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Increased Consumption of Virgin Olive Oil, Nuts, Legumes, Whole Grains, and Fish Promotes HDL Functions in Humans

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Abbreviations

CETP: cholesteryl ester transfer protein

PON1: paraoxonase-1

Keywords: “fish”, “high-density lipoprotein functionality”, “legumes and grains”, “nuts”, “virgin olive oil”

ABSTRACT

Scope. To evaluate whether increases in the consumption of cardioprotective food groups (virgin olive oil, nuts, fruits/vegetables, legumes, whole grains, fish, and wine) are associated with improvements in high-density lipoprotein (HDL) functions in high cardiovascular risk subjects.

Methods and Results. The association between 1-year changes in food group consumption and HDL functionality traits in 296 high cardiovascular risk subjects is assessed. Increases in virgin olive oil (10 g d–1) and whole grain consumption (25 g d–1) are associated with increments in cholesterol efflux capacity (+0.7%, P = 0.026, and +0.6%, P = 0.017, respectively). Increases in nut (30 g d–1) and legume intake (25 g d–1) are linked to increments in paraoxonase-1 activity (+12.2%, P = 0.049, and +11.7%, P = 0.043, respectively). Legume intake increases are also related to decreases in cholesteryl ester transfer protein activity (–4.8%, P = 0.028). Fish consumption increments (25 g d–1) are associated with increases in paraoxonase-1 activity (+3.9%, P = 0.030) and declines in cholesteryl ester transfer protein activity (–1.6%, P = 0.021), HDL cholesterol concentrations (–1.1%, P = 0.039), and functions related to HDL levels (cholesterol efflux capacity, –1.1%, P = 0.010).

Conclusion. Increases in the consumption of virgin olive oil, nuts, legumes, whole grains, and fish (achievable through a regular diet) were associated with improvements in HDL functions in high cardiovascular risk subjects.

1. INTRODUCTION

Few real-life dietary modifications have been shown to be able to improve the biological functions of high-density lipoproteins (HDLs) in humans. Only increases in the intake of polyphenol-rich virgin olive oil (25 mL d–1),1 a lycopene-rich diet,2 and a traditional Mediterranean diet3 have been reported to enhance HDL functions in clinical trials. Regarding the Mediterranean diet, our research group has demonstrated that adherence to this dietary pattern (associated with a high intake of virgin olive oil, nuts, fruit, vegetables, legumes, and whole grains, and a moderate consumption of fish and wine with meals)4 improved several HDL functions: it promoted cholesterol efflux capacity (their capacity to pick up cholesterol), HDL ability to esterify cholesterol (necessary for the effective transport of cholesterol in these lipoproteins), paraoxonase-1 activity (PON1, a key HDL-bound antioxidant enzyme), and HDL capacity to promote endothelial release of nitric oxide, and decreased the activity of the cholesteryl ester transfer protein (CETP, pro-atherogenic when excessively active).3 The food items individually responsible for such effects (within the context of a healthy dietary pattern such as this or others), however, remain to be elucidated. Our aim was to determine whether real-life increases in the intake of cardioprotective food groups (virgin olive oil, nuts, fruit and vegetables, legumes, whole grains, fish, and wine) for 1 year were linked to improvements in HDL biological functions in high cardiovascular risk subjects.

2. EXPERIMENTAL SECTION

Study population

The analyses were performed in a random sub-sample of 296 volunteers from the PREDIMED trial (PREvención con DIeta MEDiterránea)4, 5 in which HDL functions were previously assessed.3

