# Diets naturally rich in polyphenols improve fasting and postprandial dyslipidemia and reduce oxidative stress: a randomized controlled trial[1–3]

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## ABSTRACT

**Background:** The postprandial triglyceride-rich lipoprotein (TRL) concentration is a recognized independent cardiovascular disease risk factor. Diet is the natural approach for these postprandial alterations. Dietary polyphenols and long chain n−3 polyunsaturated fatty acids (LCn3s) are associated with a lower cardiovascular disease risk.

**Objective:** This randomized controlled study evaluated, in persons with a high risk of cardiovascular disease, the effects of diets naturally rich in polyphenols and/or marine LCn3s on plasma TRLs and urinary 8-isoprostane concentrations, a biomarker of oxidative stress.

**Design:** According to a $2 \times 2$ factorial design, 86 overweight/obese individuals with a large waist circumference and any other component of the metabolic syndrome were randomly assigned to an isoenergetic diet *1*) poor in LCn3s and polyphenols, *2*) rich in LCn3s, *3*) rich in polyphenols, or *4*) rich in LCn3s and polyphenols. The diets were similar in all other components. Before and after the 8-wk intervention, fasting and postmeal TRLs and 8-isoprostane concentrations in 24-h urine samples were measured.

**Results:** Dietary adherence was good in all participants. Polyphenols significantly reduced fasting triglyceride concentrations (2-factor ANOVA) in plasma ($P = 0.023$) and large very-low-density lipoproteins (VLDLs) ($P = 0.016$) and postprandial triglyceride total area under the curve in plasma ($P = 0.041$) and large VLDLs ($P = 0.004$). LCn3s reduced postprandial chylomicron cholesterol and VLDL apolipoprotein B-48. The concentrations of urinary 8-isoprostane decreased significantly with the polyphenol-rich diets. Lipoprotein changes induced by the intervention significantly correlated with changes in 8-isoprostane.

**Conclusions:** Diets naturally rich in polyphenols positively influence fasting and postprandial TRLs and reduce oxidative stress. Marine LCn3s reduce TRLs of exogenous origin. Through their effects on postprandial lipemia and oxidative stress, polyphenols may favorably affect cardiovascular disease risk. This trial was registered at clinicaltrials.gov as NCT00781365.     *Am J Clin Nutr* 2014;99:463–71.

## INTRODUCTION

Cardiovascular disease (CVD)[4] is the leading cause of morbidity and mortality in industrialized countries (1). In addition to the established CVD risk factors, other relevant factors include fasting and postprandial alterations in lipoprotein metabolism, endothelial dysfunction, oxidative stress, and subclinical inflammation (2). In particular, postprandial metabolic abnormalities may play an important role considering that, in our society, people spend most of their time in the absorptive condition (3). Diet is the natural approach for postprandial alterations, and, because of its pleiotropic action, it may modulate several major and emerging CVD risk factors at the same time, with favorable and multiplicative effects, as suggested by prospective studies (4, 5). Of the dietary components that might positively influence the risk of chronic diseases, long-chain n−3 PUFAs (LCn3s) and, more recently, polyphenols have attracted much interest.

Epidemiologic studies have shown an association between a higher intake of polyphenols and a lower risk of CVD, diabetes mellitus, cancer, and neurodegenerative disorders (6, 7). Different mechanisms may mediate these putative protective effects, including changes in lipid metabolism, both at fasting and postprandially (8, 9). However, the few human intervention studies conducted have produced conflicting results, with some studies showing impressive positive effects and others just the opposite or no effect at all (9). Furthermore, whereas these studies

explored the effects of polyphenols contained in individual foods (tea, chocolate, red wine, etc), the effects of diets containing different natural sources of polyphenols were not investigated. This is relevant because polyphenols differ in terms of their metabolic characteristics, such as bioavailability and the activity of their intermediate metabolites (10). Given the antioxidant capacity of polyphenols—shown in vitro and in animal studies (11)—another possible cardioprotective mechanism may relate to their role in counteracting oxidative stress, which is a key point in the pathogenesis of CVD.

LCn3s have shown a cardioprotective effect in epidemiologic studies and clinical trials (12). The effects of dietary LCn3s, although widely investigated and associated with an overall favorable lipoprotein profile in terms of CVD risk (13), still need to be clarified, especially in relation to oxidative stress and postprandial lipid metabolism (14, 15). Moreover, in many human studies, marine LCn3s were given as supplements and not by increasing the consumption of food naturally rich in LCn3s. Finally, only a few in vitro (16) and acute (15) studies of the combined effects of polyphenols and LCn3s and the possible interactions between these 2 dietary components are available, which could, respectively, reduce or increase oxidative stress.

Therefore, the aim of this randomized controlled intervention study was to elucidate in individuals with features of the metabolic syndrome, and therefore at high risk of type 2 diabetes and CVD, the medium-term effects of diets naturally rich in different sources of polyphenols and/or marine LCn3s on 1) lipid metabolism, focusing on postprandial state, and 2) oxidative stress.

