European Journal of Nutrition
https://doi.org/10.1007/s00394-018-1718-x

ORIGINAL CONTRIBUTION

Gut-derived lipopolysaccharides increase post-prandial oxidative stress via Nox2 activation in patients with impaired fasting glucose tolerance: effect of extra-virgin olive oil

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Received: 30 October 2017 / Accepted: 11 May 2018

Abstract

Purpose Post-prandial phase is characterized by enhanced oxidative stress but the underlying mechanism is unclear. We investigated if gut-derived lipopolysaccharide (LPS) is implicated in this phenomenon and the effect of extra virgin olive oil (EVOO) in patients with impaired fasting glucose (IFG).

Methods This is a randomized cross-over interventional study including 30 IFG patients, to receive a lunch with or without 10 g of EVOO. Serum LPS, Apo-B48, markers of oxidative stress such as oxidized LDL (oxLDL) and soluble Nox2-derived peptide (sNox2-dp), a marker of nicotinamide-adenine-dinucleotide-phosphate oxidase isoform Nox2 activation, and plasma polyphenols were determined before, 60 and 120 min after lunch.

Results In patients not given EVOO oxidative stress as assessed by sNox2-dp and oxLDL significantly increased at 60 and 120 min concomitantly with an increase of LPS and Apo-B48. In these patients, changes of LPS were correlated with Apo-B48 (Rs = 0.542, $p = 0.002$) and oxLDL (Rs = 0.463, $p = 0.010$). At 120 min, LPS (β − 15.73, $p < 0.001$), Apo-B48 (β − 0.14, $p = 0.004$), sNox2-dp (β − 5.47, $p = 0.030$), and oxLDL (β − 42.80, $p < 0.001$) significantly differed between the two treatment groups. An inverse correlation was detected between polyphenols and oxLDL (R − 0.474, $p < 0.005$). In vitro study showed that LPS, at the same concentrations found in the human circulation, up-regulated Nox2-derived oxidative stress via interaction with Toll-like receptor 4.

Conclusions Post-prandial phase is characterized by an oxidative stress-related inflammation potentially triggered by LPS, a phenomenon mitigated by EVOO administration.

Keywords Extra-virgin olive oil · Lipopolysaccharide · Oxidative stress

Introduction

Abnormal post-prandial blood glucose levels and lipid profile, as detected by post-prandial increase of Apo-B48 levels [1], have deleterious effects on vascular disease by promoting and/or aggravating the atherosclerotic process [2]. For example, post-prandial glycaemia has been associated with oxidative stress and a higher incidence of cardiovascular events in diabetic patients vs non-diabetic ones [3, 4]. Oxidative stress is believed to play a role in the process of atherosclerosis via oxidation of LDL in the arterial wall and interference with mechanism that ultimately lead to plaque instability [5]. Thus, investigation of the mechanism accounting for post-prandial-related oxidative stress would contribute to a novel strategy to counteract it. Recent study in patients with diabetes mellitus demonstrated enhanced

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Published online: 16 May 2018

circulating levels of gut-derived bacteria lipopolysaccharide (LPS) after a high-fat meal [6]. This change may represent an important trigger for systemic post-prandial oxidative stress, as LPS is responsible for activation of Nox2, which is among the most important producer of reactive oxidant species (ROS); however, data addressing this specific issue in human are still lacking [7].

Observational and interventional studies demonstrated that extra-virgin olive oil (EVOO) improves vascular outcomes by down-regulating not only platelet activation but also oxidative stress [8]. In particular, we have previously reported that post-prandial phase of a meal together with 10 g EVOO is associated with down-regulation of Nox-2-derived oxidative stress [8]. Based on in vitro evidence that LPS up-regulates Nox2-derived oxidative stress, we speculated that EVOO could exert its post-prandial antioxidant activity by lowering circulating LPS and possibly oxidative stress.

To investigate this issue, we included patients suffering from impaired fasting glucose (IFG), who are characterized by increased oxidative stress and are at risk of cerebrovascular and cardiovascular events [9]. Moreover, in a previous work we showed that IFG patients had higher circulating levels of LPS compared to controls [10].

Therefore, aims of the study were to investigate (1) the behavior of post-prandial oxidative stress and LPS in patients with IFG; (2) the effect of EVOO administration on post-prandial LPS and oxidative stress. Furthermore, we performed in vitro study to assess the relationship between LPS and several markers of oxidative stress including Nox2 activation.

