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ARTICLE

Lipids and cardiovascular/metabolic health

Effects of a diet naturally rich in polyphenols on lipid composition of postprandial lipoproteins in high cardiometabolic risk individuals: an ancillary analysis of a randomized controlled trial

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Abstract

Background/Objectives Plasma lipoprotein composition, especially in the postprandial state, could be relevant for cardiovascular risk and could be influenced by eating habits. This study evaluated the effects of a polyphenol-rich diet on postprandial lipoprotein composition in individuals at high cardiometabolic risk.

Subjects/Methods Seventy-eight individuals with high waist circumference and at least another component of the metabolic syndrome were randomized to either a high-polyphenol (HighP) or low-polyphenol (LowP) diet. Before and after the 8-week intervention, chylomicrons, VLDL1, VLDL2, IDL, LDL, HDL particles, and their lipid concentrations were determined over a 6-h high-fat test meal with high or low-polyphenol content, according to the diet assigned.

Results VLDL1 postprandial areas under the curve (AUCs) were lower for cholesterol (Chol) (1.48 ± 0.98 vs. 1.91 ± 1.13 mmol/L × 6 h, M ± SD, $p = 0.014$) and triglycerides (Tg) (4.70 ± 2.70 vs. 6.02 ± 3.07 mmol/L × 6 h, $p = 0.005$) after the HighP than after the LowP diet, with no changes in Chol/Tg ratio. IDL Chol AUCs were higher after the HighP than after the LowP diet (1.29 ± 0.77 vs. 1.01 ± 0.51 mmol/L × 6 h, $p = 0.037$). LDL Tg AUCs were higher after the HighP than after the LowP diet (1.15 ± 0.33 vs. 1.02 ± 0.35 mmol/L × 6 h, $p < 0.001$), with a lower Chol/Tg ratio (14.6 ± 4.0 vs. 16.0 ± 3.8, $p = 0.007$). HDL Tg AUCs were lower after the HighP than after the LowP diet (1.20 ± 0.41 vs. 1.34 ± 0.37 mmol/L × 6 h, $p = 0.013$).

Conclusions A high-polyphenol diet reduces the postprandial lipid content of large VLDL and increases IDL cholesterol; it modifies the composition of LDL particles—which become richer in triglycerides, and of HDL—which become instead triglyceride poor. The overall changes in atherogenicity by these effects warrant further investigation on clinical cardiovascular outcomes.

Introduction

The postprandial state is a transient and dynamic condition characterized by different metabolic changes [1]. Among these, the modifications involving lipoprotein concentration and composition are of great relevance [2, 3]. These modifications, mainly after a fatty meal, are characterized by an increment in triglyceride-rich lipoprotein concentrations—chylomicrons, very low-density lipoproteins (VLDL)—and a slight decrease in low-density lipoproteins (LDL) and high-density lipoproteins (HDL) [4–6].

Mainly through the modulation of abdominal adiposity and insulin resistance [6], dietary habits—in particular, the increasingly more widespread high-fat/high-calorie western diets, characterized by short fasting intervals—are the major contributors to the quantitative/qualitative

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Trial registered at ClinicalTrials.gov as NCT01154478.

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alterations of postprandial lipoprotein composition [7]. Consequently, dietary modifications may represent a useful approach for modulating lipoprotein metabolism in the postprandial state.

Among the dietary components, polyphenols have gained growing interest over the last few years. Polyphenols represent a great variety of plant metabolites, and are powerful antioxidants; in addition, they have several other properties involved in immunomodulatory, anti-mutagenic, and anti-inflammatory activities [8]. There is epidemiological evidence of an inverse correlation between high-polyphenol consumption and incidence of many chronic metabolic diseases [9]. Clinical trials investigating the effects of polyphenol intake on fasting lipid profile have reported conflicting results with respect to total cholesterol, triglycerides, or lipoprotein composition. This discrepancy could be related to trial duration, sample size, amount and, importantly, specific types of polyphenol supplementation [10–12]. Only few clinical trials have evaluated the effects of polyphenol intake on postprandial lipoproteins [13]. These studies generally focused only on the cholesterol content of LDL and HDL, while triglyceride content was not evaluated [13]. In a randomized controlled trial, we observed that diets rich in polyphenols of variable origin induced a significant reduction in plasma triglyceride and cholesterol concentrations in the VLDL1 fraction, in both the fasting and postprandial state [14].

The purpose of this study—an ancillary analysis of the above trial [14]—was to investigate in individuals with abdominal adiposity, and therefore at high risk of type 2 diabetes and cardiovascular disease, the medium-term effects of a diet with a naturally high-polyphenol content of different origin on postprandial cholesterol and triglyceride composition of chylomicrons, large VLDL (VLDL1), small VLDL (VLDL2), IDL, LDL, and HDL.

