A lifestyle intervention with an energy-restricted Mediterranean diet and physical activity enhances HDL function: a substudy of the PREDIMED-plus randomized controlled trial

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Data sharing statement

The datasets generated and analyzed in the current study are not expected to be made available outside the core research group, as neither the participants' consent forms nor ethics approval included permission for open access. We do, however, follow a controlled data-sharing collaboration model, as in the informed consent participants agreed to a controlled collaboration with other investigators for research related to the project's aims. Data described in the manuscript, codebook, and analytic code will be made available upon request pending application and approval by the PREDIMED-Plus Steering Committee. Investigators who are interested in this study can contact the Committee by sending a request letter (predimed_plus_scommittee@googlegroups.com). A data-sharing agreement indicating the characteristics of the collaboration and data management will be completed for the proposals that are approved.

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Running head: Energy-restricted Mediterranean diet and HDL function

Abbreviations

ApoA-I: Apolipoprotein A-I

ApoA-IV: Apolipoprotein A-IV

ApoB: Apolipoprotein B

ApoC-III: Apolipoprotein C-III

ApoE: Apolipoprotein E

BMI: Body mass index

C3c: Complement component 3

CEC: Cholesterol efflux capacity

HDL: High-density lipoprotein

HDL-C: HDL cholesterol

HOII: HDL oxidative/inflammatory index

MedDiet: Mediterranean diet

S1P: Sphingosine-1-phosphate

SAA: Serum amyloid A

Clinical Trial Registry

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98 **ABSTRACT**

99 **Background:** Consumption of a Mediterranean diet, adequate levels of physical activity, and
100 energy-restricted lifestyle interventions have been individually associated with improvements
101 in HDL function. Evidence of intensive interventions with calorie restriction and physical
102 activity is, however, scarce.

103 **Objectives:** To determine whether an intensive lifestyle intervention with an energy-
104 restricted Mediterranean diet plus physical activity enhanced HDL function compared
105 to a non-hypocaloric Mediterranean eating pattern without physical activity.

106 **Methods:** In 391 older adults with metabolic syndrome (mean age, 65 years; mean BMI,
107 33.3 kg/m^2) from 1 of the Prevención con Dieta Mediterránea-Plus trial centers, we
108 evaluated the impact of a 6-month intervention with an energy-restricted Mediterranean
109 diet plus physical activity (intensive lifestyle, *n*=190) relative to a nonrestrictive
110 Mediterranean diet without physical activity (control; *n*=201) on a set of HDL functional
111 traits. These included cholesterol efflux capacity, HDL oxidative/inflammatory index, HDL
112 oxidation, and levels of complement component 3, serum amyloid A, sphingosine-1-
113 phosphate, triglycerides, and apolipoproteins A-I, A-IV, C-III, and E in apoB-depleted plasma.

114 **Results:** The intensive lifestyle intervention participants displayed greater 6-month
115 weight reductions (-3.83 kg [95% CI: -4.57, -3.09]), but no changes in HDL
116 cholesterol compared with control-diet participants. Regarding HDL functional traits, the
117 intensive lifestyle decreased triglyceride levels (-0.15 mg/g protein; 95% CI: -0.29 to
118 -0.014 mg/g protein) and apoC-III (-0.11 mg/g protein 95% CI: -0.18 to -0.026 mg/g protein)
119 compared to the control group diet, with weight loss being the essential mediator (proportions
120 of mediation were 77.4% and 72.1% for triglycerides and apoC-III levels in HDL,
121 respectively).

Conclusions: In older adults with metabolic syndrome, an energy-restricted Mediterranean diet plus physical activity improved HDL triglyceride metabolism Compared with a non-restrictive Mediterranean diet without physical activity.

KEYWORDS

High-density lipoprotein, physical activity, calorie restriction, Mediterranean diet, randomized controlled trial

INTRODUCTION

Raised levels of HDL cholesterol have been associated with lower risks of cardiovascular disease (1). Pharmacological interventions and Mendelian randomization studies have, however, questioned the causal association between increased HDL cholesterol concentrations and lower cardiovascular risk (2,3). Thus, HDL functional traits merit further investigation as to their possible roles in modifying such a risk (2). These include: 1) cholesterol efflux capacity (CEC), the ability of HDLs to pick up cholesterol excess from cells, such as macrophages; 2) HDL antioxidant/anti-inflammatory properties [HDL oxidative/inflammatory index (HOII); HDL oxidation status, HDL levels of acute-phase proteins such as complement component 3 (C3c) and serum amyloid A (SAA); etc.]; 3) HDL endothelial protection [related to their sphingosine-1-phosphate (S1P) content]; 4) HDL role on triglyceride metabolism; and 5) HDL-bound apolipoprotein concentrations (4–6). Reduced CEC values, pro-oxidative/pro- inflammatory HDLs (with increased HOII values and elevated levels of C3c), S1P- poor HDLs, dysfunctional HDLs on the triglyceride metabolism (enriched in disruptors of the triglyceride metabolism, such as apoC-III), and HDLs with impaired levels of apolipoproteins such as apoA-I, have been associated with greater cardiovascular risk in several cohorts (7–10). In addition, a recent Mendelian randomization study has established a potentially causal relationship between HDL quality characteristics beyond HDL cholesterol levels and coronary artery disease (11), suggesting that HDL functional/quality characteristics could act as potential therapeutic targets for cardiovascular disease.

Adequate levels of physical activity are key in the prevention of cardiovascular disease (12). Additional benefits on cardiovascular risk can be achieved when this lifestyle modification is accompanied by energy restriction, leading to sustained weight reduction (13). Regarding HDL functions, several short-term, small-scale, randomized controlled studies and noncontrolled trials have assessed the individual associations among physical activity, weight loss, and HDL functionality. In most cases, results were inconsistent or of lesser scientific quality. The relationship between physical activity and CEC has been shown to be controversial (14–18). Calorie restriction has been linked to decreases in CEC values in 2 noncontrolled studies (19,20), and studies combining both have also reported conflicting or uncontrolled findings (21–23). HDL antioxidant capacities and HDL oxidation have only been studied in noncontrolled trials, although enhancements in both have been associated with physical activity (18,23–28). Physical activity has also been linked to improvements in HDL anti-inflammatory properties in further noncontrolled studies (18,28), although findings were inconsistent in a randomized controlled trial (15). Finally, the associations between physical activity and HDL proteome (HDL levels of acute-phase proteins such as C3c, SAA, apoA-I, apoA-IV, apoC-III, and E, among many others) have also been investigated in 2 observational studies (23,29). Testing the effects of promoting physical activity and calorie restriction within the frame of a Mediterranean diet (MedDiet) would therefore be a logical next step. This dietary pattern, and some of its key foods, have been associated with improvements in CEC, the HDL cholesterol metabolism, HDL antioxidant properties, HDL oxidation, HDL-bound levels of acute-phase proteins, HDL endothelial protection, HDL´s role in triglyceride metabolism, and HDL levels of certain apolipoproteins such as ApoA-I (30–33).

The aim of this study was to determine whether a lifestyle intervention consisting of an energy-restricted MedDiet and physical activity improved HDL functional traits in individuals with metabolic syndrome, compared to a MedDiet with spontaneous caloric intake and no changes in physical activity.