Research design and methods

We enrolled 30 outpatients referring to the Day Service of Internal Medicine and Metabolic Disorders of the Policlinico Umberto I University Hospital in Rome for screening for suspected metabolic disease. All patients aged > 18 years and diagnosed with IFG according to the 2012 American Diabetes Association guidelines [11] were eligible for the study.

Patients were excluded if they had diabetes mellitus, previous cardiovascular or cerebrovascular ischemic events, active cancer, chronic infections, autoimmune disease, liver failure or chronic kidney disease. Patients taking any antioxidants or supplements were also excluded.

Thus, 30 patients were randomized to receive, in a cross-over design, a lunch with or without 10 g of EVOO. The two meals consisted of pasta with tomato sauce (100 g), baked ham (40 g), cheese (50 g), salad (100 g), apple (150 g) (Meal 1); pasta with tomato sauce (100 g), baked ham (40 g), EVOO (10 g), salad (100 g), apple (150 g) (Meal 2). The two meals were isoenergetic (~ 700 kcal) with a similar composition in proteins 16–19%, carbohydrates 53–54% and lipids 28–30% (Table 1).

Table 1 Composition of the two meals

	Carbohydrate (g)	Protein (g)	Lipid (g)	Total fiber (g)	Kcal
MEAL 1					
Pasta 100 g	79	10.9	1.4	0.0	353
Salad 100 g	2.2	1.8	0.4	1.5	19
Apple 150 g	20.5	0.45	0.5	3.0	79.5
Ham 40 g	0.4	7.9	5.9	0.0	86
Cheese 50 g	1.8	14.2	15.3	0.0	201
	52% (389 kcal)	19% (141 kcal)	28% (211 kcal) SFA 11.8 g MUFA 6.5 g PUFA 2.7 g	1.2% (9 Kcal)	750
MEAL 2					
Pasta 100 g	79.0	10.9	1.4	0.0	353
Salad 100 g	2.2	1.8	0.4	1.5	19
Apple 150 g	20.6	0.5	0.5	3.0	79.5
Ham 80 g	0.7	15.8	11.8	0.0	172
EVOO 10 g	0.0	0.0	10.0	0.0	89
	54% (384 kcal)	16% (116 kcal)	30% (216 kcal) SFA 6.2 g MUFA 11.9 g PUFA 4 g	1.2% (9 Kcal)	725 kcal

SFA saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids

Blood sample was collected in one BD Vacutainer (Franklin Lakes, New Jersey, USA) without anticoagulant (code 367615) and two BD Vacutainer with anticoagulant (0.129 M sodium citrate (code 366560), at baseline (T1) (1.00 pm), 60 (T2) and 120 (T3) minutes after the lunch by centrifugation at 300 g for 10 min. The supernatant serum and plasma samples of each individual subject were divided into aliquots and stored at − 80 °C. Every blood determination was performed blind.

The study was conformed to the declaration of Helsinki and approved by the Ethical Committee of Sapienza University of Rome.

Measurement of lipopolysaccharide

Lipopolysaccharide (LPS) levels in serum were measured using a commercial ELISA kit (Cusabio, Wuhan, China). Standards of LPS, purified from *Escherichia coli*, and samples were plated for 2 h at room temperature onto a microplate pre-coated with the antibody specific for LPS. After incubation, samples were read at 450 nm. Values were expressed as pg/ml; intra-assay and inter-assay coefficients of variation (CVs) were 8 and 10%, respectively.

Serum Apo-B48

Apo-B48 serum measurement was performed by ELISA Kit (Elabscience). Values were expressed as mg/l; intra-assay and inter-assay coefficients of variation were 3.25 and 4.12%, respectively.

Plasma total polyphenol content

Total polyphenol content in plasma samples was determined by a Folin–Ciocalteu colorimetric method modified to remove protein interferences [12]. Briefly, total phenolic concentrations in plasma samples were determined after a procedure of acid extraction/hydrolysis. For hydrolyzing the conjugated forms of polyphenols, 1.0 mol/l hydrochloride acid was added to the sample, followed by 2.0 mol/l sodium hydroxide in 75% methanol. For removing plasma proteins, 0.75 mol/l metaphosphoric acid (MPA) was used in this procedure. The final extraction of polyphenols was performed by adding 1:1 (v/v) solution of acetone:water. The results were expressed as the gallic acid equivalent (GAE) in mg/l. Intra-assay and inter-assay coefficients of variation were 3.74 and 9.14%, respectively.