Materials/subjects and methods

Subjects

Eighty-six men and women aged between 35 and 70 years, with overweight or obesity (BMI 27–35 kg/m^2), high waist circumference (above 102 cm for men or 88 cm for women), and at least one more feature of the metabolic syndrome based on the National Cholesterol Education Program/Adult Treatment Program [15], were recruited at the obesity outpatient clinic of the Federico II University Hospital. Subjects were not included if they presented diabetes, cardiovascular events in the previous 6 months, liver or renal disease, fasting plasma triglycerides ≥4.5 mmol/l, fasting cholesterol ≥7.0 mmol/l, any other chronic disease, or who were on medications influencing inflammatory state, and lipid or glucose metabolism. Inclusion and exclusion criteria were assessed by interviews, clinical examinations, and routine laboratory tests. Diabetes status was evaluated by a 75-g oral glucose tolerance test (OGTT). The study protocol—performed in accordance with the Declaration of Helsinki for clinical trials—was approved by the Ethics Committee of the Naples Federico II University, and all participants provided written informed consent before entering the study. The study is registered at ClinicalTrials.gov as NCT01154478.

Study design

After a 3-week run-in period to stabilize their habitual diet, participants were randomly allocated to an 8-week experimental diet according to a 2 × 2 factorial design, as previously described in detail [14, 16]. The experimental diets were isoenergetic and similar for macronutrient and micronutrient composition, but differed for their content in polyphenols and long-chain *n*-3 polyunsaturated fatty acids (LCn3). Each participant was randomly assigned to follow one of the following diets: (1) low in LCn3 (1.5 g/day) and polyphenols (365 mg/day); (2) rich in LCn3 (4 g/day) and low in polyphenols (363 mg/day); (3) low in LCn3 (1.4 g/day) and rich in polyphenols (2.903 mg/day); (4) rich in LCn3 (4 g/day) and polyphenols (2.861 mg/day). The high-polyphenol diet was ensured by supplying participants with decaffeinated green tea and coffee, vegetables (i.e., onions, fennels, rocket), fruits (i.e., oranges), dark chocolates, and extra-virgin olive oil. Salmon, dentex, and anchovies represented the main dietary sources of LCn3. Participants were asked to complete a 7-day food record at baseline, and at 4 and 8 weeks to improve dietary compliance, which was reinforced through dietary counseling at the clinic every week and through phone calls every 2–3 days. For the same purpose, every week participants were given meals and beverages for the whole duration of intervention, in amounts sufficient to cover overall household needs. Participants allocated to the diets rich in polyphenols or LCn3 were considered compliant with the treatment if the intake of polyphenols or LCn3, respectively, was ≥80% of that assigned; participants allocated to the diets low in polyphenols or LCn3 were considered compliant with the treatment, if the corresponding intake was not >20% of that assigned. Meals were prepared in a qualified catering service under the supervision of the team of dietitians.

Energy and nutrient composition of the diets were calculated according to the food composition tables of the Italian Institute of Nutrition, with the aid of the MetaDieta software (Meteda s.r.l., Ascoli-Piceno, Italy). The USDA [17] and Phenol-Explorer databases [18] were used to assess dietary polyphenol content of the foods consumed.

Since the specific aim of this ancillary analysis was to evaluate the medium-term effects of dietary polyphenols on postprandial cholesterol and triglyceride composition of chylomicrons, VLDL1, VLDL2, IDL, LDL, and HDL, the four dietary intervention groups in the trial were pooled into two diet groups according to their polyphenol content (HighP or LowP)—i.e., High-Polyphenol&Low-LCn3 and High-Polyphenol&High-LCn3 were combined into the HighP group, while Low-Polyphenol&Low-LCn3 and Low-Polyphenol&High-LCn3 were combined into the LowP group (Supplementary Fig. 1).

Experimental procedures

At baseline and after the 8-week intervention, anthropometric parameters (i.e., body weight, height, and waist circumference) were measured according to standardized procedures. At baseline and at the end of the intervention, after a 12-h overnight fast, the participants consumed a high-fat test meal consisting of rice, butter, cured raw beef, parmesan cheese, white bread, plus olive oil (LowP group) or extra-virgin olive oil, and decaffeinated green tea (HighP group), to achieve a low or high-polyphenol content according to the assigned diets. Blood samples were collected at fasting and 2, 4, and 6 h after the meal to measure cholesterol and triglyceride content in chylomicrons, VLDL1, VLDL2, IDL, LDL, and HDL. Apolipoprotein B-48 (Apo B-48) was measured in plasma, and VLDL1 at fasting and 4 and 6 h after the test meal in VLDL1.

Laboratory methods

Chylomicrons (Sf >400), VLDL1 (Sf 60–400), VLDL2 (Sf 20–60), IDL (Sf 12–20), and LDL (Sf 0–12) were isolated from plasma by discontinuous density-gradient ultracentrifugation, as described previously [19]. HDL were isolated from plasma by the phosphotungstic acid/magnesium chloride precipitation method. Cholesterol and triglyceride concentrations were assayed by enzymatic methods (Roche Molecular Biochemicals, Mannheim, Germany) on a Cobas Mira autoanalyser (ABX Diagnostics, Montpellier, France). Apo B-48 concentrations were analyzed in plasma and VLDL1 by ELISA (Shibayagi Co Ltd, Shibukawa, Gunma, Japan), automated on a Triturus ELISA autoanalyzer (Grifols Italia S.p.A). All analyses were performed by technicians blinded to the group assignment.