METHODS

Participants

Our study population is a subsample of 391 volunteers from the *Prevención con Dieta Mediterránea-Plus* (PREDIMED-Plus) study. The subjects were recruited in the Hospital del Mar Medical Research Institute (Barcelona, Spain) and provided plasma samples at baseline and after 6 months of intervention. The PREDIMED-Plus study is a multicenter, parallel, randomized controlled trial that aims to evaluate the effect of a lifestyle intervention with an energy-restricted MedDiet combined with physical activity and behavioral support relative to a MedDiet with a spontaneous caloric intake and without physical activity (control group), on the primary incidence of cardiovascular disease (13,34). Participants were community-dwelling males (aged 55-75 years) and females (aged 60-75 years) with a BMI between 27 and 40 kg/m^2. They presented at least 3 criteria for metabolic syndrome: 1) triglycerides ≥150 mg/dL or triglyceride-lowering medication; 2) fasting glucose ≥100 mg/dL or glucose-lowering medication; 3) systolic/diastolic blood pressure ≥130/85 mmHg or antihypertensive medication; 4) HDL cholesterol levels <40 mg/dL in males and <50 mg/dL in females; and/or 5) waist circumference ≥94 cm in males and ≥80 cm in females (35). The study protocol complied with the Declaration of Helsinki,

and is registered at the International Standard Randomized Controlled Number Registry as ISRCTN89898870. It has also been published elsewhere (35) and is available on the PREDIMED-Plus study website (https://www.predimedplus.com/en/project). This particular Sub-project was approved by the Parc de Salut Mar Clinical Research Ethics Committee. All participants provided written informed consent at the beginning of the study. The study

The study flowchart is depicted in **Figure 1**.

Exposure: lifestyle intervention

Participants were randomly allocated to a 1:1 ratio of either intensive lifestyle or nonrestrictive MedDiet intervention groups by a centrally controlled, computer-generated, random-numbered, internet-based system with stratification by center, age, and sex, as previously described (13).

Participants allocated to the control group were instructed by trained dieticians to follow a traditional MedDiet without caloric intake restrictions (the dietary intervention described in the PREDIMED study) (36). We encouraged: 1) the consumption of fruit, vegetables, legumes, nuts, and fish; 2) the use of extra-virgin olive oil as main culinary fat and for traditional food preparation techniques such as "sofrito"; and 3) a reduction in the intake of red/processed meats (by replacing them with poultry), sugary drinks, pastries, confectionery, sweets, and fatty spreads (36). Participants in the control group did not receive recommendations to increase their levels of physical activity or lose weight. The follow-up in this group consisted of an individual on-site interview at the beginning of the study and after each 6 months (35).

Participants allocated to the intensive intervention group were instructed to follow an energy-restricted MedDiet, together with physical activity recommendations, with the purpose of achieving specific weight loss goals (we aimed at an 8% weight

231 reduction or ≥5% decrease in waist circumference). Regarding physical activity,
232 subjects were encouraged to perform at least 45 minutes per day of moderate-
233 intensity aerobic activity (such as brisk walking, cycling, and swimming) and carry out
234 resistance, balance, or flexibility training. They were additionally advised to perform
235 different exercises to develop the strength of the main muscles for at least 2
236 days/week (duration: 30-40 minutes/day), as well as directed balanced activities
237 (e.g., yoga, tai chi) if they felt motivated and had access to these activities. Dietitians
238 adapted the previous recommendations to gradually achieve physical activity goals,
239 considering the participants' preferences. In addition, the energy-restricted MedDiet
240 intervention was aimed at a long-term, progressive, sustained calorie decrease of
241 approximately 30% of estimated energy requirements (about 600 kcal/day) according
242 to each participant's basal metabolic rate and physical activity levels, following the
243 Institute of Medicine equations (13). This calorie restriction was recommended within
244 the context of the previously described traditional MedDiet pattern, with some
245 particularities: 1) there were more restrictive limits for the consumption of
246 red/processed meats, fatty spreads, and sugary drinks; and 2) there were greater
247 limitations regarding the intake of refined carbohydrates (such as added sugar in
248 beverages, white bread, and refined cereals) and a promotion of whole-grain
249 consumption (35). To accomplish such goals, this intensive intervention group
250 followed a more thorough visit plan (1 face-to-face individual interview, 1group
251 session, and 1 phone call every month) (13,34,35).
252 Dietary quality, physical activity levels, and energy intake were evaluated in all
253 participants using 3 questionnaires. Adherence to the energy-restricted MedDiet
254 pattern was assessed by a 17-item questionnaire, with scores ranging from 0 (null
255 adherence) to 17 (full adherence) (35). We measured the total energy expenditure

from physical activity with the Minnesota-REGICOR (Registre GIroní del COR) leisure-time physical activity questionnaire (37). It was estimated in metabolic equivalents of task (METs) minutes per week by multiplying the METs linked to each activity collected in the questionnaires with the mean duration in minutes/week reported by the participants. Finally, we measured the intake of total energy (kcal/day) using the information gathered in a 143-item, semi-quantitative FFQ validated in an adult Spanish population (38).

Outcomes: HDL functional traits

We collected fasting EDTA plasma samples at baseline and after 6 months of the intervention and stored them at -80ºC until use. In these samples, we measured levels of glucose (Glucose HK CP, Horiba ABX), total cholesterol (Cholesterol CP, Horiba ABX), triglycerides (Triglycerides CP, Horiba ABX), and HDL cholesterol (HDL Direct CP, Horiba ABX) in an autoanalyzer ABX Pentra. LDL cholesterol was calculated using the Friedewald equation when triglycerides were <300 mg/dL.

We determined all HDL functional traits in apoB-depleted plasma, a modified preparation in which all lipoproteins except HDL are eliminated (low- and very low-density lipoproteins) by precipitation with 20% polyethylene glycol 8000 (Sigma-Aldrich) (31). CEC was measured in a human THP-1 monocyte- derived macrophage cell line incubated with 0.025 mM fluorescent 23-(dipyrrometheneboron difluoride)-24-norcholesterol (Avanti Polar Lipids) (7). The antioxidant/anti-inflammatory capacity of HDL was estimated by the HOII technique [the HDL capacity to prevent the oxidation of the fluorescent marker 2'-7'dichlorohydrofluorescein (Life Technologies) by oxidized LDLs] (7,31). HDL oxidation status [HDL content of oxidized lipids

(malondialdehyde equivalents) per unit of protein] was measured by the thiobarbituric acid reactive substances assay as previously described (31). ELISA kits were used to determine levels of SAA (*Human SAA ELISA Kit*, Life Technologies), S1P (*Sphingosine 1 Phosphate BioAssay ELISA Kit*, US Biological), and apoA-IV (*Human Apolipoprotein A-IV ELISA Kit*) (7). Finally, in an ABX Pentra autoanalyzer we determined the levels of C3c, triglycerides, apoA-I, apoC-III, ApoE, and total protein content in ApoB-depleted plasma samples [*ApoA1*, *Triglycerides CP, and Total Protein CP*; Horiba ABX); *Complement C3*, *ApoC-III*, and *ApoE*, Spinreact)] (7,31). Levels of C3c, SAA, S1P, triglycerides, apoA-I, apoA-IV, apoC-III, and apoE in apoB-depleted plasma were normalized against total protein concentration in these samples.

Interassay variability was minimized by: 1) examining the pre- and post-intervention samples from the same participant in the same experimental run; 2) analyzing the pair of samples from a participant of the intervention group followed by the samples of a participant of the control arm, according to a random sequence established prior to analyses; and 3) including in each experiment a sample pool (isolated from 20 healthy volunteers) used to calculate interassay CVs. Regarding functional tests (CEC and HOII): 1) both were assayed in duplicate and values with CVs ≥15% were eliminated; and 2) interassay variability was minimized by dividing CEC and HOII values of samples by those obtained for the control pool, providing normalized ratios without units as results (7,31). Interassay CVs and the number of missing values for all determinations are available in **Supplemental Table 1**.

