Postprandial endotoxemia linked with chylomicrons and LPS handling in obese vs lean men: a lipid dose-effect trial

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Context: Postprandial endotoxemia is a metabolic risk factor, which has been shown to originate from the intestinal absorption of gut LPS using non-physiological high-fat tests.

Objective: To determine if different realistic fat amounts can modulate postprandial dynamics and handling of LPS by varying postprandial lipidemia in humans of different BMI.

Design, setting and participants: Randomized controlled cross-over study in nutrition research center. 8 normal-weight (NW) and 8 obese age-matched men, without diabetes nor dyslipidemia, ingested breakfasts containing 10g vs 40g fat. Blood samples, leukocytes and chylomicron-rich fractions were obtained during 8h. Plasma and chylomicron-endotoxemia, plasma LPS transporters (LBP, sCD14) and IL-6, NF-κB translocation and IL-6 gene expression of immune cells were measured.

Main outcome: The postprandial fatty acid handling after ingesting 40g fat was previously published as primary outcome. The secondary outcomes were inflammatory ones including postprandial endotoxemia, LPS handling and plasma markers of inflammation after ingesting 10g or 40g fat.

Results: Chylomicronemia increased in all subjects according to ingested fat amount (P<0.01), but only obese had higher postprandial endotoxemia after 40g (P<0.05). Obese subject chylomicrons were more enriched with LPS compared to NW (P_BMI<0.01). We observed neither nuclear factor-κB translocation, nor variation of interleukin (IL)-6 expression in leukocytes. In both groups, fat amount did not modify postprandial response of plasma IL-6. However, AUC of IL-6 in obese was higher than in NW (P<0.05) parallel to higher fasting LPS-Binding Protein (LBP, P<0.05). AUC of IL-6 was correlated with LBP (P<0.01).

Conclusion: Postprandial endotoxemia is modulated by ingested fat amount in obese men. LPS handling in plasma through chylomicrons and LBP appears critical in driving the acute inflammatory response. The pathophysiological importance of repeated postprandial endotoxemia excursions and their contribution to a vicious cycle of LBP-driven low-grade inflammation deserve further investigation in the nutritional management of cardio-metabolic risk prevention.
Among lifestyle interventions for the prevention and management of metabolic diseases, nutritional strategies play a major role (1), especially those targeting major risk factors such as lipid abnormalities and inflammation. Metabolic endotoxemia, characterized by increased endotoxin activity in plasma, is strongly linked with unbalanced and high-caloric diets consumption (2). The bacterial endotoxins (lipopolysaccharides, LPS) triggering metabolic endotoxemia are gut proinflammatory compounds (3). Endotoxemia is now recognized as a significant contributing factor in the metabolic disorders of obesity and type 2 diabetes (4–6). The establishment of metabolic endotoxemia observed in a pathophysiological context could partly result from a chronic plasma exposure to LPS due to repeated daily postprandial fluctuations. Increased postprandial endotoxemia compared to the fasting condition has been reported during high-fat load digestion tests in humans and rodents (7–11). Dietary fats can promote the intestinal absorption of bacterial endotoxins from the gut into circulation, partly through LPS transport by chylomicrons (9, 12). LPS can also be carried and exchanged in plasma with other known transporters such as LPS-Binding Protein (LBP), Soluble Cluster of Differentiation 14 (sCD14) or high-density lipoprotein (HDL) (4). The overall inflammatory outcome thus appears to result from a balance between LPS concentration (notably via chylomicron formation) and its subsequent handling in plasma. Moreover postprandial hyperlipidemia, due to exogenous lipid accumulation in plasma after a meal, is a risk factor for cardiovascular diseases (13). One proposed cause is that the ingestion of high-fat meals may serve as a stimulus to raise systemic inflammatory tone, notably by leukocyte activation (14). Such stimulus would involve remnants of triglyceride-rich lipoproteins including chylomicrons, and LPS. However to our knowledge, most of the studies about postprandial endotoxemia consider high-fat loads or no fat, mostly in subjects with altered metabolic status, but a dose-response study testing nonexaggerated fat amounts before the onset of metabolic disorders is lacking. Therefore, investigating moderate fat doses is an important issue because individuals can consume foods and meals with a large variety of fat contents in everyday life.

On the basis of the above, we tested the hypotheses that (i) postprandial endotoxemia can be modified by the fat amount in the meal, namely 10 g vs 40 g, in subjects of different BMI due to chylomicron transport, (ii) differences in acute endotoxin absorption and handling would contribute to modulate postprandial systemic inflammation, notably proinflammatory cytokine IL-6 and translocation of NF-κB in plasma immune cells and (iii) these metabolic responses would be altered in obese men.

Subjects and Methods

Subjects

The subjects were those of the Lipinflox study approved by the Ethics Committee of Lyon-Sud-Est-II and AFSSAPS and registered at Clinical Trials (NCT01249378); some results of the primary outcome about postprandial dietary fatty acid handling after ingestion of 40 g fat were previously reported (15). Twenty-two men were recruited and 20 completed the study. Four subjects were not included in data analyses due to abnormal postprandial lipid metabolism (n = 2) and postprandial endotoxemia (n = 2) as they were outliers at P < .05 according to Extreme Studentized Deviate statistical method. Sixteen subjects divided in two groups, 8 NW (20 < BMI < 25 kg/m²) and 8 obese (30 < BMI < 35 kg/m² and waist circumference > 94 cm) with comparable mean age, were finally tested for the secondary outcomes. All participants were nonsmokers, consuming less than 40 g alcohol per day and chosen free of diabetes, insulin-resistance and dyslipidemia. None was taking any drugs or nutritional supplements affecting lipid metabolism, gut microbiota or inflammation. Participants gave written informed consent.