Statistical analysis

The sample size was calculated on the primary outcome, i.e., postprandial lipid response, of the original trial [14]. To detect a 30% difference between treatments in the total triglyceride areas under the curve (AUCs) in the chylomicron and VLDL fractions after a fat-rich meal, with an 80% power at a 5% significance level, 80 patients had to be studied. In view of possible dropouts, 86 participants were enrolled. Based on the reduction in 6 h-AUCs of cholesterol/triglyceride ratio in LDL (−30%), VLDL1 (−10%), and IDL (−14%) observed in a previous pharmacological study [20], a sample size of 28 participants was needed to detect differences in these variables in the present analysis.

The random allocation to the intervention, stratified by sex, age, BMI, and plasma triglycerides, was performed by a minimization method using the MINIM software (www.users.york.ac.uk). The data are expressed as mean ± SD unless otherwise specified. Variables not normally distributed were analyzed following logarithmic transformation. The ratio between cholesterol and triglycerides was evaluated as a measure of lipoprotein composition. Postprandial total AUCs were calculated by the trapezoidal method. The effects of dietary polyphenols, dietary LCn3, and the interaction between polyphenols and LCn3 were evaluated by two-way ANOVA repeated measures analysis. Since there was no interaction between the effects of dietary polyphenols and dietary LCn3, the results are presented pooling the intervention groups according to the polyphenol content of the diet: HighP diet (High-Polyphenol&Low-LCn3 plus High-Polyphenol&High-LCn3) or LowP diet (Low-Polyphenol&Low-LCn3 and Low-Polyphenol&High-LCn3) (Supplementary Fig. 1). The absolute values of AUCs and postprandial profiles at end of the study (8-week) were compared between the HighP and LowP diets adjusting for the values at the start of the dietary intervention (0-week) as covariate.

Univariate associations were assessed by Pearson's correlation. All statistical tests were two sided. Statistical significance was accepted at a p level < 0.05. SPSS Statistics version 21.0 (SPSS/PC; Chicago, IL, USA) was used to perform statistical analysis according to standard methods.

Results

Baseline data and dietary compliance

Seventy-eight of the 86 participants enrolled completed the study. Eight subjects, equally distributed between the two experimental groups, withdraw from the study either because they were unwilling to undergo further tests or for work/family-related reasons. At baseline, the LowP and HighP groups were comparable for age, body weight, body mass index, waist circumference, fasting levels of plasma cholesterol, triglycerides, Apo B-48, glucose, insulin, and HOMA-IR (Table 1). At baseline, lipoprotein

Table 1 Baseline characteristics of the two groups of participants in the dietary intervention study

	High-polyphenol group, $n = 39$	Low-polyphenol group, $n = 39$	P^a
Sex (M/F)	17/22	16/23	
Age (y)	54 ± 9	55 ± 8	0.481
Body weight (kg)	86.5 ± 11	85.5 ± 11	0.758
Body mass index (kg/m^2)	31 ± 3	32 ± 3	0.136
Waist circumference (cm)	103 ± 9	104 ± 8	0.482
Systolic blood pressure (mm Hg)	122 ± 14	121 ± 9	0.491
Diastolic blood pressure (mm Hg)	74 ± 9	75 ± 8	0.737
Fasting plasma triglycerides (mmol/L)	1.38 ± 0.6	1.46 ± 0.7	0.649
Fasting plasma cholesterol (mmol/L)	4.9 ± 0.7	4.9 ± 0.8	0.922
HDL cholesterol (mmol/L)	1.1 ± 0.2	1.0 ± 0.2	0.528
LDL cholesterol (mmol/L)	2.9 ± 0.7	3.0 ± 0.6	0.807
Apo B-48 (μg/ml)	7.6 ± 7.7	6.5 ± 4.3	0.417
Fasting plasma glucose (mmol/L)	5.6 ± 0.5	5.7 ± 0.6	0.344
Fasting plasma insulin (pmol/L)	132 ± 41	125 ± 41	0.664
HOMA-IR	4.7 ± 1.8	4.6 ± 1.8	0.883

HOMA-IR homeostasis model assessment of insulin resistance, *Apo B-48* apolipoprotein B-48

All values are means ± SDs

[a]*t* test, no significant differences between the two groups

concentrations and compositions were not significantly different between the two groups at fasting (Supplementary Table 1) and after the test meals (Supplementary Table 2). Participants' dietary compliance was adequate, as demonstrated by their 7-day food records completed during the study [14], as well as by the assessment of phenolic metabolites in their 24 h-urine collection, which were significantly higher in the participants assigned to the HighP diet [21].

Whole-plasma lipids and glucose metabolism

As previously reported [14], compared with the LowP diet, the HighP diet decreased fasting (−0.19 ± 0.4 vs. 0.03 ± 0.62 mmol/L, $p = 0.023$) and postprandial whole-plasma triglycerides (−1.6 ± 3.36 vs. 0.13 ± 0.06 mmol/L, $p = 0.041$), while no significant changes were observed in the fasting state (−0.14 ± 0.59 vs. −0.13 ± 0.57 mmol/L, $p = 0.041$), and postprandial whole-plasma cholesterol (−0.76 ± 3.18 vs. −1.01 ± 2.66 mmol/L, $p = 0.699$). No significant differences in fasting whole-plasma concentrations of Apo B-48 were observed between the HighP and LowP diets (−0.10 ± 5 and −0.63 ± 4.8 μg/ml, respectively, $p = 0.178$). As previously reported [16], glucose tolerance and early insulin secretion during an oral glucose tolerance test were improved by polyphenols, while no significant differences between the HighP and LowP diets were detected in plasma glucose and insulin responses to the high-fat meal.