## SUBJECTS AND METHODS

### Patients

Eighty-six individuals of both sexes, aged 35–70 y, with a large BMI (in kg/m$^2$; 27–35) and waist circumference (men, >102 cm; women, >88 cm) were recruited from patients referred to the obesity outpatient clinic of the Federico II University Hospital. Health status and medical history were assessed by interviews, clinical examinations, and routine laboratory tests, and glucose tolerance was evaluated with a 75-g oral-glucose-tolerance test. In addition to a large BMI and waist circumference, subjects had to have at least one or more components of the metabolic syndrome per the Adult Treatment Panel III (17). Exclusion criteria were as follows: CVD events (myocardial infarction or stroke) in the past 6 mo, diabetes mellitus, fasting plasma triglycerides ≥400 mg/dL or cholesterol >270 mg/dL, regular intensive physical activity, renal failure (serum creatinine >1.7 mg/dL), liver disease, anemia, any other chronic disease, or use of drugs able to influence glucose and lipid metabolism or inflammation. Eight subjects, who were equally distributed among the 4 experimental groups, were not evaluated at the end of the intervention because of an unwillingness to repeat the tests or for job or family reasons (see Supplemental Figure 1 under "Supplemental data" in the online issue). The clinical characteristics of the 78 participants who completed the dietary intervention are shown in **Table 1**. The study protocol was approved by the Federico II University Ethics Committee. All participants gave their written informed consent to participate in the study.

### Study design

According to a 2 × 2 factorial design, participants were randomly assigned to one of the following nutritional iso-energetic interventions for 8 wk: 1) control diet, low in LCn3s and polyphenols; 2) diet rich in LCn3s and low in polyphenols; 3) diet rich in polyphenols and low in LCn3s; or 4) diet rich in LCn3s and polyphenols. During the 3-wk run-in period, participants were stabilized with a diet that reflected their eating habits. As shown in **Table 2**, the assigned diets differed only in LCn3 and polyphenol contents and were similar in all other characteristics, including macronutrient composition and content of micronutrients that could possibly affect outcomes, especially for their antioxidant properties. Dietary composition was derived from the tables of the Italian National Research Institute for Food and Nutrition (18), whereas polyphenol contents and the Oxygen Radical Absorbance Capacity index were calculated according to USDA tables (19). The polyphenol content in tea, coffee, chocolate, and blueberry jam was measured directly (20). The main dietary sources of polyphenols were decaffeinated green tea, decaffeinated coffee, dark

**TABLE 1**
Baseline characteristics of the 4 groups of participants in the dietary intervention study[1]

| | Control | High LCn3s | High polyphenols | High polyphenols and high LCn3s | P value (ANOVA) |
|---|---|---|---|---|---|
| Sex (M/F) | 8/12 | 8/11 | 9/11 | 8/11 | |
| Age (y) | 54 ± 9[2] | 56 ± 8 | 53 ± 9 | 55 ± 9 | 0.645 |
| Body weight (kg) | 87 ± 10 | 86 ± 12 | 87 ± 11 | 84 ± 12 | 0.858 |
| BMI (kg/m$^2$) | 33 ± 3 | 32 ± 4 | 32 ± 3 | 30 ± 3 | 0.126 |
| Waist circumference (cm) | 104 ± 7 | 105 ± 10 | 104 ± 9 | 101 ± 8 | 0.601 |
| Systolic blood pressure (mm Hg) | 120 ± 7 | 121 ± 12 | 126 ± 16 | 119 ± 9 | 0.231 |
| Diastolic blood pressure (mm Hg) | 76 ± 8 | 74 ± 7 | 76 ± 9 | 73 ± 8 | 0.663 |
| Fasting plasma triglycerides (mg/dL) | 120 ± 47 | 138 ± 68 | 120 ± 60 | 125 ± 78 | 0.787 |
| Fasting plasma cholesterol (mg/dL) | 194 ± 38 | 191 ± 26 | 194 ± 34 | 193 ± 27 | 0.992 |
| HDL cholesterol (mg/dL) | 43 ± 10 | 41 ± 11 | 43 ± 9 | 44 ± 14 | 0.855 |
| LDL cholesterol (mg/dL) | 118 ± 30 | 114 ± 22 | 117 ± 26 | 112 ± 30 | 0.874 |
| Fasting plasma glucose (mg/dL) | 104 ± 12 | 104 ± 12 | 100 ± 9 | 103 ± 11 | 0.498 |
| Urinary 8-isoprostane (ng/24 h) | 1176 ± 485 | 1037 ± 475 | 1433 ± 752 | 1385 ± 660 | 0.169 |

[1] LCn3, long-chain n−3 PUFA.
[2] Mean ± SD (all such values).