Determination of oxLDL in serum and platelet supernatant

Oxidized LDL (oxLDL) was measured in serum and platelet samples using ELISA commercial kit (DRG International). Values are expressed as mU/ml. Intra-assay and inter-assay coefficients of variation were 6.3 and 4.7%, respectively.

Serum and platelet Nox2

Serum Nox2 was measured as soluble Nox2-derived peptide (sNOX2-dp) with an ELISA method, which was partly modified in comparison to that previously reported [13].

The assay is based on: (1) coating reference standards of known concentrations of sNox2-dp and of serum samples (1 µg of protein) into ELISA 96-well plate overnight at 4 °C, (2) washing away unbound materials, (3) blocking any free binding site for 120 min at room temperature, (4) washing away unbound materials, (5) addition in each well of anti-sNox2dp–horseradish peroxidase (HRP) monoclonal antibody against the amino acidic sequence of the extra membrane portion of Nox2, and (6) quantification of immobilized antibody enzyme conjugates by monitoring HRP activities in the presence of the substrate 3,3′,5,5′-tetramethylbenzidine (TMB). The enzyme activity is measured spectrophotometrically by the increased absorbency at 450 nm after acidification of formed products (2 M sulphuric acid). Since the increase in absorbency is directly proportional to the amount of sNox2dp of the unknown sample, the latter can be derived by interpolation from a reference curve generated in the same assay with reference standards of known concentrations of sNox2dp (0–200 pg/ml). Values were expressed as pg/ml; intra-assay and inter-assay coefficients of variation were 8.95 and 9.01%, respectively.

Determination of conjugated dienes in vitro

The standard oxidation assay was performed using a Perkin Elmer Lambda 4B UV/VIS spectrometer (Norwalk, CT, USA). Measurement of the 234 nm absorption was read at intervals of 2 min for a period of 2 h, as described, and expressed as micromoles of conjugated dienes [14]. Intra-assay and inter-assay coefficients of variation were 1.85 and 4.36%, respectively.

Isolation of plasma low-density lipoprotein

Blood obtained into tubes containing 7.2 mg EDTA was centrifuged at 1500 g for 10 min at 4 °C. Next, 250 µl of phosphate-buffered saline (PBS) containing 0.25 mM EDTA was stratified on 750 µl of plasma and tubes were centrifuged at 100,000 g for 7 min (Sorvall Discovery 90SE Ultra Centrifuges; rotor, TH-641). The upper 250 µl were removed to eliminate chylomicrons and another 250 µl of PBS containing 0.25 mM EDTA was added. Samples were centrifuged at 100,000 g for 2.5 h. Next, 250 µl of the upper layer was removed and 150 µl of KBr 50% (w/v) was added to obtain a density of 1.063 g/ml. Samples were centrifuged at

100,000 rpm for 5 h and 200 µl was recovered as the fraction containing LDL. Finally, the fraction of LDL was dialyzed with PBS containing EDTA.

In vitro study of stimulated platelets

Citrated blood samples were taken between 8 and 9 a.m. from healthy subjects (HS, $n=5$, males 3, females 2, age 44.4 ± 3.2) who had fasted for at least 12 h and aged between 20 and 50 years. Subjects were excluded if they had previous clinical illness or were taking drugs within 24 h before blood collection. To obtain platelet-rich plasma (PRP), citrated blood samples were centrifuged for 15 min at 180 g. To avoid leukocyte contamination, only the top 75% of the PRP was collected. Before activation, PRP samples were pre-incubated (20 min at 37 °C) with a Nox2 inhibitor (Nox2-tat; Santa Cruz Biotechnology, 50 µM), TLR4 inhibitor peptide or control peptide (CP) as negative control (Novus Biologicals, 4 µM). After incubation, samples were treated with lipopolysaccharides from *E. coli* 0111:B4 (20–80 pg/ml) in the presence or not of LDL (50 µg/ml) and stimulated with subthreshold (STC) concentration of collagen (Mascia Brunelli, 0.25 µg/ml) for 10 min at 37 °C according to Nocella et al. [7]. Supernatants were stored at − 80 °C for analysis of sNox2-dp, conjugated dienes and oxLDL.