The following information5 was collected: 1) clinical variables (age, sex, weight, height, blood pressure, and biochemical profile); 2) use of cardiovascular drugs; 3) consumption of 137 foods by a validated food frequency questionnaire; 4) adherence to a traditional Mediterranean diet by a validated 14-item score; 5) levels of physical activity with a validated Minnesota Leisure Time Physical Activity questionnaire; and 6) smoking habit. Body mass index was calculated as the ratio between weight (kg) and the height squared (m2), and three categories were established: normoweight (18.5–24.9 kg m–2), overweight (25.0–29.9 kg m–2), and obesity (≥30.0 kg m–2). Hypercholesterolemia was defined as the presence of total cholesterol ≥200 mg dL–1 or the use of statins; hypertriglyceridemia as the presence of triglycerides ≥150 mg dL–1 and/or the use of fibrates or pharmacological doses of omega-3 PUFAs; type-II diabetes mellitus as the presence of an altered glucose metabolism or the use of antidiabetic drugs; and hypertension as systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg, or the use of antihypertensive agents.5 Finally, the consumption of food groups was computed from the results of the food frequency questionnaire as follows: 1) “virgin olive oil” as the sum of all virgin and extra virgin olive oils consumed; 2) “nuts” as the sum of the intake of walnuts, almonds, pistachios, hazelnuts, and pine nuts; 3) “fruit and vegetables” as the sum of the consumption of green leafy vegetables, tomatoes and tomato soup (gazpacho), peppers, carrots, allium plants, cucurbits, cruciferous plants, green beans, asparagus, other vegetables, fruits of the Rosacea and Citrus families, berries, bananas, melons, watermelons, pineapples, kiwis, grapes, and other minor fruits; 4) “legumes” as the sum of consumed lentils, chickpeas, beans, and peas; 5) “whole grains” as the sum of the intake of whole grain bread and biscuits; 6) “fish” as the sum of the consumption of all lean and fatty fish (fresh or preserved naturally or in oil); and 7) “wine” as the sum of all red, rosé, white, sparkling, and sweet wine consumed.5 Average intake of all food items was expressed as g d–1 (excepting wine, expressed as mL d–1).

Study participants provided written informed consent before joining the trial. The study protocol was approved by local Research and Ethics Committees and registered with the International Standard Randomized Controlled Trial Number ISRCTN35739639. Details have been published elsewhere. 4, 5.

HDL functionality determinations

HDL particles were first isolated from volunteers' plasma samples by density gradient ultracentrifugation (isolated HDL fraction)1, 3 and polyethylene glycol-induced precipitation of apolipoprotein B-containing lipoproteins (apolipoprotein B-depleted plasma),3 and the samples were stored at –80 °C until use. The participants' HDL cholesterol levels were analyzed in an ABX-Pentra 400 autoanalyzer (Horiba ABX, Montpellier, France).3 The following were determined: 1) cholesterol efflux capacity in a model of human THP-1 monocyte-derived macrophages with 3H-cholesterol treated with 5% apolipoprotein B-depleted plasma samples as previously described;3 2) the ability of HDLs to esterify cholesterol as the ratio between the percentage of esterified cholesterol (in isolated HDL samples obtained by ultracentrifugation) and lecithin cholesterol acyltransferase concentration in serum samples;3 3) the activities of CETP and PON1 enzymes in plasma and serum samples, respectively, by commercial kits;1, 3 and 4) HDL capacity to promote endothelial release of nitric oxide in vitro in a human umbilical vein endothelial cell model treated with apolipoprotein B-depleted plasma samples.3

Sample size

Accepting a type-I error of 0.05, a type-II error of 0.2, and a 1% loss rate in a two-sided test, a sample size of 196 subjects provided sufficient statistical power to determine that Pearson's correlation coefficients ≥0.2 were significantly different from zero. Sample size was incremented by 50% (up to 294 volunteers) to allow adjustments for different covariates.

106 **Statistical analyses**

107 The 1-year differences in HDL functionality variables were computed as percentage
108 changes to simplify data interpretation ((1-year value – baseline value)/baseline value
109 × 100), the 1-year differences in dietary variables as linear differences (1-year value –
110 baseline value), and the distribution of continuous variables was assessed using
111 normality plots and histograms. The association between the changes in consumption
112 of food groups and changes in HDL functions by multivariate linear regression models
113 was determined, without any adjustment and adjusted for: age (continuous); gender;
114 study site; study intervention group (three categories4, 5); adherence to a traditional
115 Mediterranean diet (continuous); 1-year changes in the status of dyslipidemia
116 (hypercholesterolemia + hypertriglyceridemia), type-II diabetes, hypertension, and
117 tobacco use; body mass index category at baseline, energy intake at baseline
118 (continuous), and physical activity at baseline (tertiles). These analyses were repeated
119 after stratifying subjects in quartiles according to baseline HDL cholesterol levels, and
120 whether there were linear trends in the association coefficients when increasing along
121 quartiles by Pearson's tests was assessed. Any two-sided P-value <0.05 was accepted
122 as significant and the previous analyses in R Software, version 3.4.1, using the “lme4”
123 package were executed.6, 7
124