Covariates and other variables

Trained staff collected data on the following variables at the baseline visit: age, sex, educational level, glucose-lowering, cholesterol-lowering, and antihypertensive drug use, and smoking habit. Qualified health-care providers measured weight and height using calibrated weight scales and stadiometers, and waist circumference (midway between the lowest rib and the iliac crest) using an anthropometric tape. BMI was calculated as weight divided by height squared (kg/m^2). Blood pressure was measured using a calibrated automated oscillometer (35). Type-2 diabetes was defined as described in the PREDIMED-Plus protocol (35); hypercholesterolemia was described as presenting with total cholesterol levels ≥200 mg/dL or using cholesterol-lowering mediation; and hypertension was described as presenting with systolic blood pressure ≥140 mmHg, presenting with diastolic blood pressure ≥90 mmHg, or using antihypertensive drugs.

Sample size

A sample size of 190 participants per group allowed ≥80% power to detect differences of 0.019 units in normalized CEC between pre- and post-intervention values, and of 0.026 units between the 2 interventions, considering a 2-sided type I error of 0.05, a loss rate of 5%, and the SD of the differences in CEC reported after an analogous dietary intervention in individuals at high cardiovascular risk (SD, 0.089) (31).

Statistical analyses

We described normally distributed continuous variables by means and SDs, nonnormally distributed continuous variables by medians (1st to 3rd quartile),

and categorical variables by proportions.

As main analyses, we assessed whether there were differences in the post-intervention values in lifestyle variables, continuous cardiovascular risk factors, and HDL functional traits in the energy-restricted MedDiet + physical activity group relative to the nonrestrictive MedDiet arm by multivariable linear regressions adjusted for: baseline levels of each outcome parameter (continuous), age (continuous), sex, educational level (primary/secondary/greater/unavailable), HDL cholesterol (continuous), triglycerides (continuous), prevalence of type 2 diabetes mellitus (yes/no), hypercholesterolemia (yes/no), hypertension (yes/no), smoking habit (current/former/never smoker), BMI (continuous), physical activity (continuous), and total energy intake (continuous). Multicollinearity among covariates was ruled out by checking their variance inflation factor values in all regression models, and normal distribution of all model residuals was confirmed by their quartile-quartile Q-Q plots. Models were fitted using the “lme4” package in R Software (R Foundation for Statistical Computing) (39). We also calculated the mediating effect of the 6-month weight loss on the associations between the intervention and the changes in HDL functionality traits using the “mediation” package in R Software (40). The proportion of mediation was calculated as the ratio between the effect size of the association through the 6-month BMI changes and the total effect size. Finally, as exploratory analyses, we assessed the average change across groups relative to preintervention values. We analyzed whether there were differences relative to baseline in all study participants by paired t-tests in normally distributed continuous variables and Wilcoxon signed rank tests in nonnormally distributed variables. These analyses were also performed within-group when the intergroup differences were significant. We did not perform any multiple testing adjustment because our analyses

were hypothesis driven and the phenotypes of interest were correlated and not independent (**Supplemental Figures 1** and **2**).

Analyses were performed using R Software version 3.6.1 (41).

RESULTS

Study participants

Participants were 391 older adults (mean age, 65.5 ± 4.64 years; 52% women) with excess body weight (19% of the population presented BMI values of 27.0-29.9 kg/m^2, and the remaining 81% presented values between 30.0-40.0 kg/m^2) and a high prevalence of cardiovascular risk factors (85% hypertension, 69% hypercholesterolemia, 35% diabetes, 9% current smokers). No differences at baseline between intervention and control groups were found for these characteristics, adherence to the MedDiet, and leisure-time physical activity levels (**Table 1**).

Lifestyle modifications

All participants increased their estimated total energy expenditure in physical activity and decreased their calorie intake relative to baseline values. However, participants in the intensive-lifestyle intervention displayed a greater increase (relative to the control arm) in physical activity (+726 METs-min/week; 95% CI: 294, 1160 METs-min/week) and a modest but greater decrease in energy intake (-75.8 kcal/day; 95% CI: -147 to -4.5 kcal/day; **Supplemental Table 2**). Both intervention arms, based on MedDiets, were associated relative to baseline with increases in the consumption of virgin olive oil, vegetables, legumes, nuts, whole grains, poultry, and white and fatty fish and

decreases in the intake of refined grains, red meat, processed meat, and alcoholic beverages (all P values <0.001). Adherence to the energy-restricted MedDiet pattern was, however, greater in the intensive lifestyle intervention group (+1.43 score points; 95% CI: 0.93-1.93 scores points). This intervention arm presented higher increases in the consumption of legumes, nuts, and poultry and decreases in the intake of refined grains (a marginal reduction in the consumption of red meat was also suggested) (**Supplemental Table 2**). No changes in smoking status were observed (**Supplemental Table 3**).

Changes in continuous cardiovascular risk factors

Irrespective of the study group, relative to baseline all participants had decreases in fasting glucose values, total and LDL cholesterol levels, systolic and diastolic blood pressure, body weight, BMI, and waist circumference, and increases HDL cholesterol concentrations. However, those allocated to the intensive lifestyle group, compared to the control group, experienced greater 6-month reductions in fasting glucose (-4.71 mg/dL; 95% CI: -9.06 to -0.35 mg/dL), triglycerides (-21.1 mg/dL; 95% CI: -30.5 to -11.6 mg/dL), systolic blood pressure, (-4.36 mmHg; 95% CI: -6.87 to -1.84 mmHg), diastolic blood pressure (-3.57 mmHg; 95% CI: -5.26 to -1.89 mmHg), body weight (-3.83 kg; 95% CI: -4.57 to -3.09 kg), BMI (-1.43 kg/m^2; 95% CI: -1.71 to -1.16 kg/m^2), and waist circumference (-3.44 cm; 95% CI: -4.28 to -2.61 cm). No intergrdifferences in total, HDL, and LDL cholesterol levels were observed (**Supplemental Table 4).**

Changes in HDL functional traits

Compared to participants in the control group, those in the intensive-intervention group had greater 6-month reductions in levels of triglycerides (-0.15 mg/g protein; 95% CI: -0.29 to -0.014 mg/g protein) and apoC-III (-0.11 mg/g protein; 95% CI: -0.18 to -0.026 mg/g protein) in apoB-depleted plasma (**Table 2**). Intergroup differences in both parameters were substantially mediated by 6-month weight changes (triglycerides: proportion of mediation=77.4% (95% CI: 22.3%-382%; *P*-value=0.016); apoC-III: proportion of mediation=72.1% (95% CI: 30.3%-265%; *P*-value=0.006); (**Supplemental Table 5**). Intergroup differences, stratified by sex and baseline prevalence of diabetes, are available in **Supplemental Tables 6** and **7**. No intergroup differences in 6-month changes were detected in the remaining HDL functional traits. Nevertheless, we observed decreases in HDL oxidative/inflammatory potential, HDL oxidation, and concentrations of C3c, SAA, and S1P and increases in apoA-I relative to baseline

values across groups (**Table 2**).

DISCUSSION

An intervention with an energy-restricted MedDiet plus physical activity improved HDL functionality on triglyceride metabolism in older adults with metabolic syndrome compared with a nonrestrictive MedDiet without physical activity.

HDLs are intimately related to triglyceride metabolism. High triglyceride levels in HDLs destabilize their structure and function (42) and, in turn, have been causally linked to greater coronary artery disease (11). Moreover, HDLs carry lipoproteins involved in triglyceride metabolism, such as apoC-III which inhibits lipoprotein lipase activity and the hepatic clearance of triglyceride-rich lipoproteins (43), and is directly linked to coronary heart disease risk (44). In our study, intervention with an energy-restricted MedDiet plus physical activity was able to decrease both apoC-III and the triglyceride content of HDLs, mainly through the associated weight loss. This factor could partially explain decreases in these parameters, as obesity is related to greater HDL content of apoC-III and triglycerides (42,45). ApoC-III synthesis is also exacerbated in impaired glucose metabolism states (46) which may diminish after weight loss. Finally, the molecular effects of physical activity and energy restriction may additionally contribute to decreasing triglyceride levels. Aerobic physical activity and caloric restriction have been shown to be able to stimulate AMP-activated protein kinase, which, in turn, decreases the activation of lipogenic transcription factors involved in triglyceride synthesis in the liver (47). A synergistic effect between these lifestyle modifications and some MedDiet bioactive compounds could additionally be present. Phenolic compounds and SCFAs derived from the bacterial

metabolism of dietary fiber in the intestine have been reported to be able to boost AMP-activated protein kinase through alternative metabolic pathways (48,49).