Statistical analysis

Data are expressed as mean ± standard deviation or percentages. Each outcome (LPS, Apo-B48 and OxLDL, polyphenols, sNox2-dp) is modeled separately by means of a linear mixed effects model where treatment and measurement-occasion indicator are treated as fixed effects. A random effect corresponding to each patient is included to take into account dependence arising from repeated measurements on the same subject. We do so by including a subject-specific random intercept, which is assumed to be normally distributed. All significance levels are Bonferroni adjusted to take into account multiplicity of comparisons. Changes of variables at each time point were evaluated through Spearman's correlation and related test. We report adjusted p values, so that differences or changes with a $p<0.05$ can be interpreted as statistically significant after Bonferroni correction. All analyses were performed through the software R, version 3.3.3.

Sample size

For computing the sample size, we have identified sNox2-dp as primary endpoint. Assuming a variation at 120 min of 5 pg/ml and a standard deviation of 8 pg/ml a sample size of 27 patients guarantees a power of 90% to reject the null hypothesis of zero mean variation.

Results

Characteristics of study population are reported in (Table 2).

At baseline serum LPS, Apo-B48, oxLDL and sNox2-dp were 31.8 ± 6.8 pg/ml, 0.72 ± 0.1 mg/l, 35.4 ± 11.5 mU/ml and 27.3 ± 2.8, respectively. EVOO showed a significant effect on LPS (β − 7.01 95% CI − 9.14, − 4.88, $p<0.001$), Apo-B48 (β − 0.07 95% CI − 0.11, − 0.04, $p<0.001$), oxLDL (β − 21.19 95% CI − 26.82, − 15.55, $p<0.001$) and sNox2-dp (β − 7.48, 95% CI − 11.19, − 4.64, $p<0.001$).

Within-group differences of post-prandial oxidative stress-related markers

LPS

Compared to baseline (T1), LPS significantly increased at T2 (T2 vs T1 β 6.87, 95% CI 3.95, 9.78, $p<0.001$) and T3 (T3 vs T1 β 9.23, 95% CI 6.32, 12.15, $p<0.001$) in patients not receiving EVOO (Fig. 1, Panel A). In these patients, changes of LPS were correlated with Apo-B48 (Rs = 0.542, $p=0.002$), and oxLDL (Rs = 0.463, $p=0.010$).

In patients treated with EVOO, LPS remained stable at T2 (T2 vs T1 β 1.70, 95% CI − 1.40, 4.94, $p=0.825$), and significantly decreased at T3 (T3 vs T1 β − 6.50, 95% CI − 9.67, − 3.33, $p<0.001$) (Fig. 1, Panel A). Changes of LPS levels were significantly correlated with Apo-B48 (Rs = 0.616, $p=0.001$).

Apo-B48

Serum levels of Apo-B48 significantly increased after meal patients not given EVOO both at T2 (T2 vs T1 β 0.06, 95% CI 0.02, 0.10, $p=0.01$) and T3 (T3 vs T1 β 0.17, 95% CI 0.13, 0.21, $p<0.001$) (Fig. 1, Panel B).

Table 2 Clinical characteristics of the population

Total patients ($n=30$)	
Age (years)	58.1 ± 11.4*
Males/females (n)	17/13
Body mass index (kg/m^2)	31.1 ± 4.3* **(23.4–37.8)****
Waist circumference (cm)	107.8 ± 4.3*
Systolic blood pressure (mmHg)	122 ± 15*
Diastolic blood pressure (mmHg)	83 ± 9*
Smoking (n)	0
Statin (n)	9
Aspirin (n)	1