**TABLE 2**
Mean composition of the diets assigned per protocol and the diets followed in the 4 experimental groups[1]

| | Control (n = 20) | | High LCn3s (n = 19) | | High polyphenols (n = 20) | | High polyphenols and high LCn3s (n = 19) | |
|---|---|---|---|---|---|---|---|---|
| | Assigned[2] | Followed[3] | Assigned[2] | Followed[3] | Assigned[2] | Followed[3] | Assigned[2] | Followed[3] |
| Total energy (kcal) | 2524 ± 366 | 2345 ± 472 | 2718 ± 283 | 2602 ± 248 | 2622 ± 456 | 2539 ± 459 | 2507 ± 452 | 2407 ± 471 |
| Proteins (% of energy) | 15.7 ± 0.0 | 16.0 ± 1.2 | 15.7 ± 0.0 | 15.9 ± 0.6 | 15.7 ± 0.0 | 15.8 ± 0.5 | 15.7 ± 0.0 | 15.8 ± 0.6 |
| Total fat (% of energy) | 33.6 ± 0.0 | 32.5 ± 1.7 | 33.6 ± 0.0 | 33.2 ± 1.9 | 33.6 ± 0.0 | 33.9 ± 1.4 | 33.6 ± 0.0 | 33.8 ± 1.5 |
| SFA (% of energy) | 7.2 ± 0.1 | 7.2 ± 0.3 | 7.3 ± 0.0 | 7.3 ± 0.4 | 7.1 ± 0.0 | 7.3 ± 0.4 | 7.2 ± 0.3 | 7.4 ± 0.5 |
| MUFA (% of energy) | 21.0 ± 0.2 | 20.0 ± 1.6 | 19.9 ± 0.2 | 19.4 ± 1.5 | 19.2 ± 0.3 | 19.3 ± 1.1 | 18.6 ± 0.4 | 18.8 ± 1.0 |
| n−6 PUFAs (% of energy) | 3.1 ± 0.1 | 3.1 ± 0.2 | 2.7 ± 0.0 | 2.7 ± 0.2 | 2.8 ± 0.2 | 2.9 ± 0.3 | 2.7 ± 0.1 | 2.7 ± 0.2 |
| n−3 PUFAs (% of energy) | 0.5 ± 0.0 | 0.5 ± 0.0 | 1.5 ± 0.0 | 1.4 ± 0.1* | 0.5 ± 0.4 | 0.5 ± 0.1 | 1.5 ± 0.1 | 1.5 ± 0.2* |
| EPA (% of energy) | 0.02 ± 0.01 | 0.02 ± 0.01 | 0.43 ± 0.02 | 0.40 ± 0.06* | 0.04 ± 0.01 | 0.04 ± 0.01 | 0.47 ± 0.02 | 0.46 ± 0.06* |
| DHA (% of energy) | 0.01 ± 0.01 | 0.01 ± 0.01 | 0.58 ± 0.02 | 0.53 ± 0.08* | 0.01 ± 0.0 | 0.01 ± 0.01 | 0.60 ± 0.03 | 0.59 ± 0.08* |
| Cholesterol (mg) | 191 ± 3 | 184 ± 27 | 195 ± 1 | 192 ± 32 | 178 ± 6 | 187 ± 16 | 198 ± 8 | 197 ± 24 |
| Total CHO (% of energy) | 50.7 ± 0.0 | 51.5 ± 1.0 | 50.7 ± 0.0 | 50.9 ± 1.9 | 50.7 ± 0.0 | 50.4 ± 1.4 | 50.7 ± 0.0 | 50.3 ± 1.9 |
| Fiber (g) | 28.7 ± 0.2 | 26.8 ± 4.4 | 28.4 ± 0.2 | 27.8 ± 3.9 | 29.0 ± 0.9 | 27.5 ± 2.2 | 28.4 ± 0.3 | 27.2 ± 2.8 |
| Vitamin C (mg) | 279 ± 0.0 | 261 ± 24 | 284 ± 0.0 | 256 ± 38 | 279 ± 0.0 | 256 ± 21 | 275 ± 0.0 | 257 ± 24 |
| Vitamin E (mg) | 17.3 ± 2.0 | 15.8 ± 3.2 | 18.1 ± 1.5 | 17.5 ± 2.2 | 18.7 ± 2.5 | 18.0 ± 2.7 | 18.6 ± 3.0 | 17.7 ± 3.0 |
| Polyphenols (mg) | 365 ± 3 | 336 ± 79 | 363 ± 2 | 377 ± 55 | 2903 ± 19 | 2776 ± 234§ | 2861 ± 42 | 2667 ± 400§ |

[1] All values are means ± SDs. Comparisons between the assigned and followed diets were not significantly different, $P > 0.05$ for all dietary components ($t$ test). *Significantly different from the control and high-polyphenol groups, $P < 0.0001$ (ANOVA and least-significant-difference post hoc test). §Significantly different from the control and high-LCn3 groups, $P < 0.0001$ (ANOVA and least-significant-difference post hoc test). CHO, carbohydrate; LCn3, long-chain n−3 PUFA.
[2] Data for the assigned diets include the changes made in different visits during the trial to keep body weight stable.
[3] Data for the followed diets were calculated from the 7-d food records at weeks 4 and 8.

chocolate, blueberry jam, artichokes, onions, spinach, rocket, and extra-virgin olive oil (*see* Supplemental Table 1 under "Supplemental data" in the online issue). The distribution of different classes of polyphenols is shown elsewhere (*see* Supplemental Table 2 under "Supplemental data" in the online issue). The main sources of LCn3s were salmon, dentex, and anchovies (*see* Supplemental Table 1 under "Supplemental data" in the online issue). The initial assigned energy intake of the diet was determined based on the individual's habitual energy intake evaluated by a 7-d food record, adjusted for body weight and clinical judgment of the dietitians, in order to take care of a possible underreporting, common in overweight/obese individuals.