Lipoprotein composition

Chylomicrons. After the dietary intervention, no differences were observed in chylomicron cholesterol and triglyceride concentrations, and cholesterol/triglyceride (Chol/Tg) ratio, between the HighP and LowP diets either at fasting and after the test meal (Fig. 1, Table 2).

VLDL1. Postprandial VLDL1 cholesterol concentrations were lower after the HighP than after the LowP diet ($p = 0.022$, time × polyphenol interaction, Fig. 1; AUCs difference, $p = 0.014$, Table 2), as were VLDL1 triglyceride concentrations ($p = 0.007$, time × polyphenol interaction, Fig. 1; AUCs difference, $p = 0.005$, Table 2). Chol/Tg ratio was not significantly different between the HighP and LowP diets (Fig. 1, Table 2). Similarly, no significant differences in apo B-48 AUCs in VLDL1 were observed between the HighP and LowP diets (12.9 ± 9.9 vs. 16.2 ± 12.0 μg/ml·6 h, $p = 0.178$).

VLDL2. No differences were observed in VLDL2 cholesterol and triglyceride concentrations, and Chol/Tg ratio, after the dietary intervention between the HighP and LowP diets (Fig. 1, Table 2).

IDL. Postprandial IDL cholesterol concentrations were higher after the HighP than after the LowP diet ($p = 0.037$, time × polyphenol interaction, Fig. 2; AUCs difference, $p = 0.037$, Table 2). No differences were observed in IDL triglyceride concentrations and Chol/Tg ratio (Fig. 2, Table 2).

LDL. No differences were observed in postprandial LDL cholesterol concentrations after the dietary intervention

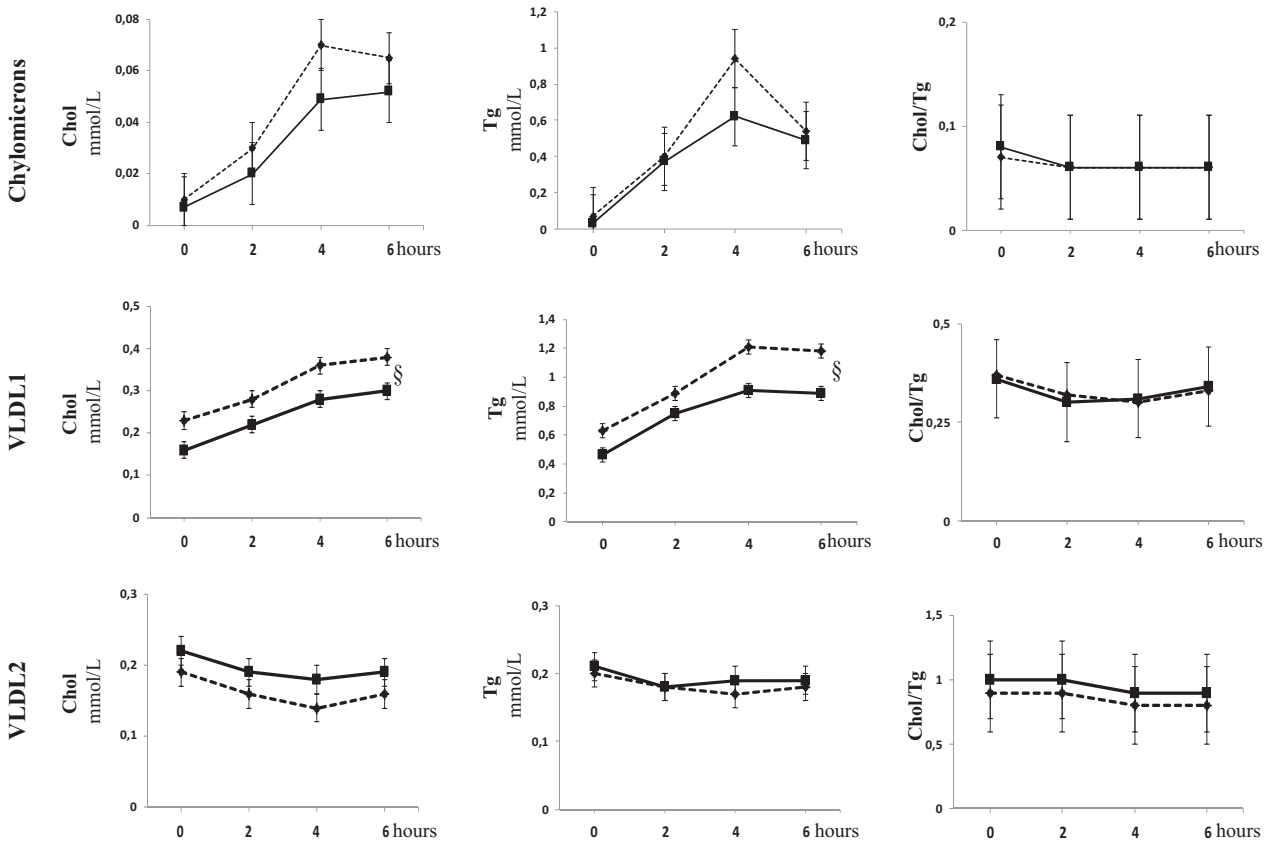

Fig. 1 Cholesterol (Chol) and triglycerides (Tg) concentrations, and Chol/Tg ratio in Chylomicrons, VLDL1, and VLDL2 fractions over a 6-h test meal after the 8-week intervention in the high- (—■—) and low- (--◆--) polyphenol groups. $^{§}p < 0.05$ GLM repeated measures