125 **3. RESULTS**

126

127 Characteristics of study participants are available in Table S1, Supporting Information.
128 Associations between 1-year changes in food intake and variations in HDL function
129 are depicted in Table 1. Regarding the fully adjusted model, increases in the daily
130 consumption of a serving of virgin olive oil (10 g, one spoonful) and whole grains (25
131 g) were independently associated with increments in cholesterol efflux capacity of
132 0.7% (p = 0.026) and 0.6% (p = 0.017), respectively. Increases in the consumption of
133 nuts in 30 g d–1 (a fistful) and legumes in 25 g d–1 (≈2 servings per week) was

independently linked to increments of 12.2% (p = 0.049) and 11.7% (p = 0.043) in PON1 antioxidant activity, respectively. The increase in legume intake was also linked to a 2.6% rise in HDL cholesterol levels (p = 0.036) and a 4.8% decrease in CETP activity (p = 0.028). Increments of fish consumption in 25 g d–1 (≈2 servings per week) were associated with a 3.9% promotion of PON1 antioxidant activity (p = 0.030) and a protective 1.6% decline in CETP activity (p = 0.021), together with a 1.1% decrease in HDL cholesterol concentrations (p = 0.039), and in those functions possibly related to HDL levels (such as cholesterol efflux capacity, –1.1%, p = 0.010). When studying fish subtypes, only augmentations in fresh fatty fish consumption (25 g d–1) were linked to a greater decrease in CETP activity (–2.3%, p = 0.043). Finally, when stratifying the previous analyses according to baseline HDL cholesterol concentrations, we observed that the associations of increasing nuts or fish consumption with increments in PON1 antioxidant activity were particularly present in those subjects with high HDL cholesterol levels (Table S2, Supporting Information). (**Supplemental Table 2**).
No significant differences in HDL properties were associated with changes in the consumption of fruit/vegetables and wine. Raw baseline data have already been described in a previous publication.3

4. DISCUSSION

Our results show that 1-year increases in the consumption of virgin olive oil, nuts, legumes, whole grains (and fish, in a more ambiguous way) were associated with improvements in HDL functions in high cardiovascular risk individuals. Such enhancements were unrelated to other lifestyle- and cardiovascular-related variables.

Specifically, we have confirmed the protective capacity of increasing the consumption of virgin olive oil on cholesterol efflux capacity. 1, 3, an essential measurement of HDL

function that is inversely related to the incidence of coronary events,8 and observed that incrementing the consumption of a serving of whole grains (25 g d–1, a slice of whole bread) induces a similar protective effect. Such foods are a key source of fiber, polyphenols, and other bioactive components that could contribute to explaining our results.9, 10 To the best of our knowledge, this is the first time that the effect of increasing whole grain consumption on HDL function has been reported in humans.

Our data also showed that legumes strongly modulated HDL functional traits in our data: they promoted HDL antioxidant function and moderated CETP function. The enhancement of PON1 activity after increasing the consumption of 25 g d–1 of legumes (≈2 servings per week; +11.7%) was similar to that achieved after incrementing the consumption of nuts to a portion per day (+12.2%). The richness in fiber and antioxidants of these food items may account for their cardiovascular benefits.10, 11 To date, no association between legume consumption and HDL functionality has been reported.