To improve dietary adherence, meals and beverages were provided to the participants for the whole study period in amounts sufficient to cover their household consumption. Meals were prepared in a qualified catering service under the surveillance of the dietitians. Adherence to diets was evaluated by a 7-d dietary record at baseline, 4 wk, and 8 wk and was reinforced by the dietitians through counseling every week and phone calls every 2–3 d. Participants allocated to high-polyphenol diets or high-LCn3 diets were considered compliant with the treatment if intakes were ≥80% of those assigned; participants allocated to low-polyphenol or low-LCn3 diets were considered compliant if intakes were not >20% of those assigned. For all other dietary components, participants were considered compliant if, for each component, the intake was within ±20% of that assigned.

## Procedures

At baseline and after the 8-wk intervention, body weight, height, and waist circumference were measured according to standardized procedures (21). After a 12-h overnight fast, the participants consumed a 1000-kcal test meal composed of rice, butter, parmesan cheese, bresaola, and white bread, with intakes of olive oil, extra-virgin olive oil, salmon, and decaffeinated green tea differing in order to obtain a similar composition as the assigned diet (*see* Supplemental Table 3 under "Supplemental data" in the online issue). Before and 2, 4, and 6 h after the meal, blood samples were collected for the measurement of plasma concentrations of cholesterol and triglycerides and triglyceride-rich lipoproteins (TRLs) (chylomicrons and large VLDLs), and apolipoprotein B-48 (apo B-48) in large VLDLs. In addition, 24-h urine samples were collected on the day after the test meal, to measure 8-isoprostane concentrations, after adequately training the study participants on proper collection and storage procedures.

## Laboratory methods

Chylomicrons (Svedberg flotation unit >400) and large VLDLs (Svedberg flotation unit 60–400) were isolated from plasma by discontinuous density-gradient ultracentrifugation, as previously described (22). HDLs were isolated from plasma by using the phosphotungstic acid/magnesium chloride precipitation method. LDL cholesterol was calculated by using the Friedewald formula. Cholesterol, triglyceride, and glucose concentrations were assayed by enzymatic methods (Roche Molecular Biochemicals) on a Cobas Mira autoanalyser (ABX Diagnostics). Concentrations of apo B-48 were analyzed in large VLDLs by ELISA (Shibayagi Co Ltd), automated on a Triturus ELISA autoanalyzer (Grifols Italia S.p.A).

For isoprostane analysis, after collection, 10-mL urine samples were centrifuged for 5 min (3000 rpm, at 4°C). After removal of the sediment, they were fractionated into 500-$\mu$L aliquots with the addition of 0.005% butylated hydroxytoluene in ethanol and stored at $-80°$C. Immediately before the assay, one aliquot was thawed in ice, centrifuged, and diluted 10 times. Concentrations of 8-isoprostane were measured in triplicate with a Cayman 8-isoprostane enzyme immunoassay kit, performed manually, and read through with DiaSorin EtiStar Spectrophotometry. Concentrations are expressed as ng isoprostanes released in 24 h. The interassay CV was 14.3%, and the intraassay CV was 8.2%. All evaluations were performed before and after the 8-wk intervention by personnel who were blind to the assignment.

## Statistical analysis

To detect a 30% difference between treatments in the total AUCs of triglyceride concentrations 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. This degree of change after treatment is clinically based, corresponding to the differences observed between patients with type 2 diabetes and healthy control subjects in a previous study (22). The random allocation to the intervention—stratified for sex, age, BMI, and plasma triglycerides—was performed by using the minimization method with MINIM software (www.users.york.ac.uk).

The data are expressed as means ± SDs unless otherwise stated. Variables not normally distributed were analyzed after logarithmic transformation. Postprandial total AUCs and incremental AUCs were calculated by using the trapezoidal method. Data were analyzed by intention to treat for all subjects with completed outcomes. An analysis for protocol would yield the same results because all participants were compliant with treatment. Differences between the 4 groups at baseline were tested by 1-factor ANOVA and post hoc analysis with Bonferroni correction for multiple comparisons. The effects of dietary polyphenols, dietary LCn3s, and the interaction between polyphenols and LCn3s were evaluated by 2-factor ANOVA of absolute values at 8 wk adjusted for baseline values as covariate. The effects in each intervention group were compared with the control group by ANOVA and a least-significant-difference post hoc analysis, when a significant interaction effect was detected by 2-factor ANOVA. Bivariate associations were assessed by Pearson's correlation. Statistical analysis was performed according to standard methods by using the SPSS statistics version 15.0 (SPSS/PC).

## RESULTS

### Compliance with dietary intervention

The composition of the diets strictly reflected the dietary composition assigned per protocol in all groups (Table 2). As expected, the diets were significantly different in polyphenol and LCn3 contents. The absolute contents of n−3 PUFAs were 1.40 ± 0.29, 4.08 ± 0.52, 1.35 ± 0.15, and 3.94 ± 0.76 g/d in the control, high-LCn3, high-polyphenol, and high-polyphenol and high-LCn3 groups, respectively. The Oxygen Radical Absorbance Capacity indexes were 6415 ± 1181, 6530 ± 723, 24,985 ± 2665, and 23,159 ± 4621 Trolox equivalents in the same 4 groups, respectively. No differences in macronutrients, fiber, and vitamin

contents were observed between the 4 dietary groups. All subjects were within the ranges of intakes defined for good compliance for each dietary component.