*Mean and standard deviation, ** BMI range

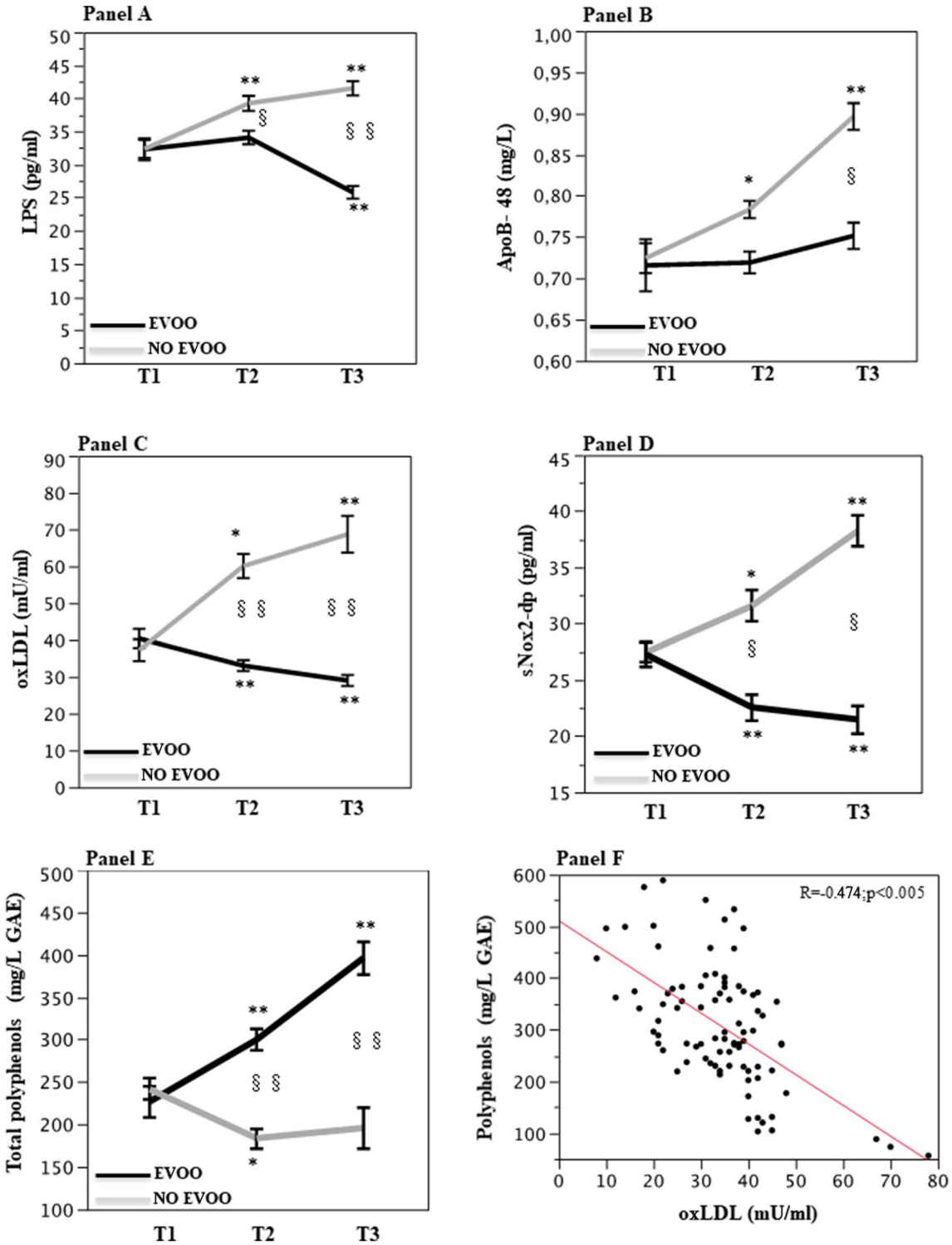

Fig. 1 Post-prandial effect of EVOO interventional study: LPS (Panel **A**), ApoB-48 (Panel **B**), oxLDL (Panel **C**), sNox2-dp (Panel **D**) and plasma polyphenols (Panel **F**) before (T1), at 60 (T2) and at 120 min (T3) after a meal with (black line) or without (gray line) extra-virgin olive oil (EVOO). Linear correlation between polyphenols and oxLDL (Panel **F**) (*$p < 0.05$ compared to T1; **$p < 0.001$ compared to T1; $^{\S}p < 0.05$ between groups at T2 or T3; $^{\S\S}p < 0.001$ between groups at T2 or T3)

In patients receiving a meal containing EVOO, the levels of Apo-B48 did not change at T2 (T2 vs T1 β 0.00, 95% CI −0.05, 0.06, $p=1.000$) and T3 (T3 vs T1 β 0.04, 95% CI −0.02, 0.09, $p=0.844$), compared to baseline (Fig. 1, Panel B).

OxLDL

Serum levels of oxLDL significantly increased after meal not containing EVOO, both at T2 (T2 vs T1 β 0.06, 95% CI 0.02, 0.10, $p=0.01$) and T3 (T3 vs T1 β 0.17, 95% CI (0.13, 0.21, $p<0.001$) (Fig. 1, Panel C).