between the HighP and LowP diets. Instead, LDL triglyceride concentrations were higher after the HighP than after the LowP diet ($p < 0.001$, time × polyphenol interaction, Fig. 2; AUCs difference, $p < 0.001$, Table 2). Therefore, Chol/Tg ratio was lower after the HighP than after the LowP diet ($p = 0.005$, time × polyphenol interaction, Fig. 2; AUCs difference, $p = 0.007$, Table 2).

HDL. No significant difference was observed in HDL cholesterol concentrations after the dietary intervention between the HighP and LowP diets (Fig. 2, Table 2), while HDL triglyceride concentrations significantly decreased after the HighP than after the LowP diet ($p = 0.012$ for time × polyphenol interaction, Fig. 2; AUCs difference, $p = 0.013$, Table 2). Postprandial Chol/Tg ratio was higher after the HighP than after the LowP diet ($p = 0.038$ time × polyphenol interaction, Fig. 2). The difference was not statistically significant when expressed as total Chol/Tg ratio AUC ($p = 0.189$, Table 2).

All these differences were still significant after adjusting for the absolute changes of total plasma triglycerides or cholesterol reported in the original trial (VLDL1 cholesterol AUCs difference, $p = 0.010$; VLDL1 triglyceride AUCs difference, $p = 0.015$; IDL cholesterol AUCs difference, $p = 0.044$; LDL triglyceride AUCs difference, $p < 0.001$; LDL cholesterol/triglyceride ratio AUCs difference, $p < 0.001$; HDL triglyceride AUCs difference, $p = 0.030$).

Correlation analyses

To further elucidate the plausible mechanisms involved in postprandial lipoprotein composition changes, correlation analysis (Pearson) between lipoprotein postprandial AUC changes after the dietary interventions was performed, and the more relevant correlations are reported in Fig. 3.

Changes in Chylomicrons correlated with those occurring in VLDL1 ($r = 0.623$, $p < 0.001$ for cholesterol; $r = 0.601$, $p < 0.001$ for triglycerides). Changes in VLDL2 directly correlated with those occurring in IDL ($r = 0.545$, $p < 0.001$ for cholesterol, Fig. 3a; $r = 0.424$, $p < 0.001$ for triglycerides, Fig. 3b; $r = 0.556$, $p < 0.001$ for Chol/Tg ratio, Fig. 3c). Changes in IDL directly correlated with those occurring in LDL ($r = 0.275$, $p = 0.016$ for cholesterol; $r = 0.511$, $p < 0.001$ for triglycerides), while changes in IDL Chol/Tg ratio inversely correlated with those occurring in LDL cholesterol ($r = -0.204$, $p = 0.007$, Fig. 3d). Changes in HDL triglycerides directly correlated with triglyceride

Table 2 Postprandial lipoprotein AUCs of cholesterol, triglycerides, and cholesterol/triglyceride ratio after the dietary intervention (8 weeks)

	High-polyphenol group, $n = 39$	Low-polyphenol group, $n = 39$	P^a
Chylomicrons			
Cholesterol (mmol/L × 6 h)	0.21 ± 0.17	0.29 ± 0.20	0.224
Triglycerides (mmol/L × 6 h)	2.55 ± 2.14	3.27 ± 2.20	0.301
Cholesterol/triglyceride ratio	0.08 ± 0.02	0.08 ± 0.05	0.219
VLDL1			
Cholesterol (mmol/L × 6 h)	1.48 ± 0.98	1.91 ± 1.13	**0.014**
Triglycerides (mmol/L × 6 h)	4.70 ± 2.70	6.02 ± 3.07	**0.005**
Cholesterol/triglyceride ratio	0.31 ± 0.05	0.32 ± 0.04	0.992
VLDL2			
Cholesterol (mmol/L × 6 h)	1.16 ± 0.80	0.97 ± 0.51	0.841
Triglycerides (mmol/L × 6 h)	1.17 ± 0.59	1.14 ± 0.48	0.511
Cholesterol/triglyceride ratio	0.99 ± 0.21	0.85 ± 0.14	0.114
IDL			
Cholesterol (mmol/L × 6 h)	1.29 ± 0.77	1.01 ± 0.51	**0.037**
Triglycerides (mmol/L × 6 h)	0.35 ± 0.18	0.30 ± 0.12	0.088
Cholesterol/triglyceride ratio	3.68 ± 1.22	3.36 ± 0.81	0.386
LDL			
Cholesterol (mmol/L × 6 h)	16.80 ± 4.19	16.36 ± 3.28	0.131
Triglycerides (mmol/L × 6 h)	1.15 ± 0.33	1.02 ± 0.35	**<0.001**
Cholesterol/triglyceride ratio	14.60 ± 4.01	16.03 ± 3.81	**0.007**
HDL			
Cholesterol (mmol/L × 6 h)	5.74 ± 1.42	5.87 ± 1.52	0.074
Triglycerides (mmol/L × 6 h)	1.20 ± 0.41	1.34 ± 0.37	**0.013**
Cholesterol/triglyceride ratio	4.78 ± 1.50	4.37 ± 1.52	0.189