### Baseline data

At baseline, the 4 groups were comparable in age, body weight, BMI, waist circumference, and blood pressure (Table 1). Fasting plasma concentrations of triglycerides, total cholesterol, HDL cholesterol, LDL cholesterol, and glucose were not significantly different between the groups.

#### Anthropometric measures

Body weight was relatively stable during the intervention, with small decreases at 8 wk ($-0.11 \pm 0.67$, $-1.14 \pm 1.30$, $-0.57 \pm 0.80$, and $-0.32 \pm 0.64$ kg in the control, high-LCn3, high-polyphenols, and high-polyphenols and high-LCn3 groups, respectively), which were statistically significant in the group assigned to LCn3s ($P = 0.041$). Waist circumference did not change significantly during the intervention ($-0.8 \pm 1.4$, $-1.1 \pm 1.5$, $-0.6 \pm 1.5$, and $-1.2 \pm 1.6$ cm in the same 4 groups, respectively).

#### Fasting lipids and lipoproteins

The 8-wk interventions with polyphenols, LCn3s, or their combination, appeared to decrease fasting plasma triglycerides compared with the control diet (**Table 3**). By 2-factor ANOVA, only the effect of polyphenols was statistically significant ($P = 0.023$); no significant effects for LCn3s or their interaction were found. Fasting total and LDL-cholesterol concentrations were not significantly affected by the dietary interventions. Polyphenols significantly decreased fasting concentrations of triglyceride and cholesterol in large VLDL and HDL fractions; no significant effects for LCn3s or their interaction were found.

#### Postprandial lipids and lipoproteins

The absolute changes after the intervention (8 wk minus baseline) in postprandial total AUC for lipids and apo B-48 are shown in **Figure 1**. The total AUC for plasma triglyceride after the test meal appeared to decrease in the high-polyphenols, high-LCn3, and high-polyphenols and high-LCn3 groups. By

2-factor ANOVA, only the effect of polyphenols was statistically significant ($P = 0.041$); no significant effects for LCn3 or their interaction were found. Plasma total cholesterol AUC did not change significantly with the experimental diets (data not shown). Chylomicron triglyceride and cholesterol total AUC appeared to decrease in the LCn3 group, but not significantly so by 2-factor ANOVA. Total AUCs for large VLDL triglycerides and cholesterol decreased in the high-polyphenols groups. By 2-factor ANOVA the effect of polyphenols was statistically significant ($P = 0.004$ and $P = 0.013$ for triglycerides and cholesterol, respectively); no significant effects for LCn3s or their interaction were found. Total AUCs for large VLDL apo B-48 decreased significantly in the high-LCn3 group and the high-polyphenols group ($P < 0.05$ for both compared with the control diet), and the interaction was significant ($P = 0.023$). The absolute changes after the intervention (8 wk minus baseline) in postprandial incremental AUCs for lipids and apo B-48 are shown in **Table 4**. A decrease in chylomicron cholesterol incremental AUCs was observed in the LCn3 group ($P < 0.05$ compared with the control diet) in the presence of a significant negative interaction between LCn3 and polyphenols ($P = 0.047$, 2-factor ANOVA).

#### Urinary 8-isoprostane

At baseline, urinary 8-isoprostane concentrations were similar in the 4 groups (Table 1). The concentrations of 8-isoprostane decreased in the high-polyphenols groups (**Figure 2**). By 2-factor ANOVA, the effect of polyphenols was statistically significant ($P = 0.012$); no significant effects for LCn3s or the interaction of polyphenols with LCn3s were found.

#### Relation between dietary effects on lipoproteins and isoprostanes

Dietary effects on urinary 8-isoprostane correlated significantly with changes in fasting and postprandial plasma lipoproteins, as shown elsewhere for total AUCs for triglycerides and cholesterol in large VLDLs (*see* Supplemental Figure 2 under "Supplemental data" in the online issue). The strength of these associations was mainly based on the changes observed in the polyphenol-rich groups.

**TABLE 3**
Absolute changes after the intervention (8 wk minus baseline) in fasting plasma lipid concentrations[1]

| | Control (n = 20) | High LCn3s (n = 19) | High polyphenols (n = 20) | High polyphenols and high LCn3s (n = 19) | LCn3 effect | Polyphenol effect | Polyphenol-LCn3 interaction |
|---|---|---|---|---|---|---|---|
| Plasma triglycerides (mg/dL) | $19 \pm 49$ | $-14 \pm 56$ | $-18 \pm 31$ | $-15 \pm 40$ | 0.261 | 0.023 | 0.095 |
| Plasma total cholesterol (mg/dL) | $-2 \pm 23$ | $-8 \pm 21$ | $-9 \pm 21$ | $-1 \pm 24$ | 0.836 | 0.859 | 0.175 |
| Large VLDL triglycerides (mg/dL) | $7.2 \pm 32.0$ | $-9.4 \pm 38.2$ | $-14.8 \pm 27.0$ | $-11.9 \pm 26.0$ | 0.872 | 0.016 | 0.210 |
| Large VLDL cholesterol (mg/dL) | $1.61 \pm 6.34$ | $-1.90 \pm 7.40$ | $-2.19 \pm 4.88$ | $-1.78 \pm 4.96$ | 0.821 | 0.045 | 0.211 |
| LDL cholesterol (mg/dL) | $-4.07 \pm 16.2$ | $-2.06 \pm 14.6$ | $-6.50 \pm 18.4$ | $5.20 \pm 16.4$ | 0.120 | 0.088 | 0.835 |
| HDL triglycerides (mg/dL) | $1.35 \pm 2.76$ | $-0.58 \pm 8.83$ | $-4.60 \pm 6.42$ | $-1.42 \pm 4.02$ | 0.910 | 0.017 | 0.229 |
| HDL cholesterol (mg/dL) | $0.2 \pm 3.7$ | $0.4 \pm 4.1$ | $-2.4 \pm 3.9$ | $-3.5 \pm 5.7$ | 0.503 | 0.005 | 0.675 |