Contrary to what was observed for HDL's role in the triglyceride metabolism, we did not observe any intergroup difference in HDL properties related to oxidative status and low-grade inflammation, because there was a decrease in these properties relative to baseline in both study arms. Both were based in antioxidant-rich dietary patterns (50), and previous human studies have indicated that dietary antioxidants are able to bind to HDLs and possibly induce a local antioxidant effect (30,31,51). In addition, a MedDiet has been shown to decrease the levels of circulating pro-inflammatory cytokines (52), probably due to the ability of dietary antioxidants to modulate various transcriptomic mechanisms (53), which in turn could be associated with reduced adhesion of these molecules to the surface of HDL. These findings agree with previous evidence, since an improvement in HDL antioxidant/anti-inflammatory properties has been reported after a 1-year intervention with a MedDiet in individuals with a high cardiovascular risk (31,33). Finally, the 2 intervention arms failed to increase CEC. In a prior study comparing a traditional MedDiet intervention with a low-fat diet, no intergroup difference was observed in CEC values, although they increased in the MedDiet intervention groups relative to baseline (31). A weight-loss intervention based on a healthy dietary pattern [Dietary Approaches to Stop Hypertension (DASH diet] plus physical activity was also linked to increased CEC levels in an observational study (29). Such divergent findings might be due to: 1) differing proportions of individuals prone prone to lower CEC values (likely to benefit from the intervention), such as participants with type-2 diabetes or excess weight (54); 2) distinct intervention lengths (6 months in the present study, 12 months in our prior work, 3 months for the DASH diet); 3) different magnitudes of weight loss among studies; and 4) the techniques used to quantify CEC (in the present study we worked with a fluorescent-labeled cholesterol probe,

whilst in the others a radiolabeled cholesterol analog was used).

Our study has some strengths. As far as we know, this is the largest to address the effect of a whole-lifestyle intervention on a comprehensive, hypothesis-driven set of HDL functional traits. Its sample size, together with its randomized design, provide high quality evidence and minimize the influence of confounding and bias. There are, however, a number of limitations. First, results were obtained in older adults with metabolic syndrome and excess body weight, and cannot therefore be extrapolated to other populations. Second, as expected, we only found moderate differences between intervention arms, given that we used an active comparator as a control group (a healthy, traditional MedDiet), and the intensive intervention consisted of real-life changes of diet and physical activity, adapted to the participants' clinical conditions. Third, whilst a substantial increase in the physical activity levels of the participants in the intensive lifestyle intervention arm was observed, the intergroup differences in energy intake were of a lower magnitude. Nevertheless, the aimed decrease in energy consumption is ambitious and intended to be achieved throughout the whole study. Currently, we are only considering the 6 first months of the intervention. Fourth, 16 participants from the 407 recruited individuals in our center were lost to follow-up after 6 months of the study. This may represent a potential source of bias in our analyses. Fifth, our study design compares an intensive intervention based on the combination of calorie restriction, physical activity, and a Mediterranean dietary pattern relative to a control arm based on a non-hypocaloric Mediterranean diet exempt of physical activity recommendations. Our design does not allow us to discriminate between the individual effects of calorie restriction or physical activity, nor to examine their interactions. Possible synergistic or additive effects should be further explored in more specific designs. Sixth, our study is based on a

hypothesis-driven approach and investigates secondary outcomes of the PREDIMED-Plus study (which are correlated and not independent). Thus, we did not correct our results according to multiple testing, and the *P* values reported in our findings should be interpreted with caution. Finally, the results of the mediation analyses presented wide Cis due the limited sample size and should also be interpreted carefully.

In conclusion, in older adults with metabolic syndrome, an intensive-lifestyle intervention with an energy-restricted MedDiet and physical activity improved HDL functions on the triglyceride metabolism relative to a nonrestrictive MedDiet control group. Our findings suggest that a healthy lifestyle may have a positive impact on HDL functionality. Further prospective studies examining whether these improvements mediate the cardiovascular benefits of the lifestyle modifications investigated in our work are warranted.

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CONFLICTS OF INTEREST

J.S.-S. reports being a board member and receiving personal fees from the Instituto Danone Spain; being a board member and obtaining grants from the International Nut and Dried Fruit Foundation; receiving personal fees from Aguas Font Vella Lanjarón, and Danone S.A; and obtaining grants from Eroski Distributors. R.E. reports being a board member of the Research Foundation on Wine and Nutrition, the Beer and Health Foundation, and the European Foundation for Alcohol Research; receiving personal fees from KAO Corporation; lecture fees from Instituto Cervantes, Fundación Dieta Mediterranea, Cerveceros de España, Lilly Laboratories, AstraZeneca, and Sanofi; and grants from Novartis, Amgen, Bicentury, and Grand Fountaine. E.R. reports obtaining personal fees, grants, and nonfinancial support from the California Walnut Commission and Alexion; personal fees and nonfinancial support from Danone; and nonfinancial support from the International Nut Council. The rest of the authors have nothing to disclose.

AUTHORS' CONTRIBUTIONS

AH and MF designed the research. AS conducted research. MAMG, JSS, DC, RE, FJT, ER, and MF conducted the clinical trial and provided study databases. AS, IS, and AH analyzed data. AS, AH, and MF wrote the manuscript draft. MTSF, OC, IS, CL, MAMG, JSS, DC, RE, FJT, and ER reviewed and edited the text. AH and MF have primary responsibility for final content. All authors read and approved the final manuscript.

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TABLES

Table 1. Baseline characteristics of participants

	All participants (*n*=391)	**Control group (*n*=201)**	**Intensive intervention (*n*=190)**
Age (years), mean ± SD	65.5 ± 4.64	65.3 ± 4.61	65.7 ± 4.68
Female sex, n (%)	204 (52.2)	105 (52.2)	99 (52.1)
Type-2 diabetes, n (%)	137 (35.0)	72 (35.8)	65 (34.2)
Glucose-lowering medication, n (%)	89 (22.8)	53 (26.4)	36 (18.9)
Hypercholesterolemia, n (%)	270 (69.4)	142 (70.6)	128 (68.1)
Cholesterol-lowering medication, n (%)	182 (46.5)	95 (47.3)	87 (45.8)
Hypertension, n (%)	334 (85.4)	170 (84.6)	164 (86.3)
Antihypertensive medication, n (%)	299 (76.5)	152 (75.6)	147 (77.4)
Status according to BMI:			
BMI between 27.0-29.9 kg/m^2, n (%)	76 (19.4)	32 (15.9)	44 (23.2)
BMI between 30.0-40.0 kg/m^2, n (%)	315 (80.6)	169 (84.1)	146 (76.8)
Abdominal obesity, n (%)	377 (97.2)	196 (98.5)	181 (95.8)
Smoking status:			
Never smokers, n (%)	193 (49.4)	91 (45.3)	102 (53.7)
Current smokers, n (%)	36 (9.21)	16 (7.96)	20 (10.5)
Former smokers, n (%)	162 (41.4)	94 (46.8)	68 (35.8)
Educational level:			
Elementary school, n (%)	163 (41.6)	86 (42.8)	77 (40.5)
High school, n (%)	137 (35.0)	73 (36.3)	64 (33.7)