All values are means ± SDs

[a] *t* test of absolute value at 8 weeks adjusted for baseline value as covariate

Significant differences ($p < 0.05$) are indicated in bold

change in Chylomicrons ($r = 0.302$, $p = 0.007$), VLDL1 ($r = 0.600$, $p < 0.001$, Fig. 3e), and VLDL2 ($r = 0.425$, $p < 0.001$). Moreover, changes in HDL Chol/Tg ratio inversely correlated with Chylomicron cholesterol ($r = -0.259$, $p = 0.009$), Chylomicron triglycerides ($r = -0.343$, $p = 0.002$), VLDL1 cholesterol ($r = -0.566$, $p < 0.001$), and VLDL1 Chol/Tg ratio ($r = -0.453$, $p < 0.001$, Fig. 3f).

Discussion

Our study shows that a high-polyphenol diet, compared with one low in polyphenols, significantly modifies postprandial lipoproteins by (a) reducing cholesterol and triglycerides in VLDL1, (b) increasing cholesterol in IDL, (c) increasing triglycerides in LDL thus reducing the cholesterol/triglyceride ratio, and (d) reducing triglycerides in HDL thus increasing the cholesterol/triglyceride ratio profile.

Interestingly, these significant changes still remain significant after further adjusting for the absolute changes of plasma lipid (cholesterol or triglycerides) reported in the original trial [14], suggesting a qualitative rearrangement of lipid concentration in lipoprotein behind the quantitative reduction of total circulating lipid.

To the best of our knowledge, this ancillary analysis investigated for the first time in a randomized controlled trial the effects of dietary polyphenols on postprandial lipoprotein lipid composition—a not predefined outcome—in individuals at high cardiometabolic risk.

The first finding of our study is that a diet with a naturally high-polyphenol content equally reduced cholesterol and triglycerides in VLDL1. Different mechanisms may underlie this effect. Polyphenols could reduce lipid availability at the intestinal level by inhibiting pancreatic lipase and reducing the synthesis of triglyceride-rich lipoproteins, also influenced by a lower availability of cholesterol and short fatty acids in the liver [10, 22]. The absence of a reduction in fasting total Apo B-48 as well as in Apo B-48 AUCs with the HighP diet supports the hypothesis that the number of endogenous lipoproteins of intestinal origin was not reduced. Moreover, in addition to decreasing lipid