Finally, the effects of increasing fish consumption on HDL functions were more ambiguous in our work. We observed an association between a 25 g d–1 increase in fish intake with higher PON1 activity (+3.9%) and lower CETP function (–1.6%), in parallel to lower concentrations of HDL cholesterol (–1.1%) and some HDL functional properties that could be possibly related to HDL quantity (such as cholesterol efflux capacity, which decreased to the same extent as HDL cholesterol, –1.1%). In this regard, the relationship between omega-3 PUFAs in fish and HDL is still controversial. Some authors have reported that their consumption promotes the cholesterol content of large HDLs only, others indicate that they may increase the catabolic rate of apolipoprotein A-I, and according to a comprehensive review of the topic there is as yet no consensus on their effects.12 Nevertheless, we observed that the decrease in CETP activity was the only significant association with increases in the consumption of fresh fatty fish. In addition, increases in the intake of the other omega-3-rich food item (nuts)

baseline. We hypothesize that, whether HDL cholesterol concentrations of these subjects are higher, they may also present greater levels of the enzyme in circulation and be particularly sensitive to potential functional benefits. Finally, the improvements in PON1 and CETP activities that we have reported were of greater magnitude than the decline in HDL cholesterol levels, and concur with earlier evidence indicating that omega-3 PUFAs may promote PON1 function and decrease CETP activity.13, 14

This study has strengths and limitations. Regarding our strengths, we have reported associations between prospective data (changes in food consumption and the promotion of HDL functions), provided a quantitative measurement of beneficial effects (percentage changes in HDL functions), and used standardized protocols in a large sample size to comprehensively study key HDL functions. However, it also presents limitations. First, this was a prospective change analysis in a high cardiovascular risk population and our conclusions should be confirmed in randomized controlled trials and could only be extrapolated to high cardiovascular risk subjects. To increase the generalizability of our conclusions, our regression models have been fully adjusted for several co-variates that may affect HDL function (such as age, sex, cardiovascular risk factors, energy intake, and physical activity) and are independent from the effect of the diet as a whole (our results are also adjusted for the allocation of the volunteers to Mediterranean diets or a low-fat one and their adherence to a traditional Mediterranean diet). Second, as expected, the changes observed in this work were modest because they were associated with moderate real-life diet modifications. Third, several HDL functions were determined in cellular models that, while noninvasive, may not reflect possible counter-regulatory mechanisms affecting the final outcome. Finally, CETP and PON1 activities, and HDL capacity to promote endothelial release of nitric oxide could not be measured due to sample availability and technical issues in 67 and 50 volunteers, respectively.

In conclusion, we report that real-life increases in the 1-year consumption of virgin olive oil, nuts, legumes, whole grains, and fish may lead to relevant improvements in HDL functions in high cardiovascular risk subjects. This study describes for the first time an association between incrementing the consumption of legumes and whole grains and enhancements in HDL function. It also confirms the beneficial effects of virgin olive oil, nuts, and fish on these properties, and reinforces the idea that a healthy diet may promote HDL functionality. Further randomized controlled trials are warranted to investigate whether these dietary modifications may contribute to promoting HDL function in humans.

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lipoproteins, lecithin:cholesterol acyltransferase, and lipid transfer protein activity in humans. *Arteriosclerosis* **1990**, *10*, 85–94.

AUTHOR CONTRIBUTIONS

A.H. and M.Fitó designed the study. A.H. acquired the data. M.A.M.-G., E.R., X.P., R.E., J.S.-S., D.C., A.M.A.G., L.S.-M., M.Fiol, J.L., R.M.L.-R., and M.Fitó contributed with biological samples and/or participated in the design and development of the clinical trial. A.H. and A.S. wrote the manuscript which was critically reviewed by O.C., M.A.M.-G., E.R., X.P., R.E., J.S.-S., D.C., A.M.A.G., L.S.-M., M.Fiol, J.L., R.T, R.M.L.-R., and M.Fitó. All authors approved the final version of the manuscript.

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CONFLICT OF INTEREST

Emilio Ros reports receiving grants/research support through his Institution from the California Walnut Commission, being a nonpaid member of its Scientific Advisory Committee, and receiving lecture fees from Nuts for Life and Danone. Ramón Estruch reports serving on the board of and receiving lecture fees from the Research Foundation on Wine and Nutrition (FIVIN), serving on the boards of the Beer and Health Foundation and the European Foundation for Alcohol Research (ERAB), and receiving lecture fees from Cerveceros de España. Jordi Salas-Salvadó reports receiving grants/research support through his Institution from the International Nut and Dried Fruit Foundation (in whose Scientific Committee he is a nonpaid member) and the American Pistachio Growers, receiving honoraria from Nuts for Life, Danone and

313 Eroski, and being a member of the executive committee of the Instituto Danone Spain.
314 Rosa-María Lamuela-Raventós reports serving on the board of and receiving lecture
315 fees from FIVIN, receiving lecture fees from Cerveceros de España, and receiving
316 lecture fees and travel support from PepsiCo. No other potential conflict of interest
317 relevant to this article has been reported.