Undergraduate education, n (%)	40 (10.2)	15 (7.46)	25 (13.2)
Graduate or postgraduate, n (%)	48 (12.3)	27 (13.4)	21 (11.1)
Unavailable information, n (%)	3 (0.77)	0 (0.00)	3 (1.58)
Adherence to the MedDiet (score), mean ± SD	7.32 ± 2.46	7.17 ± 2.38	7.48 ± 2.55
Leisure-time physical activity (metabolic equivalents of task-minute/week), median (1^{st}-3^{rd} quartile)	1,958 (895-3,413)	1,734 (895-3,413)	2,168 (899-3,378)

Table 2. Differences in 6-month changes in HDL functionality traits between control and intervention groups

	Non-restrictive MedDiet, control group		**Energy-restricted MedDiet + physical activity**		**Average change across groups**	**Inter-group difference**	
	Pre-interv. values	**Post-interv. values**	**Pre-interv. values**	**Post-interv. values**	***P*-value**	**Adjusted difference [95% CI]**	***P*-value**
Cholesterol efflux capacity, ratio	1.08 ± 0.17	1.09 ± 0.17	1.05 ± 0.15	1.05 ± 0.15	0.372	-0.006 [-0.028; 0.016]	0.616
HDL oxidative/inflammatory index, ratio	0.89 ± 0.20	0.83 ± 0.19	0.91 ± 0.17	0.87 ± 0.18	<0.001	0.022 [-0.005; 0.048]	0.107
HDL oxidation, µg MDA/g protein	10.1 ± 2.24	9.92 ± 2.33	10.3 ± 2.34	9.92 ± 2.20	<0.001	-0.095 [-0.31; 0.12]	0.386
Complement component 3 in apoB-depleted plasma, mg/g protein	4.15 ± 1.18	3.94 ± 1.15	3.92 ± 1.18	3.75 ± 1.02	<0.001	-0.040 [-0.21; 0.13]	0.646
Serum amyloid A in apoB-depleted	477	398	437	347	<0.001	76.5	0.327

plasma, µg/g protein	(249-993)	(212-883)	(264-870)	(208-893)		[-76.4; 229]	
Sphingosine-1-phosphate in apoB-depleted plasma, µg/g protein	3.65 ± 1.20	3.63 ± 1.21	4.00 ± 1.35	3.76 ± 1.27	0.028	-0.11 [-0.32; 0.11]	0.327
Triglycerides in apoB-depleted plasma, mg/g protein	3.85 ± 0.96	3.67 ± 0.90[1]	3.83 ± 1.02	3.54 ± 0.97[1]	<0.001	-0.15 [-0.29; -0.014]	0.032
Apolipoprotein A-I in apoB-depleted plasma, mg/g protein	26.6 ± 4.10	26.8 ± 4.17	25.8 ± 3.91	26.3 ± 4.17	0.009	0.15 [-0.32; 0.62]	0.531
Apolipoprotein A-IV in apoB-depleted plasma, µg/g protein	139 (97.3-208)	134 (99.5-192)	130 (101-171)	129 (94.6-175)	0.187	2.74 [-8.75; 14.2]	0.641
Apolipoprotein C-III in apoB-depleted plasma, mg/g protein	1.00 (0.66-1.50)	1.02 (0.62-1.40)	0.98 (0.61-1.42)	0.82 (0.55-1.31)[1]	<0.001	-0.11 [-0.18; -0.026]	0.009
Apolipoprotein E in apoB-depleted plasma, mg/g protein	0.31 ± 0.16	0.31 ± 0.17	0.29 ± 0.16	0.30 ± 0.16	0.699	0.010 [-0.008; 0.028]	0.277

[1]: *P*-value <0.05 (post- versus pre-intervention values: paired t-test for normally distributed variables, Wilcoxon signed-rank test for non-normally distributed variables)

Pre- and post-intervention values are presented as means ± standard deviations for normally distributed variables or medians (1^{st}-3^{rd} quartile) for non-normally distributed variables. Inter-group comparisons in post-intervention values were assessed by multivariable linear regression models adjusted for: baseline levels of the parameter, age, sex, educational level, HDL-C, triglycerides, type-II diabetes, hypercholesterolemia, hypertension, smoking, body mass index, physical activity, and total energy intake. Average change across groups was assessed in the whole study population by paired t-tests in normally distributed variables and Wilcoxon signed-rank test in non-normally distributed variables.

FIGURES

Figure 1. Study flowchart

ON-LINE SUPPLEMENTARY MATERIAL

A lifestyle intervention with an energy-restricted Mediterranean diet and physical activity enhances HDL function: a sub-study of the PREDIMED-plus randomized controlled trial

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Supplemental Table 1. Inter-assay coefficients of variability and number of missing values

	Inter-group coefficient of variation (%)	**Missing data, *n* (%)**
Determinations in plasma		
Glucose	2.73%	0 (0%)
Total cholesterol	2.64%	0 (0%)
HDL cholesterol	3.47%	0 (0%)
Triglycerides	4.45%	0 (0%)
Determinations in apolipoprotein B-depleted plasma		
Cholesterol efflux capacity	7.21%	19 (2.43%)
HDL oxidative/inflammatory index	5.65%	2 (0.26%)
HDL oxidation	4.24%	12 (1.53%)
Complement component 3	4.08%	7 (0.90%)
Serum amyloid A	16.0%	6 (0.77%)
Sphingosine-1-phosphate	10.9%	16 (2.04%)
Triglycerides	1.65%	3 (0.38%)
Apolipoprotein A-I	1.78%	9 (1.15%)
Apolipoprotein A-IV	11.3%	4 (0.51%)
Apolipoprotein C-III	6.15%	4 (0.51%)
Apolipoprotein E	3.23%	6 (0.77%)

Supplemental Table 2. Differences in 6-month changes in lifestyle and dietary parameters between control and intensive intervention groups

	Non-restrictive MedDiet, control group		Energy-restricted MedDiet + physical activity		Average change across groups	Inter-group difference	
	Pre-interv. Values	Post-interv. values	Pre-interv. values	Post-interv. values	*P*-value	Adjusted diff. [95% CI]	*P*-value
Adherence to an energy-restricted MedDiet (score points)	7.17 ± 2.38	10.3 ± 2.64[1]	7.48 ± 2.55	11.7 ± 2.40[1]	<0.001	1.43 [0.93; 1.93]	<0.001
Leisure-time physical activity (METs-min/week)	1730 (895-3410)	2240 (1120-3480)	2170 (899-3380)	2970 (1710-5010)[1]	<0.001	726 [294; 1160]	0.001
Energy intake (kcal/day)	2420 (2110-2730)	2300 (2070-2570)[1]	2300 (2050-2620)	2190 (2020-2440)[1]	<0.001	-75.8 [-147; -4.48]	0.038
Carbohydrates (g/day)	225 (188-256)	199 (172-241)[1]	209 (179-250)	190 (172-210)[1]	<0.001	-11.0 [-20.2; -1.77]	0.020
Proteins (g/day)	105 (91.9-118)	107 (97.8-117)	101 (87.9-113)	108 (97.1-117)	<0.001	1.45 [-1.69; 4.58]	0.367
Total fat (g/day)	115 (99.4-135)	116 (100-128)	110 (96.1-131)	110 (98.2-123)	0.251	-2.51 [-6.51; 1.49]	0.220
Saturated fatty acids (g/day)	29.4 (24.6-35.1)	24.1 (20.6-29.1)[1]	28.5 (22.9-33.8)	22.3 (19.7-25.8)[1]	<0.001	-2.19 [-3.35; -1.04]	<0.001
Monounsaturated fatty acids (g/day)	59.6 (51.9-69.2)	62.4 (52.4-72.5)	57.3 (50.0-67.2)	63.1 (53.2-72.6)	<0.001	1.06 [-1.86; 3.98]	0.478
Polyunsaturated fatty acids (g/day)	17.0 (13.8-21.8)	22.6 (17.4-25.2)	16.0 (13.4-20.7)	22.6 (18.9-25.0)	<0.001	0.72 [-0.29; 1.73]	0.162
Omega-3 polyunsaturated fatty acids (g/day)	0.92 (0.71-1.55)	1.49 (0.80-1.65)	0.91 (0.69-1.55)	1.51 (0.85-1.66)	<0.001	0.064 [-0.032; 0.16]	0.193
Dietary fiber (g/day)	24.0 (20.3-29.0)	30.3 (25.4-35.1)[1]	24.4 (19.9-29.5)	32.2 (26.8-36.7)[1]	<0.001	1.51 [0.088; 2.93]	0.038
Alcohol (g/day)	4.98 (1.37-12.7)	2.17 (0.69-10.3)[1]	3.50 (0.69-11.9)	1.46 (0.00-5.14)[1]	<0.001	-1.84 [-3.18; -0.50]	0.007
Virgin olive oil (g/day)	25.0 (10.0-50.0)	50.0 (50.0-50.0)	25.0 (10.0-50.0)	50.0 (50.0-50.0)	<0.001	0.20 [-2.18; 2.57]	0.872