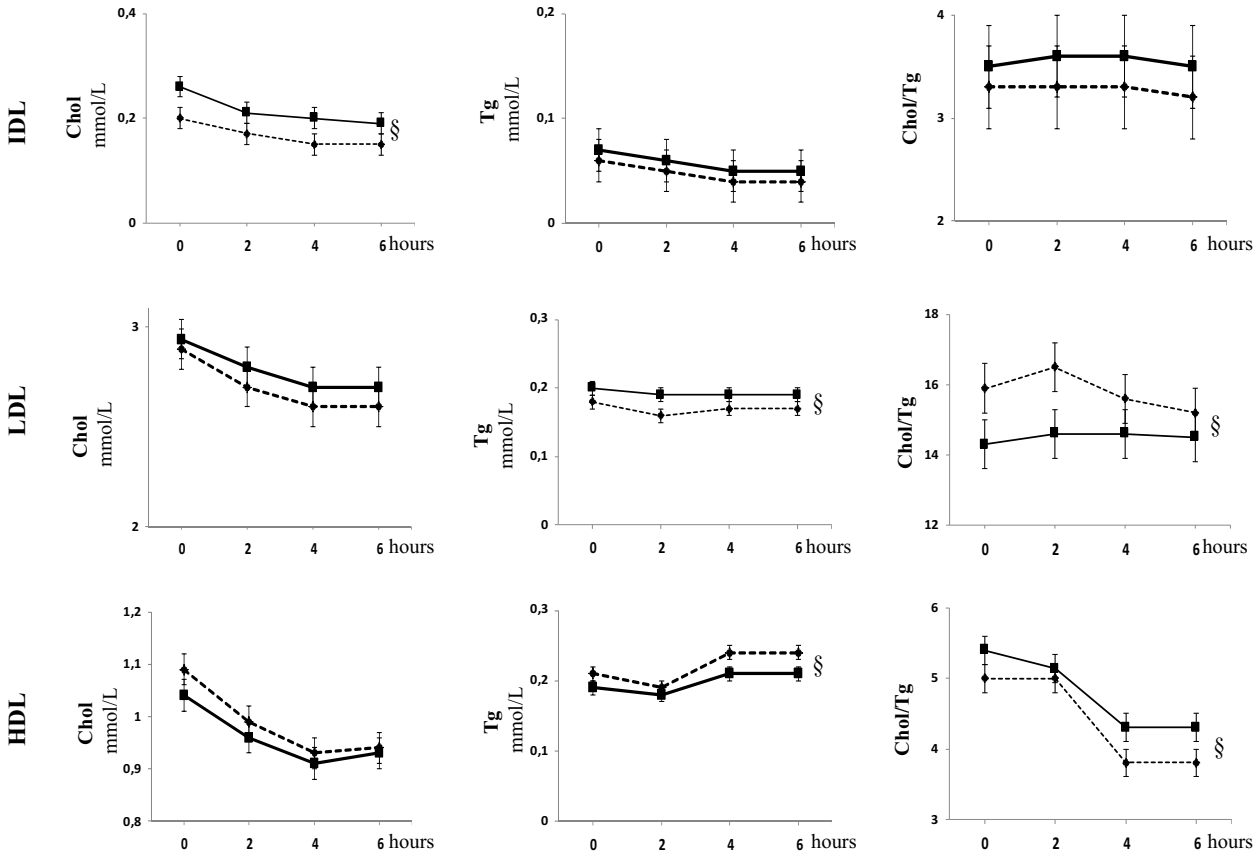

Fig. 2 Cholesterol (Chol) and triglycerides (Tg) concentrations, and Chol/Tg ratio in IDL, LDL, and HDL fractions over a 6-h test meal after the 8-week intervention in the high- (—■—) and low- (- -◆- -) polyphenol groups. $^{§}p < 0.05$ GLM repeated measures

availability in hepatocytes, polyphenols may decrease acyl-CoA cholesterol acyltransferase, inhibit microsomal triglyceride transfer protein, and increase fatty acid oxidation [23, 24]. This could explain the reduction in VLDL1 lipid content [25, 26]. The strong positive correlation between postprandial changes in chylomicron and VLDL1 lipids after a dietary intervention suggests common pathways in intestinal and hepatic metabolism.

The decreased availability of lipids in the liver could lead to a preferential secretion of VLDL2 [27]. The delipidation of these lipoproteins would lead to the IDL richer in cholesterol observed with the HighP diet. This is supported by the strong direct correlation between postprandial AUC changes in VLDL2 and IDL lipids after the dietary intervention.

Another finding of our study is the increase in LDL triglyceride concentration with a reduction in cholesterol/triglyceride ratio. This effect could be related to the cholesterol enrichment of IDL. In fact, it has been observed that the IDL richer in triglycerides—deriving from VLDL1—seem to give origin to intermediate LDL particles, while IDL richer in cholesterol—deriving from VLDL2—are the precursor of large LDL particles, rich in triglycerides [27–29]. This is supported by the positive correlation between postprandial AUC changes in IDL and LDL lipids, and the inverse correlation between changes in Chol/Tg ratio in IDL and LDL after the dietary intervention.

Polyphenols could induce the changes in LDL and HDL triglyceride and LDL cholesterol/triglyceride ratio by inhibiting the cholesteryl ester transfer protein (CETP) [30]. It has been reported that the increased activity of CETP in the postprandial state induces an HDL enrichment in triglycerides [31]. Similarly, LDL cholesterol lowering in the postprandial state could be related to an enhanced formation of small dense LDL particles poor in triglycerides, mediated by CETP [4, 32]. The possible inhibition of CETP activity by polyphenols could induce a less active triglyceride/cholesterol exchange between HDL particles and both triglyceride-rich lipoproteins and LDL. This mechanism is supported by the inverse relation found in our study between changes in Chol/Tg ratio in HDL and VLDL1.

Despite a plausible CETP inhibition, we failed to observe a significant increase in the cholesterol content of HDL. This might suggest an improvement in reverse cholesterol