TABLES

Table 1. Association between increases in the consumption of different food items and changes in HDL-related traits[a] (in %).

Variables	↑ 10 g/day of virgin olive oil		↑ 30 g/day of nuts		↑ 25 g/day of legumes		↑ 25 g/day of whole grains		↑ 25 g/day of fish	
	Raw model	*Adjusted model*	*Raw model*	*Adjusted model*	*Raw model*	*Adjusted model*	*Raw model*	*Adjusted model*	*Raw model*	*Adjusted model*
Change in HDL cholesterol concentrations (%)	-0.057 [-0.70; 0.59]	0.005 [-0.76; 0.77]	1.43 [-1.03; 3.90]	1.66 [-1.31; 4.62]	3.13* [0.70; 5.58]	2.60* [0.18; 5.03]	0.25 [-0.40; 0.90]	0.26 [-0.39; 0.91]	-1.17* [-2.20; -0.15]	-1.14* [-2.21; -0.065]
Change in cholesterol efflux capacity (%)	0.54* [0.036; 1.03]	0.68* [0.084; 1.27]	2.03 [-0.043; 4.11]	1.36 [-1.32; 4.05]	0.59 [-1.50; 2.65]	0.82 [-1.31; 2.95]	0.53* [0.018; 1.05]	0.64* [0.12; 1.16]	-0.93* [-1.73; -0.12]	-1.11* [-1.96; -0.27]
Change in HDL capacity to esterify cholesterol (%)	0.33 [-1.01; 1.67]	-0.068 [-1.70; 1.57]	-3.90 [-9.84; 2.04]	-2.03 [-9.93; 5.85]	-0.13 [-7.00; 6.75]	0.78 [-6.55; 8.13]	-0.49 [-1.72; 0.74]	-0.35 [-1.63; 0.93]	-0.46 [-2.65; 1.73]	-0.36 [-2.75; 2.04]
Change in cholesteryl ester transfer protein activity (%)	0.003 [-0.76; 0.76]	0.54 [-0.40; 1.48]	0.63 [-2.75; 4.02]	0.37 [-4.29; 5.01]	-3.35 [-7.25; 0.53]	-4.80* [-9.03; -0.57]	0.26 [-0.45; 0.97]	0.24 [-0.52; 0.99]	-1.41* [-2.63; -0.18]	-1.63* [-3.00; -0.27]
Change in paraoxonase-1 antioxidant activity (%)	2.56* [0.62; 4.51]	2.09 [-0.33; 4.51]	3.48 [-5.37; 12.4]	12.2* [0.13; 24.2]	14.6* [4.25; 24.9]	11.7* [0.44; 22.8]	0.17 [-1.67; 2.01]	-0.13 [-2.08; 1.82]	3.18* [-0.003; 6.33]	3.93* [0.40; 7.45]
Change in HDL capacity to promote endothelial release of nitric oxide (%)	0.26 [-0.99; 1.51]	-0.28 [-1.79; 1.23]	2.07 [-2.69; 6.81]	-1.79 [-7.80; 4.20]	1.37 [-3.53; 6.25]	2.02 [-2.93; 6.95]	0.064 [-1.27; 1.40]	-0.28 [-1.65; 1.08]	1.29 [-0.70; 3.28]	1.88 [-0.19; 3.95]

a) Adjusted models have been adjusted for: age; sex; study site; PREDIMED intervention group; changes in the status of type-II diabetes, dyslipidemia (hypercholesterolemia and hypertriglyceridemia), hypertension, and tobacco use; and baseline values of body mass index category, adherence to a Mediterranean diet, and physical activity (tertiles). *: $P<0.05$.