The $P^2$ header spans the LCn3 effect, Polyphenol effect, and Polyphenol-LCn3 interaction columns.

[1] All values are means ± SDs. LCn3, long-chain n−3 PUFA.
[2] Two-factor ANOVA of absolute values at 8 wk adjusted for baseline values as covariate. $P < 0.05$ indicates a significant difference.
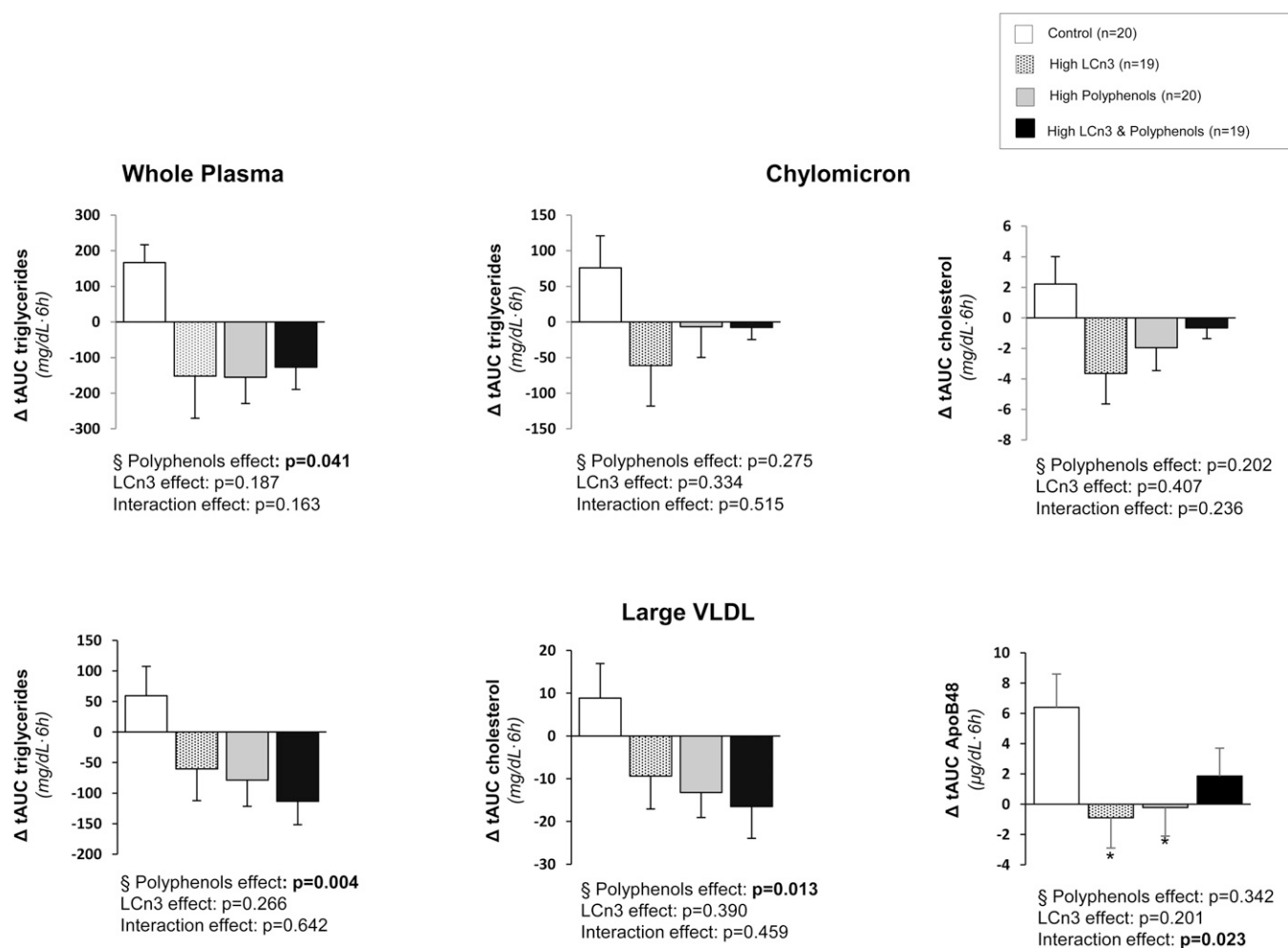
**FIGURE 1**. Mean (±SEM) absolute changes after the intervention (8 wk minus baseline) in postprandial tAUCs of whole plasma triglycerides, chylomicron triglycerides and cholesterol, and large VLDL triglycerides, cholesterol, and ApoB48 in the 4 experimental groups. *Significantly different from the control diet, $P < 0.05$ (ANOVA and least-significant-difference post hoc analysis). §Two-factor ANOVA of absolute values at 8 wk adjusted for baseline values as covariate. ApoB48, apolipoprotein B-48; LCn3, long-chain n−3 PUFA; tAUC, total AUC.