Vegetables (g/day)	322 (244-414)	376 (300-460)	331 (274-413)	382 (313-482)	<0.001	13.7 [-12.8; 40.1]	0.312
Fruits (g/day)	341 (240-460)	335 (250-439)	339 (218-441)	352 (288-429)	0.233	15.8 [-14.2; 45.8]	0.304
Legumes (g/day)	20.6 (12.6-25.1)	25.1 (20.6-29.7)[1]	17.1 (12.0-25.1)	25.7 (21.1-29.7)[1]	<0.001	2.67 [0.76; 4.58]	0.006
Nuts (g/day)	12.6 (2.00-25.7)	32.0 (25.6-49.1)[1]	9.42 (4.00-25.7)	38.6 (30.0-53.6)[1]	<0.001	7.08 [2.70; 11.4]	0.002
Refined grains (g/day)	109 (72.3-157)	44.5 (20.5-99.9)[1]	99.7 (57.2-139)	42.3 (20.5-57.8)[1]	<0.001	-11.4 [-21.2; -1.55]	0.024
Whole grains (g/day)	8.33 (0.00-75.0)	75.0 (32.1-82.2)	8.33 (0.00-75.0)	75.0 (58.9-82.1)	<0.001	1.48 [-8.68; 11.6]	0.775
Dairy products (g/day)	346 (257-452)	346 (275-538)	308 (233-412)	336 (268-404)	0.028	-8.85 [-44.9; 27.2]	0.630
Eggs (g/day)	25.7 (25.7-25.7)	25.7 (25.7-25.7)	25.7 (12.9-25.7)	25.7 (25.7-25.7)	0.113	0.20 [-1.26; 1.66]	0.787
Poultry and rabbit (g/day)	64.3 (42.8-85.7)	74.3 (64.3-85.7)[1]	74.3 (52.8-85.7)	85.7 (74.3-85.7)[1]	<0.001	10.2 [4.21; 16.2]	<0.001
Red meat (g/day)	64.3 (31.4-85.7)	41.4 (31.1-64.3)	52.8 (31.4-84.3)	31.4 (21.4-42.8)	<0.001	-4.66 [-10.1; 0.81]	0.096
Processed meat (g/day)	36.2 (26.7-47.1)	30.3 (24.2-39.5)	35.5 (26.4-41.8)	29.0 (22.0-35.0)	<0.001	-2.18 [-4.93; 0.58]	0.122
White fish (g/day)	64.3 (25.4-68.3)	64.3 (25.4-68.3)	64.3 (25.4-68.3)	64.3 (30.0-68.3)	<0.001	0.88 [-3.43; 5.18]	0.691
Fatty fish (g/day)	32.8 (21.9-62.8)	59.0 (25.7-62.8)	30.1 (21.9-62.7)	59.0 (25.7-62.8)	<0.001	2.18 [-2.33; 6.68]	0.345
Seafood (g/day)	30.6 (26.6-45.9)	30.6 (30.1-45.9)	30.6 (21.9-45.9)	30.6 (26.6-45.9)	0.809	1.01 [-2.84; 4.87]	0.606
Wine (mL/day)	20.0 (6.66-70.4)	13.3 (2.50-44.5)[1]	14.3 (6.66-54.5)	6.66 (0.00-42.8)[1]	<0.001	-9.22 [-18.2; -0.22]	0.045
Beer (mL/day)	22.0 (0.00-141)	22.0 (0.00-47.1)[1]	22.0 (0.00-47.1)	0.00 (0.00-22.0)[1]	<0.001	-36.3 [-55.6; -17.0]	<0.001

[1]: *P*-value <0.05 (post- versus pre-intervention values: paired t-test for normally distributed variables, Wilcoxon signed-rank test for non-normally distributed variables)

Pre- and post-intervention values are presented as means ± standard deviations for normally distributed variables or medians (1^{st}-3^{rd} quartile) for non-normally distributed variables. Inter-group comparisons in post-intervention values were assessed by multivariable linear regression models adjusted for: baseline levels of the parameter, age, sex, educational level, HDL-C, triglycerides, type-II diabetes, hypercholesterolemia, hypertension, smoking, body mass index, physical activity, and total energy intake. Average change across groups was assessed in the whole study population by paired t-tests in normally distributed variables and Wilcoxon signed-rank test in non-normally distributed variables.

Supplemental Table 3. Changes in the proportion of non-smokers or ever smokers after 6 months of intervention

Non-smokers vs. smokers				
		Post-intervention		McNemar's test *P*-value
		Non-smokers	Smokers	
Pre-intervention	Non-smokers	350 (89.5%)	5 (1.28%)	0.724
	Smokers	3 (0.77%)	33 (8.44%)	
Never smokers vs. ever smokers				
		Post-intervention		McNemar's test *P*-value
		Never smokers	Ever smokers	
Pre-intervention	Never smokers	181 (46.3%)	12 (3.07%)	0.361
	Ever smokers	18 (4.60%)	180 (46.0%)	

Supplemental Table 4. Differences in 6-month changes in clinical parameters between control and intensive intervention groups

	Non-restrictive MedDiet, control group		Energy-restricted MedDiet + physical activity		Average change across groups	Inter-group difference	
	Pre-interv. Values	Post-interv. values	Pre-interv. values	Post-interv. values	*P*-value	Adjusted diff. [95% CI]	*P*-value
Glucose, mg/dL	110 (100-132)	106 (97-124)	110 (102-130)	106 (97-118)[1]	<0.001	-4.71 [-9.06; -0.35]	0.035
Total cholesterol, mg/dL	218 ± 41.4	215 ± 40.9	222 ± 40.7	217 ± 37.2	0.012	0.20 [-5.51; 5.91]	0.946
HDL cholesterol, mg/dL	54.4 ± 11.1	55.4 ± 11.9	52.5 ± 11.2	55 ± 12.5	<0.001	1.10 [-0.34; 2.54]	0.136
LDL cholesterol, mg/dL	134 ± 35.3	129 ± 33.7	139 ± 37.1	136 ± 33.3	0.010	2.61 [-2.26; 7.48]	0.294
Triglycerides, mg/dL	144 (107-187)	136 (99-183)[1]	131 (103-179)	118 (91-155)[1]	<0.001	-21.1 [-30.5; -11.6]	<0.001
Systolic blood pressure, mmHg	140 ± 12.5	138 ± 14.5[1]	141 ± 12.0	135 ± 14.1[1]	<0.001	-4.36 [-6.87; -1.84]	<0.001
Diastolic blood pressure, mmHg	75 ± 10.2	74 ± 9.96	76 ± 8.84	72 ± 9.73[1]	<0.001	-3.57 [-5.26; -1.89]	<0.001
Body weight, kg	89.0 ± 13.8	86.3 ± 13.7[1]	87.4 ± 14.0	81.0 ± 12.8[1]	<0.001	-3.83 [-4.57; -3.09]	<0.001
Body mass index, kg/m^2	33.6 ± 3.49	32.6 ± 3.61[1]	33.1 ± 3.5	30.7 ± 3.42[1]	<0.001	-1.43 [-1.71; -1.16]	<0.001
Waist circumference, cm	111 ± 9.59	109 ± 9.70[1]	110 ± 9.71	104 ± 9.30[1]	<0.001	-3.44 [-4.28; -2.61]	<0.001