EVOO significantly decreased oxLDL at T2 (T2 vs T1 β −7.33, 95% CI −12.35, −2.32, $p=0.01$) and at T3 (T3 vs T1 β −11.33, 95% CI −16.35, −6.32, $p<0.001$) (Fig. 1, Panel C).

sNox2-dp

Serum levels of sNox2-dp significantly increased after meal not containing EVOO (T2 vs T1, β 4.1, 95% CI 7.6, 0.56, $p=0.025$; T3 vs T1, β 10.7, 95% CI 14.3, 7.2; $p<0.001$). Conversely, EVOO did significantly decrease sNox2-dp at T2 (T2 vs T1 β −4.70, 95% CI −7.74, −1.65, $p=0.002$) and T3 (T3 vs T1 β −5.77, 95% CI −8.54, −2.96, $p=0.002$) (Fig. 1, Panel D).

Between-group differences of post-prandial oxidative stress-related markers

At T2, a significant difference between patients treated or not with EVOO was found for LPS (LPS-T2 β −5.10, 95% CI −9.41, −0.79, $p=0.041$), sNox2-dp (β −7.63 95% CI −12.52, −2.75, $p=0.002$), and oxLDL (β −30.16 95% CI −41.55, −18.78, $p<0.001$), but not for Apo-B48 (β −0.06 95% CI −0.12, 0.01, $p=0.464$) (Fig. 1, Panels A–D).

At T3, LPS (β −15.73 95% CI −20.04, −11.43, $p<0.001$), Apo-B48 (β −0.14 95% CI −0.20, −0.07, $p=0.004$), sNox2-dp (β −5.47 95% CI −10.35, −0.58, $p=0.030$), and oxLDL (β −42.80 95% CI −54.18, −31.42, $p<0.001$) significantly differed between the two groups (Fig. 1, Panels A–D).

Polyphenol plasma levels

After a meal not containing EVOO, polyphenol plasma levels significantly decreased at T2 (β −57.73, $p=0.002$) compared to T1, with a trend for reduction also at T3 (β −47.73, $p=0.058$). Conversely, after a meal containing EVOO, polyphenol plasma levels increased at T2 (β 58.23, $p<0.001$) and T3 (β 154.67, $p<0.001$). The difference between the two meals was significant both at T2 ($p<0.001$) and T3 ($p<0.001$) (Fig. 1, Panel E). The variations of polyphenols were significantly and inversely correlated with those of oxLDL (Rs −0.474, $p<0.005$) (Fig. 1, Panel F).

In vitro study of stimulated platelets

Platelets from healthy volunteers ($n=5$, males 3, females 2, age 44.4 ± 3.2) were incubated with scalar concentrations of LPS up to 80 pg/ml in the presence or not of LDL (50 µg/ml). The choice of this concentration was based on serum LPS values detected in human circulation in the post-prandial phase in patients not given EVOO (41.5 ± 5.9 pg/ml). LDL-treated platelets stimulated with scalar concentrations of LPS showed a significant increase of conjugated dienes, oxLDL and Nox2 activation compared to LDL-treated platelets alone (Fig. 2, Panels A–C); all these effects were significantly inhibited by blocking Nox2 activation with Nox2-tat (50 µM) (Fig. 2, Panels A–C). To further investigate the underlying mechanism, we pre-treated platelets with TLR4 inhibitor peptide (4 µM), which significantly decreased conjugated dienes, oxLDL formation and Nox2 activation elicited by LDL-treated platelets stimulated with LPS (Fig. 2, Panels A–C).

Discussion and conclusion

The study provides evidence that LPS is implicated post-prandial oxidative stress via up-regulation of Nox2-derived oxidative stress. EVOO administration modulates this phenomenon by reducing post-prandial LPS and eventually oxidative stress.

LPS circulates in the blood at concentration between 1 and 200 pg/ml as a consequence of its translocation with dietary fat from the gut to systemic circulation [15]; thus, experimental study showed that LPS absorption by gut is mediated by chylomicron transport from the gut [16]. Evidence is emerging that post-prandial phase is associated with an increase of circulating LPS, which has powerful inflammatory properties and is implicated in the athero-thrombotic process process [17]. In accordance with this report, circulating levels of LPS increased in the post-prandial phase coincidentally with serum Apo-B48, which is a protein synthetized by intestinal cells to transport chylomicrons in the peripheral circulation [18], therefore, reinforcing the hypothesis that LPS translocation from gut is mediated by chylomicrons.