## DISCUSSION

This was the first randomized controlled trial with an adequate sample size that investigated medium-term dietary effects of diets naturally rich in polyphenols and/or LCn3s in individuals at high risk of type 2 diabetes and CVD. The results indicate that *1*) diets rich in polyphenols of different sources significantly reduce

**TABLE 4**
Absolute changes after the intervention (8 wk minus baseline) in postprandial incremental AUCs of plasma and lipoprotein triglycerides, cholesterol, and apo B-48[1]

| | Control (*n* = 20) | High LCn3s (*n* = 19) | High polyphenols (*n* = 20) | High polyphenols and high LCn3s (*n* = 19) | LCn3 effect | Polyphenol effect | Polyphenol-LCn3 interaction |
|---|---|---|---|---|---|---|---|
| | | | | | | $P^2$ | |
| Plasma triglyceride (mg/dL · 6 h) | 52 ± 294 | −69 ± 275 | −47 ± 236 | −40 ± 149 | 0.310 | 0.526 | 0.252 |
| Plasma total cholesterol (mg/dL · 6 h) | −16 ± 42 | −2 ± 72 | 14 ± 58 | −10 ± 52 | 0.816 | 0.476 | 0.187 |
| Chylomicron triglycerides (mg/dL · 6 h) | 54 ± 173 | −53 ± 220 | −3.4 ± 194 | −1.7 ± 74 | 0.189 | 0.939 | 0.174 |
| Chylomicron cholesterol (mg/dL · 6 h) | 1.4 ± 6.8 | −2.7 ± 7.5* | −1.8 ± 5.7 | −0.4 ± 2.8 | 0.351 | 0.742 | 0.047 |
| Large VLDL triglycerides (mg/dL · 6 h) | 16 ± 130 | −3.8 ± 100 | 9.8 ± 136 | −42 ± 63 | 0.169 | 0.384 | 0.542 |
| Large VLDL cholesterol (mg/dL · 6 h) | 1.0 ± 23 | −2.8 ± 21 | 0.1 ± 21 | −5.8 ± 14 | 0.297 | 0.676 | 0.825 |
| Large VLDL apo B-48 (μg/mL · 6 h) | 1.53 ± 12 | 0.27 ± 9.83 | 0.29 ± 7.86 | 0.12 ± 4.80 | 0.741 | 0.750 | 0.802 |

[1] All values are means ± SDs. *Significantly different from the control group, $P < 0.05$ (ANOVA and least-significant-difference post hoc test). apo B-48, apolipoprotein B-48; LCn3, long-chain n−3 PUFA.
[2] Two-factor ANOVA of absolute values at 8 wk adjusted for baseline values as covariate. $P < 0.05$ indicates a significant difference.
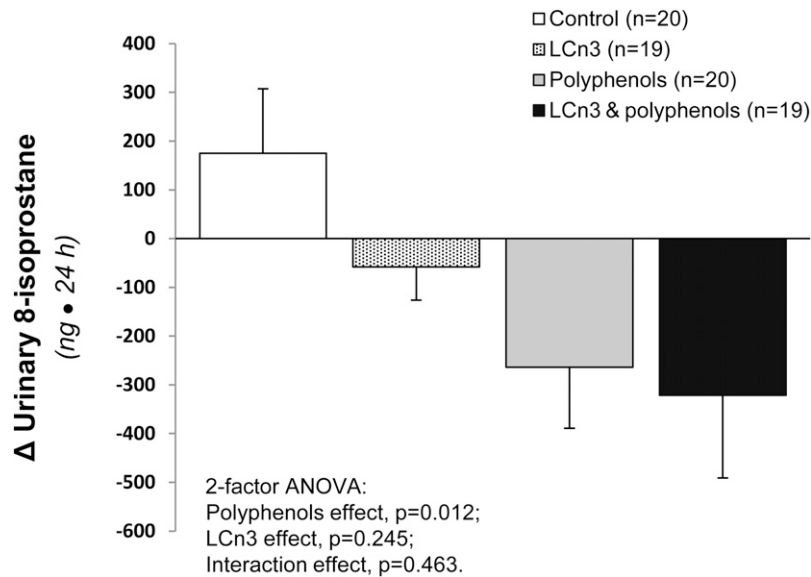
**FIGURE 2**. Mean (±SEM) absolute changes after the intervention (8 wk minus baseline) in 24-h urinary concentrations of 8-isoprostane in the 4 experimental groups. Two-factor ANOVA of absolute values at 8 wk adjusted for baseline values as covariate. LCn3, long-chain n−3 PUFA.

plasma triglyceride concentrations, mainly in the large VLDL fraction, in both the fasting and postprandial states; 2) diets rich in LCn3s of marine origin reduce postprandial TRLs, mainly chylomicron remnants; and 3) diets rich in polyphenols reduce oxidative stress.