[1]: *P*-value <0.05 (post- versus pre-intervention values: paired t-test for normally distributed variables, Wilcoxon signed-rank test for non-normally distributed variables)
Pre- and post-intervention values are presented as means ± standard deviations for normally distributed variables or medians (1^{st}-3^{rd} quartile) for non-normally distributed variables. Inter-group comparisons in post-intervention values were assessed by multivariable linear regression models adjusted for: baseline levels of the parameter, age, sex, educational level, HDL-C, triglycerides, type-II diabetes, hypercholesterolemia, hypertension, smoking, body mass index, physical activity, and total energy intake. Average change across groups was assessed in the whole study population by paired t-tests in normally distributed variables and Wilcoxon signed-rank test in non-normally distributed variables

Supplemental Table 5. Proportion of inter-change differences mediated by 6-month decreases in body mass index

	Proportion of mediation [95% CI]
Cholesterol efflux capacity, ratio	1.52 [-582; 454]
HDL oxidative/inflammatory index, ratio	5.67 [-182; 235]
HDL oxidation, µg MDA/g protein	48.5 [-761; 704]
Complement component 3 in apoB-depleted plasma, mg/g protein	137 [-2350; 2900]
Serum amyloid A in apoB-depleted plasma, µg/g protein	-29.6 [-594; 375]
Sphingosine-1-phosphate in apoB-depleted plasma, µg/g protein	127 [-1450; 1140]
Triglycerides in apoB-depleted plasma, mg/g protein	77.4 [22.3; 382]
Apolipoprotein A-I in apoB-depleted plasma, mg/g protein	52.5 [-761; 1330]
Apolipoprotein A-IV in apoB-depleted plasma, µg/g protein	8.69 [-547; 566]
Apolipoprotein C-III in apoB-depleted plasma, mg/g protein	72.1 [30.3; 265]
Apolipoprotein E in apoB-depleted plasma, mg/g protein	61.4 [-687; 637]

Analyses were adjusted for baseline levels of the parameter, age, sex, educational level, HDL-C, triglycerides, type-II diabetes, hypercholesterolemia, hypertension, smoking, body mass index, physical activity, and total energy intake.

Supplemental Table 6. Sex-stratified inter-group analyses

	Women		**Men**		**Interaction (*P*-value)**
	Inter-group diff. [95% CI]	*P*-value	Inter-group diff. [95% CI]	*P*-value	
Cholesterol efflux capacity, ratio	0.005 [-0.024; 0.033]	0.753	-0.009 [-0.045; 0.026]	0.600	0.547
HDL oxidative/inflammatory index, ratio	0.004 [-0.033; 0.042]	0.824	0.036 [-0.003; 0.074]	0.073	0.330
HDL oxidation, µg MDA/g protein	-0.013 [-0.31; 0.28]	0.934	-0.13 [-0.44; 0.19]	0.422	0.606
Serum amyloid A in apoB-depleted plasma, µg/g protein	116 [-114; 347]	0.323	-25.7 [-224; 173]	0.800	0.369
Complement component 3 in apoB-depleted plasma, mg/g protein	-0.007 [-0.26; 0.25]	0.958	-0.097 [-0.34; 0.15]	0.434	0.484
Sphingosine-1-phosphate in apoB-depleted plasma, µg/g protein	-0.065 [-0.37; 0.24]	0.683	-0.15 [-0.45; 0.15]	0.330	0.419
Triglycerides in apoB-depleted plasma, mg/g protein	-0.13 [-0.35; 0.085]	0.237	-0.20 [-0.37; -0.026]	0.025	0.749
Apolipoprotein A-I in apoB-depleted plasma, mg/g protein	0.35 [-0.35; 1.05]	0.330	-0.11 [-0.75; 0.52]	0.726	0.622
Apolipoprotein A-IV in apoB-depleted plasma, µg/g protein	4.18 [-13.2; 21.5]	0.638	1.31 [-13.8; 16.4]	0.865	0.804
Apolipoprotein C-III in apoB-depleted plasma, mg/g protein	-0.073 [-0.18; 0.030]	0.165	-0.15 [-0.27; -0.026]	0.019	0.367
Apolipoprotein E in apoB-depleted plasma, mg/g protein	0.002 [-0.024; 0.028]	0.858	0.019 [-0.006; 0.044]	0.133	0.410

Inter-group comparisons in post-intervention values were assessed by multivariable linear regression models adjusted for: baseline levels of the parameter, age, educational level, HDL-C, triglycerides, type-II diabetes, hypercholesterolemia, hypertension, smoking, body mass index, physical activity, and total energy intake. We tested whether there was a significant association between the intervention group and sex on post-intervention HDL functional properties by applying a likelihood ratio test between the regression models with and without the interaction product-term “intervention group x sex”.

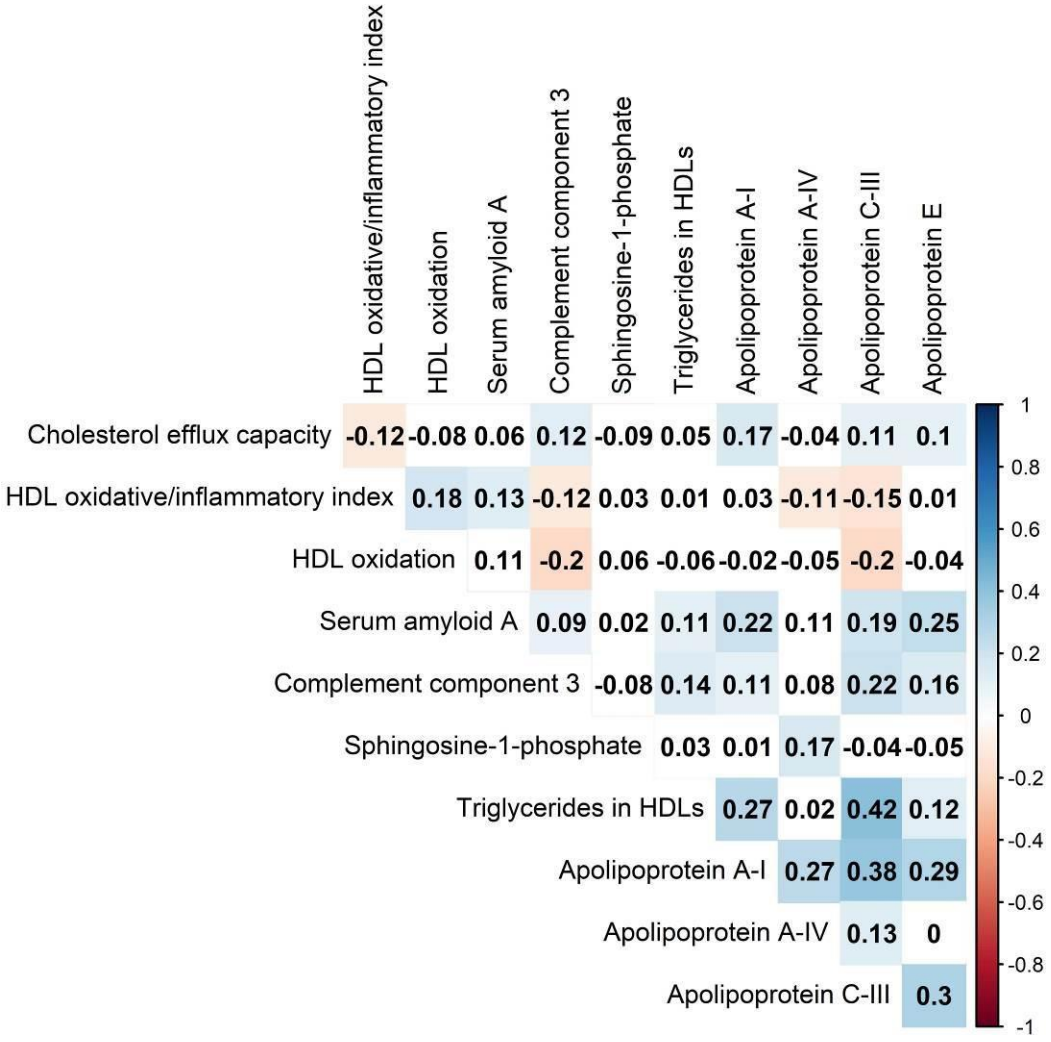
Supplemental Table 7. Diabetes-stratified inter-group analyses