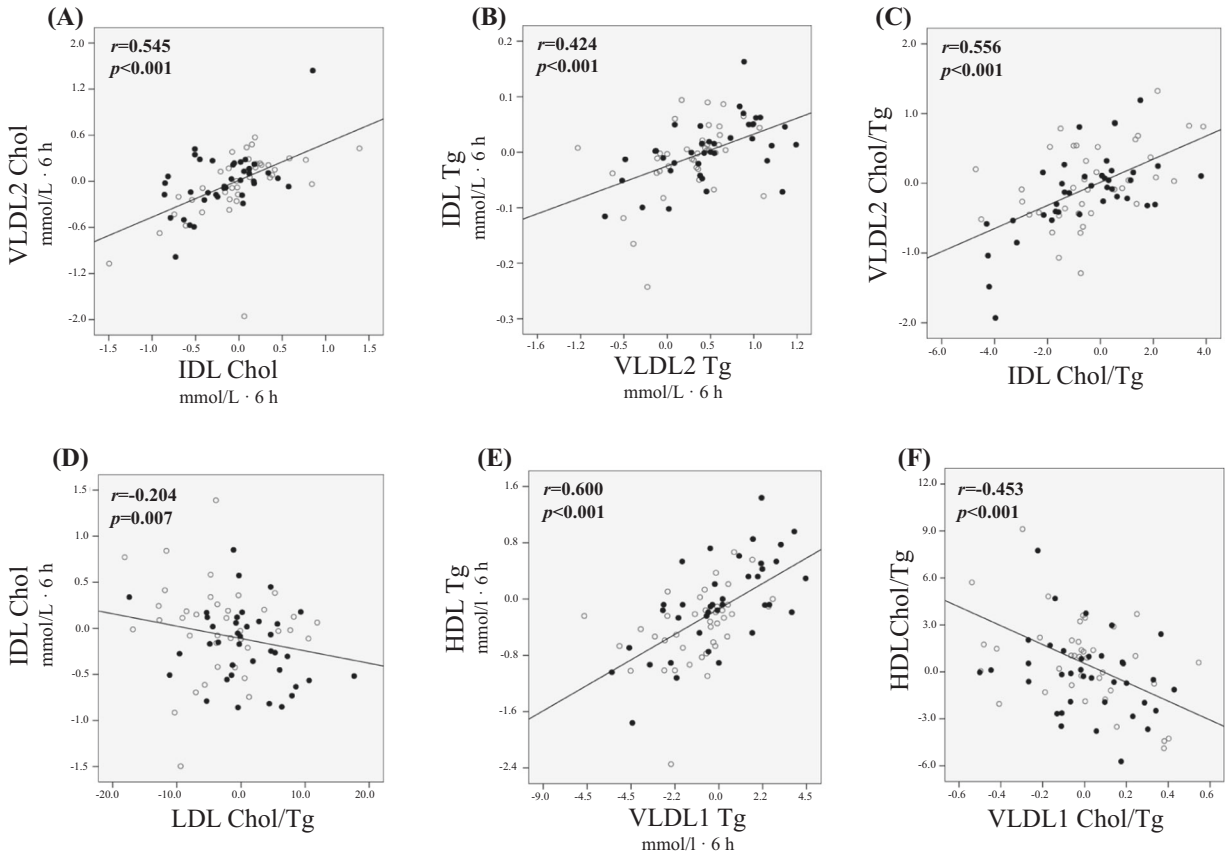

Fig. 3 Pearson's correlations between changes from baseline in total postprandial AUC of cholesterol (Chol), triglycerides (Tg), and Chol/Tg ratio in the lipoprotein fractions after the dietary intervention (○ high-polyphenol group; ● low-polyphenol group)

transport in agreement with some trials in which polyphenol intake increased the expression of ATP-binding cassette transporter-A1 and scavenger receptor-B [33, 34].

It is difficult to evaluate how the effects of the polyphenols observed in our study could influence cardiovascular risk. Since "postprandial dyslipidaemia" is considered an independent cardiovascular risk factor [2, 3], our results may be clinically relevant, as they show that a diet with a naturally high content of different classes of polyphenols, in addition to its positive effects on lipoprotein lipid concentrations [14], induces modifications in the postprandial lipoprotein composition that, at least for some of them (LDL richer in Tg), may be considered "less atherogenic" [35–38]. The data on HDL composition and cardiovascular risk are more controversial. In fact, recent epidemiological evidence has provided inconsistent results on the prognostic value of small cholesterol-enriched HDL and large triglyceride-enriched HDL for predicting cardiovascular risk [39–43].

Our trial presents some strengths. First of all, our study was a well-controlled intervention trial sufficiently long to evaluate the effects of dietary polyphenol on lipoprotein composition. Second, the sources of polyphenols in our experimental diet were easily reached with natural foods commonly used in different gastronomic traditions. Furthermore, the use of natural foods rich in different polyphenols gave us the opportunity to have, in the high-polyphenol diet, various types of bioactive polyphenol compounds, such as anthocyanidins, flavones, flavonoids, phenolic acids, flavans, flavanones, and flavonols [21, 44].

Finally, dietary compliance was assessed by the participants' 7-day food records completed during the dietary intervention and assaying their phenolic metabolites profile in 24-h urine collections.

Our study has some limitations. Although we measured phenolic metabolites profile in 24-h urine collections, the plasma bioavailability of phenolic metabolites was not investigated. Furthermore, the activities of some enzymes involved in postprandial lipoprotein composition—CETP, lipoprotein lipase, and hepatic lipase—were not measured. Information on these activities would certainly help clarify the mechanisms involved in the changes of postprandial lipoprotein metabolism. Another limitation is that we studied middle-aged men and women presenting abdominal obesity and at least one more feature of the metabolic

syndrome and, consequently, the results cannot be extrapolated to other populations.

In conclusion, consuming a diet naturally rich in different sources of polyphenols promotes changes in the lipid composition of postprandial lipoproteins. These changes may help understand the dynamic modifications occurring in the postprandial state. From a clinical point of view, particularly in relation to cardiovascular risk, it may be relevant that the effects of polyphenol on lipid concentrations, especially the reduction of VLDL1 lipids, are associated with modifications in lipid composition of lipoproteins. The overall effect of these changes on atherogenicity warrants further investigation on clinical cardiovascular outcomes.

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Author contributions LB, GA, GR, and AAR designed the experiment; LB, GDP, and CV collected and analyzed the data; GDP and CV interpreted the data and wrote the paper. GDP, CV, GC, MV, AM, PC, and LP collected the data. GA, GR, and AAR critically revised the paper and contributed to the conclusions. AAR is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors read and approved the final paper.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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