A novel finding of the present study is the significant association between post-prandial LPS and several markers of oxidative stress such as LDL oxidation and sNox2-dp, suggesting that LPS may be a trigger of Nox2-derived oxidative stress. To corroborate this hypothesis we performed in vitro study using platelets as source of reactive oxidant species and demonstrated that LPS increases oxidative stress

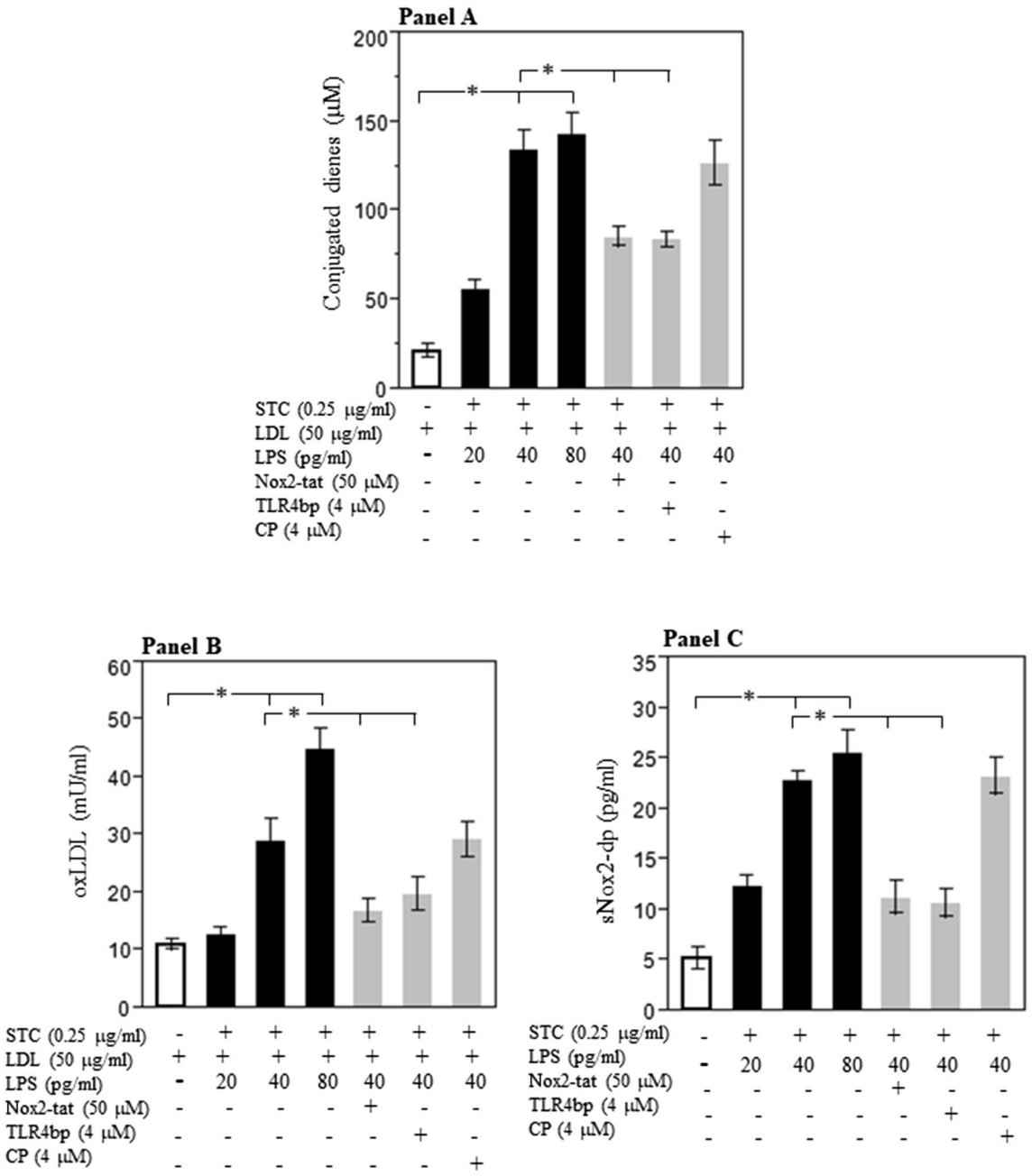

Fig. 2 In vitro study conjugated dienes (Panel **A**) and oxLDL (Panel **B**) formation were evaluated in platelets incubated with LDL (50 μg/ml) and added with scalar concentrations of LPS (20–80 pg/ml) before stimulation with or without subthreshold concentration (STC) of collagen (0.25 μg/ml), in the presence or less of TLR4 inhibitor (4 μM), Nox2-tat (50 μM) or control peptide (CP) (4 μM) ($n=5$) (*$p<0.05$). Nox2 activation (Panel **C**) was evaluated in platelets incubated with scalar concentrations of LPS (20–80 pg/ml) and stimulated with or without subthreshold concentration (STC) of collagen (0.25 μg/ml) in the presence or less of TLR4 inhibitor (4 μM), Nox2-tat (50 μM) or control peptide (CP) (4 μM). ($n=5$) (*$p<0.05$ for paired analyses; $p<0.001$ as an overall analysis)

by stimulating platelets via activation of Nox2; this effect was counteracted by platelet incubation with a specific inhibitor of TLR4 indicating that oxidative stress was triggered upon LPS interaction with its specific receptor.

Recent study from our group demonstrated that post-prandial oxidative stress is counteracted by EVOO intake with meal, which gives further insight into the mechanism potentially accounting for the beneficial effect of EVOO on cardiovascular prevention [19]; however, the mechanism accounting for this antioxidant effect was not clarified. As previous study showed that an olive oil containing different concentrations of phenols lowers post-prandial LPS

[20], we explored if EVOO could counteract oxidative stress via reduction of LPS. Here we report that EVOO administration is associated with lowered post-prandial LPS concomitantly with reduced oxidative stress suggesting a potential cause–effect relationship. The fact that EVOO reduced post-prandial LPS along with serum apoB-48 may lead to hypothesize that the post-prandial decrease of LPS by EVOO reflects an impaired biosynthesis of chylomicron with lowered LPS translocation from gut to systemic circulation. This hypothesis could be supported by the fact that EVOO up-regulates GLP1 via DPP4 inhibition, an effect that results in impaired Apo-B48 and eventually chylomicron biosynthesis [21].

