087.8	0.023#	0.748
	Currant	2136.9 ± 1861.0	825.0 ± 721.9	0.002#	
Visfatin (ng mL^{-1})	Control	8.4 ± 1.8	4.7 ± 3.2	0.066	0.233
	Currant	9.6 ± 5.3	9.2 ± 3.4	0.696	
Leptin (ng mL^{-1})	Control	63.5 ± 48.6	55.2 ± 39.4	0.090	0.794
	Currant	95.9 ± 81.6	85.2 ± 76.8	0.190	
IL-6 (pg mL^{-1})	Control	1.7 ± 3.2	1.3 ± 1.4	0.322#	0.032#
	Currant	1.6 ± 1.4	0.9 ± 0.5	0.009#	
oxLDL (U L^{-1})	Control	125.7 ± 26.1	115.5 ± 26.1	0.072	0.828
	Currant	137.5 ± 29.8	125.1 ± 33.6	0.160	
TNF-α (pg mL^{-1})	Control	1.3 ± 1.0	0.8 ± 0.5	0.004	0.066
	Currant	0.9 ± 1.0	1.3 ± 1.4	0.063	
Serum oxidizability (s)	Control	6033.6 ± 1046.5	6145.1 ± 946.2	0.569	0.851
	Currant	5579.0 ± 658.1	5745.9 ± 896.6	0.462	

Data are mean values ± standard deviation (SD). TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides; SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamic pyruvic transaminase; SGOT/SGPT, SGOT to SGPT ratio; γGT, γ-glutamyl transpeptidase; ALP, alkaline phosphatase; TBIL, total bilirubin; DBIL, direct bilirubin; HbA_{1C}, glycated hemoglobin; CRP, C-reactive protein; IL-6, interleukin-6; oxLDL, oxidized LDL; TNF-α, tumor necrosis factor α. *P* values: comparison with the baseline values by the paired samples *t* test # or the Wilcoxon test, difference was considered significant at $P < 0.05$. **P* values indicate significant differences in the change of risk factors in the raisin group as compared with the change in the Control group, applying the independent samples *t* test # or the Mann–Whitney test, difference was considered significant at $P < 0.05$.

NAFLD, however it has some limitations. Apart from the study size, liver biopsies were not performed, precluding our ability to comment on any histologic alterations. Additionally, there is substantial potential for observing effects due to the multiple tests included herein. Also, an important limitation of the study is that the CV% are in some parameters very high suggesting that some of the measures are not of sufficient precision to yield unquestioning results. On the other hand, these limitations were compensated in part by the very tight control of the Currant and Control groups to assess the potential effects of the daily consumption of currants by patients with NAFLD. Nevertheless, both mechanistic and larger-scale clinical trials are essential to determine the effect of polyphenol-rich currants on the health of patients with NAFLD and the potential mechanisms underlying any effect.

Table 4 Change in energy and in nutrient intakes in NAFLD patients participating in the Currant or Control arm after 24 weeks in the trial protocol. Nutritional data were derived from food records; MedDiet score is also presented

Variable	Arm	Week 0	Week 24	*P* value	**P* value
MedDietScore	Control	32.0 ± 4.58	35.1 ± 5.0	0.005	0.044
	Currant	29.8 ± 4.3	35.6 ± 5.3	<0.001	
Energy (kcal)	Control	1981.1 ± 521.7	1831.8 ± 618.7	0.429	0.988
	Currant	1923.1 ± 683.5	1777.4 ± 590.0	0.343	
Carbohydrates (g)	Control	205.5 ± 70.1	205.3 ± 76.5	0.992	0.577
	Currant	200.5 ± 75.8	185.6 ± 58.6	0.363	
Sugars (g)	Control	60.8 ± 22.0	68.8 ± 35.7	0.367	0.575
	Currant	62.6 ± 27.8	65.2 ± 27.4	0.551	
Protein (g)	Control	82.6 ± 30.2	76.5 ± 35.7	0.545	0.874
	Currant	78.3 ± 31.7	74.2 ± 28.8	0.576	
Fat total (g)	Control	99.5 ± 38.0	80.3 ± 26.1	0.047	0.313
	Currant	89.8 ± 41.3	84.9 ± 41.8	0.642	
SFA (g)	Control	28.0 ± 15.1	22.7 ± 9.2	0.130	0.353
	Currant	31.9 ± 12.4	22.6 ± 11.0	0.003	
PUFA (g)	Control	14.8 ± 6.9	12.9 ± 6.4	0.274#	0.442
	Currant	12.5 ± 9.6	13.0 ± 8.2	0.503#	
MUFA (g)	Control	46.0 ± 18.3	36.3 ± 16.0	0.031	0.054
	Currant	35.8 ± 21.6	41.7 ± 26.9	0.350	
Insoluble fiber (g)	Control	2.0 ± 2.8	1.2 ± 1.4	0.287#	0.461
	Currant	0.9 ± 1.4	0.6 ± 1.4	0.099#	
Crude fiber (g)	Control	2.8 ± 1.4	4.3 ± 4.6	0.170#	0.642
	Currant	3.0 ± 3.8	3.9 ± 2.9	0.144#	

Data are mean values ± standard deviation (SD). SFA, saturated fatty acid; PUFA, polyunsaturated fatty acid; MUFA, monounsaturated fatty acid. *P* values: comparison with the baseline values by the paired samples *t* test # or the Wilcoxon test, difference was considered significant at $P < 0.05$. **P* values indicate significant differences in the change of risk factors in the raisin group as compared with the change in the Control group, applying the independent samples *t* test, difference was considered significant at $P < 0.05$.

5 Conclusions

NAFLD has the potential for major economic impact on healthcare costs because of liver-related morbidity and mortality and it already represents an important economic burden for European countries. For example, patients with a sonographic fatty liver disease and increased serum liver enzymes have been shown to incur 26% higher overall health-care costs at a 5-year follow-up.[59] NAFLD patients of non-significant fibrosis can take advantage of the adoption of a balanced diet, while a minimal lifestyle change, such as changes in snacking with the increase of bioactive phytochemicals, can potentially ameliorate fasting glucose, inflammation and fibrosis stage. The quantity of raisins consumed herein by the volunteers corresponds to two fruit servings, a realistic dose to be incorporated in the diet of these patients. This modification in diet related to medium calorie restriction and to snacks rich in antioxidant phytochemicals could be an effective strategy for treating NAFLD patients with no significant fibrosis.

Author contributions

Andriana C Kaliora and Alexander Kokkinos had the concept and designed this study; Anastacia Diolintzi, Aristea Gioxari, Maria Stoupaki, Jiannis Vlachogiannakos and Constantinos Revenas were the investigators who recruited and treated patients; George V. Z. Dedoussis, Spyros D. Ladas and Vaios T. Karathanos took part in trial coordination; Andriana C. Kaliora, Anastacia Diolintzi, Panagiotis T. Kanellos and Aristea Gioxari collected, managed, analyzed and interpreted the data; Anastacia Diolintzi, Panagiotis T. Kanellos and Aristea Gioxari contributed to the statistical analyses; Andriana C. Kaliora and Anastacia Diolintzi drafted the manuscript; Andriana C. Kaliora made the final version of the manuscript to be published, approved also by Alexander Kokkinos.

Conflict of interest

No potential conflicts of interest relevant to this article were reported.

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