Most previous evidence on dietary polyphenols comes from in vitro and animal studies, wherein selected types of polyphenols induced triglyceride reductions in plasma, chylomicrons, and VLDLs and increased HDL-cholesterol concentrations (9). Results in humans were extremely conflicting; polyphenols showed only a trend to better outcomes for LDL and HDL cholesterol and extremely diverging data on triglyceride metabolism (8). Moreover, very few data were available on the effects of polyphenols on postprandial lipids (23). Possible explanations for these inconsistencies include the use of different single sources of polyphenols and poor available knowledge of the many factors that affect the bioavailability of dietary polyphenols in humans (10). Our controlled study showed that the reduction in triglyceride mainly concerns postprandial TRLs, which are a recognized independent CVD risk factor (24).

Different potential sites of action of polyphenols on lipid metabolism have been proposed, particularly reduced triglyceride absorption, with consequent lower circulating apolipoprotein B/TRLs and, eventually, remnant lipoproteins (9). Other possible mechanisms include increased energy expenditure, reduced lipogenesis, reduced fat mass, reduced inflammation, and reduced oxidative stress (25). The effects of polyphenols in our study were not mediated by changes in body weight or modifications in appetite, as suggested for catechins, and mostly related to the adrenergic effects of caffeine (26). In fact, body weight was unchanged during the trial, and the presence of caffeine was negligible because decaffeinated coffee and tea were used.

Although extensively investigated, the effects of LCn3s on lipid metabolism are still controversial, mainly concerning the use of natural foods and postprandial responses. In the current trial, marine LCn3s induced a decrease in the lipid content of postprandial chylomicrons and a significant reduction in chylomicron remnants. These changes in exogenous lipid metabo-

lism, which correspond to a less-atherogenic lipoprotein profile, are in line with intervention data showing main effects of n−3 PUFAs in functional foods (27), or as supplements in patients with proteinuria (28), on the chylomicron fraction. It is noteworthy that, in our study, a significant negative interaction between LCn3s and polyphenols was observed for the changes in chylomicron remnants, which suggests a possible effect of polyphenols in reducing the absorption of LCn3s together with other dietary lipids.

An unexpected finding of this trial was the reduction in HDL cholesterol observed in the groups that consumed a polyphenol-rich diet. HDL triglycerides were also decreased by polyphenols. The meaning of this reduction in HDL in terms of CVD risk is unknown, because changes in HDL composition more than pure cholesterol content seem to affect this risk (29).

Polyphenols are associated with a lower incidence of CVD and have potent antioxidant activity, as generally observed in vitro. The current trial showed a reduction in oxidative stress by dietary polyphenols in humans, as evidenced by the ∼20% decrease in urinary 8-isoprostane concentration, which is a reliable biological marker of oxidation. Even if polyphenols represent the main antioxidants in the human diet, this recognized antioxidant activity is mostly based on in vitro evidence, whereas data in vivo are few and conflicting (30). In the current trial, the relations observed between the dietary effects on TRLs and 8-isoprostane suggest that improvement of fasting and, especially, postprandial lipoprotein metabolism may reduce oxidative stress. However, an opposite direction for this process cannot be ruled out, at least with dietary polyphenols, ie, the reduction in oxidative stress results in an improved lipoprotein profile. The reduced oxidative stress could influence lipid metabolism, upregulating enzymes involved in hepatic fat oxidation or decreasing nutrient absorption (31).

The results of this trial are relevant because fasting and postprandial alterations of TRLs are an independent CVD risk factor (24). There is no recognized treatment of postprandial dyslipidemia, and current pharmacologic interventions provide limited benefit for correcting VLDL alterations. In addition, diet

is the most natural approach for postprandial alterations, besides providing other multiple beneficial effects. Oxidative stress is one of the possible mechanisms involved in the pathogenesis of the major chronic degenerative diseases. The possibility to act favorably on this mechanism with a diet naturally rich in different polyphenols is clinically relevant. In fact, an additional strength of this study was the use of natural foods rich in polyphenols or LCn3s, included in a feasible and accepted diet, as shown by the good adherence observed during the intervention. This differs from most previous data obtained by using supplements or single foods (9, 23). In addition, for the first time, the effects of the combination of these 2 dietary approaches were evaluated. Also, when combined, they showed beneficial, although nonadditive, effects on lipids, with a trend to a negative interaction for some variables (chylomicron cholesterol and apo B-48 in large VLDLs), which deserves more investigation. A further strength of this study was that the experimental diets were very similar for all characteristics other than polyphenols and LCn3 contents, including macronutrient and micronutrient compositions, ie, factors potentially influencing the outcomes of the trial. This avoided the most common confounding problem in dietary studies.

A limitation of this study was that the results apply to individuals with metabolic abnormalities; therefore, it remains unknown whether they also apply to individuals without metabolic alterations or those with more advanced stages of metabolic or CVDs.

In conclusion, this randomized controlled trial provides evidence that diets naturally rich in marine LCn3s may positively influence TRLs, mainly chylomicron metabolism, with no additive effects of polyphenols. For the first time, positive effects on fasting and postprandial TRLs were shown for diets naturally rich in polyphenols, which also reduce oxidative stress. These effects may contribute to explain the favorable associations of dietary polyphenols with CVD risk.

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