Taken together, the results of the present study demonstrated a relationship between LPS and post-prandial oxidative stress and a role for EVOO as nutrient capable of mitigating post-prandial oxidative stress via lowering post-prandial LPS. We cannot exclude, however, that the antioxidant effect by EVOO is also mediated by its polyphenol content as suggested by the inverse relationship between serum polyphenol and oxidative stress.

The study has implications and limitations. The study provides further insight into systemic inflammation occurring after lunch by showing that in patients with impaired fasting glucose post-prandial increase of LPS occurs even after a relatively low-fat meal intake. The fact that EVOO lowers post-prandial LPS adds more to its beneficial effect on cardiovascular disease, as experiments in animals documented that LPS has pro-atherogenic property and clinical study demonstrated a significant association between LPS and atherosclerotic progression [15]. Polyphenols could be another important components of EVOO potentially accounting for its antioxidant effects; it remains to be established if single or, more likely, a mixture of polyphenols [22] are responsible for such effect.

Despite this being an interventional randomized trial, the small sample size is a limitation of the study, and results should be considered as hypothesis generating. Furthermore, we tested the effect of EVOO in a specific subgroup of patients, such as those with IFG, and so the generalizability of our results to other cardio-metabolic settings is uncertain. Finally, we included only Caucasian patients in our study; thus, the results cannot be extrapolated to other ethnic groups.

In conclusion, IFG patients disclose enhanced post-prandial LPS, which is responsible for oxidative stress. This phenomenon is counteracted by EVOO indicating a role for this nutrient in mitigating systemic inflammation occurring in the post-prandial phase.

Acknowledgements We thank prof. Lorenzo Loffredo for his useful advice.

Author contributions FV designed research, interpreted the results and wrote paper; RC designed research, wrote paper and performed experiments; CN, VC, SB, MN performed experiments; DP and AF performed statistical analysis; FA and MB recruited patients. All authors approved the final version of the article, including the authorship list.

Funding This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

Compliance with ethical standards

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

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