	Non-diabetic		**Diabetic**		**Interaction (*P*-value)**
	Inter-group diff. [95% CI]	*P*-value	Inter-group diff. [95% CI]	*P*-value	
Cholesterol efflux capacity, ratio	0.012 [-0.013; 0.036]	0.356	-0.051 [-0.119; 0.018]	0.145	0.119
HDL oxidative/inflammatory index, ratio	0.017 [-0.014; 0.048]	0.273	0.019 [-0.032; 0.07]	0.472	0.894
HDL oxidation, µg MDA/g protein	-0.13 [-0.36; 0.090]	0.242	-0.010 [-0.45; 0.43]	0.963	0.675
Serum amyloid A in apoB-depleted plasma, µg/g protein	20.1 [-176; 216]	0.841	103 [-167; 373]	0.454	0.455
Complement component 3 in apoB-depleted plasma, mg/g protein	0.011 [-0.20; 0.22]	0.917	-0.12 [-0.43; 0.20]	0.473	0.273
Sphingosine-1-phosphate in apoB-depleted plasma, µg/g protein	-0.12 [-0.40; 0.16]	0.401	-0.076 [-0.43; 0.28]	0.677	0.590
Triglycerides in apoB-depleted plasma, mg/g protein	-0.18 [-0.36; 0.005]	0.057	-0.087 [-0.32; 0.14]	0.457	0.660
Apolipoprotein A-I in apoB-depleted plasma, mg/g protein	-0.12 [-0.68; 0.45]	0.679	0.65 [-0.23; 1.53]	0.149	0.115
Apolipoprotein A-IV in apoB-depleted plasma, µg/g protein	-11.1 [-26.7; 4.39]	0.161	19.3 [1.70; 36.8]	0.033	0.021
Apolipoprotein C-III in apoB-depleted plasma, mg/g protein	-0.16 [-0.27; -0.061]	0.002	-0.011 [-0.14; 0.12]	0.873	0.031
Apolipoprotein E in apoB-depleted plasma, mg/g protein	0.002 [-0.023; 0.026]	0.882	0.026 [$-4 \cdot 10^{-4}$; 0.052]	0.056	0.088

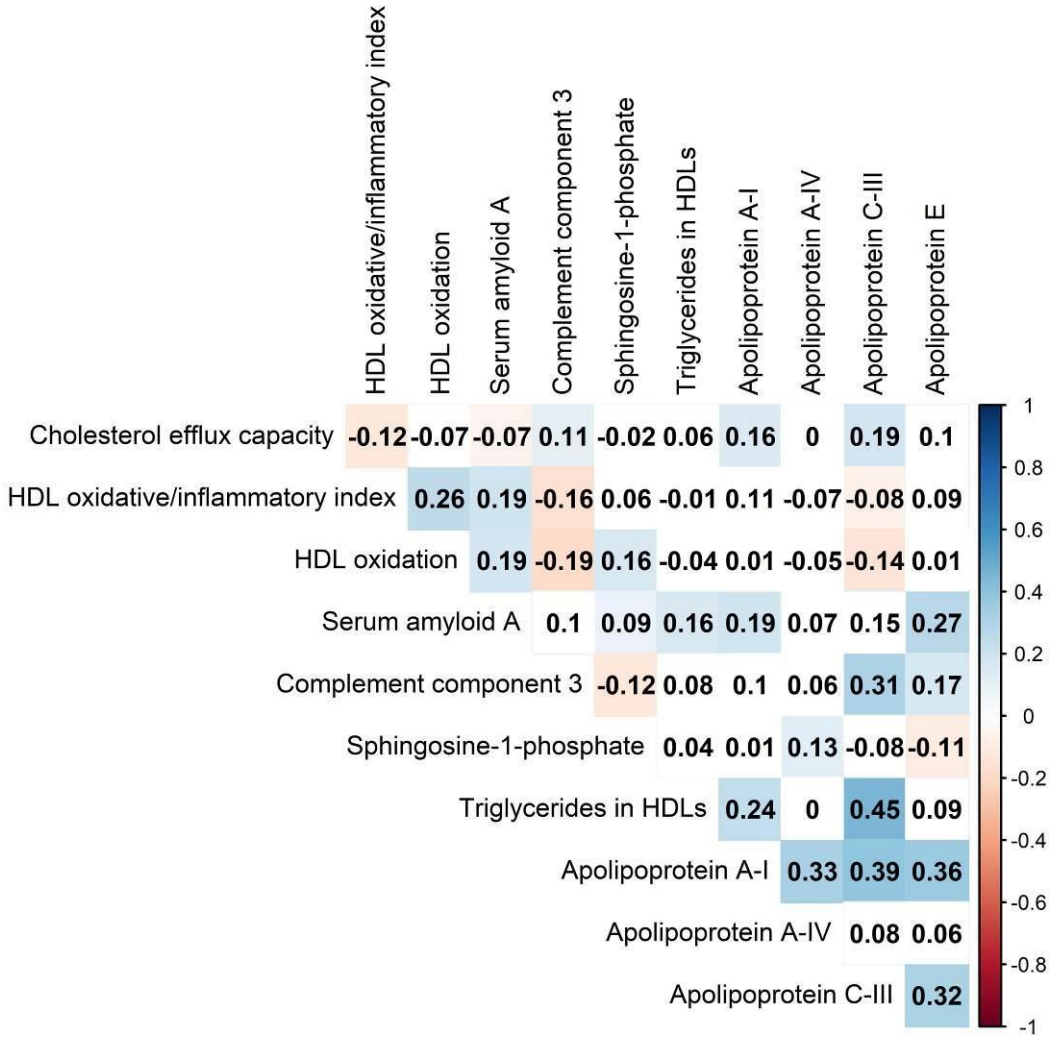
Inter-group comparisons in post-intervention values were assessed by multivariable linear regression models adjusted for: baseline levels of the parameter, age, sex, educational level, HDL-C, triglycerides, hypercholesterolemia, hypertension, smoking, body mass index, physical activity, and total energy intake. We tested whether there was a significant association between the intervention group and diabetes on post-intervention HDL functional properties by applying a likelihood ratio test between the regression models with and without the interaction product-term “intervention group x prevalence of diabetes at baseline”.

SUPPLEMENTAL FIGURES

Supplemental Figure 1. Correlation matrix among baseline HDL functionality parameters.



Supplemental Figure 2. Correlation matrix among post-intervention HDL functionality parameters.



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