Study design

We investigated in normal-weight (NW) and noninsulin resistant obese subjects the postprandial responses to 2 mixed meals differing only by fat amount, 10 g vs 40 g, using a cross-over design. The first dose of 10 g (i) could be of interest for dietary advice purpose and (ii) consistent with the mean lipid amount usually consumed at breakfast by the studied subjects in their daily life. The second dose of 40 g (i) is known to induce a significant postprandial lipid response while remaining within a nutritional range and (ii) represents the maximum fat amount ingested at breakfast by some of the subjects.

After 48 h of specific dietary recommendations and an overnight fast, the subjects came to the Human Nutrition Research Center Rhône-Alpes (Lyon, France) and ingested (i) a test breakfast containing 10 g or 40 g of anhydrous milk fat with bread and a glass of skim milk (282 kcal and 551 kcal, respectively) and (ii) a standardized lunch 5 h later providing 713 kcal (15). Metabolic explorations were divided in postprandial phases including a first period of 5 h (0 to 300 minutes) postbreakfast, a second period of 3 h postlunch (300 to 480 minutes) and the entire exploration day (0 to 480 minutes). Blood samples were collected at baseline and at regular intervals along postprandial periods from an antecubital arm vein through a catheter.

Dietary intake of the subjects was assessed from three 5-day dietary records, analysis of which indicated that the subjects typically consumed 9.0 ± 2.3 g of lipids at breakfast (0–48 g). All subjects performed the clinical study including measurements of the following secondary outcomes at fasting and postprandially: plasma and chylomicron endotoxemia, plasma LPS transporters (LBP, sCD14) and IL-6, NF-κB translocation and IL-6 gene expression of immune cells.

Chylomicron-rich fraction (CMRF) isolation and characterization

The CMRF were collected after ultracentrifugation, as described previously (15). For endotoxemia measurements, an adapted method using pyrogen-free water and supplies was used as described below. TAG concentrations of CMRF were measured using a lipase glycerokinase method. Hydrodynamic di-
ameter of CMRF was measured by dynamic light scattering (ZetaSizer-NanoS, Malvern, UK; CV < 10%).

### Endotoxemia analysis

Endotoxemia in plasma and CMRF was determined using the Limulus Amebocyte Lysate assay in kinetic chromogenic conditions (Biögénic, Perols, France) (16). Extreme care was taken to avoid contamination with exogenous LPS by using single-use non pyrogenic supplies, ie, PS tubes (Becton Dickinson), Maxyum Recovery tubes (Axygen, VWR) and pyrogen-free pipet tips (Eppendorf, WVR). For all tests, standard curves presented a correlation coefficient of 0.99 and water was validated as pyrogen-free. In addition, to ensure that no inhibition/activation occurred, a spike of 0.05 EU/mL was added to each sample. Spike recovery was 132 ± 2% and 115 ± 4% for plasma and CMRF samples, respectively, which met standard quality criteria (50%–200%).

### LPS transporters, hsIL-6 and metabolite measurements in plasma

To measure plasma concentrations of circulating LBP and sCD14, plasma samples were assayed using sandwich ELISA kits (CliniSciences and R&D Systems, France) following the manufacturer’s instructions. Plasma hsIL-6 levels were measured using a sandwich Ultrasensitive ELISA kit (Invitrogen, France). Serum CRP concentrations were measured using an immunoturbidimetric method (AU2700 Beckman Coulter®, O’Cllagan’s Mills, Ireland). HDL cholesterol concentration in plasma was measured with a cholesterol esterase/oxidase method (AU2700 Beckman Coulter).

### Leukocytes isolation and nuclear translocation of NF-κB

Leukocytes were extracted from fresh whole blood samples at baseline and 300 minutes after breakfast. Whole blood was vigorously mixed in a hypotonic buffer to lyse red blood cells. Two successive washes with the same buffer were then made by centrifugation to remove the cellular fragments of red blood cells. The last wash was made with PBS and the last leukocyte fraction obtained after centrifugation was frozen immediately and kept at −80°C until analysis. Nuclear translocation of NF-κB was then assessed by measuring the free p65 subunit of NF-κB using a kit (ActivELISATM-Kit, Imgenex-CliniSciences, France) according to the manufacturer’s instructions.

### Whole blood RNA extraction and real-time PCR

Fresh blood was collected in PAXgene Blood RNA tubes (PreAnalytiX, Qiagen, France) at baseline and 300 minutes after breakfast. Total RNA was isolated using a PAXgene RNA-kit according to manufacturer’s instructions (Qiagen). Reverse transcription was performed on 250 ng total RNA. Real-time PCR assays were performed using a Rotor-Gene 6000 (Qiagen). Values were normalized to expression of the housekeeping gene hypoxanthine guanine phosphoribosyltransferase (HPRT).

### Circulating zonulin measurement

Serum zonulin concentrations were measured at baseline by zonulin ELISA Kit (K5600, Immundiagnostik AG, Bensheim, Germany), which detects the active (uncleaved) form.

### Statistical analysis

All data are presented as means ±SEM (n=8 per group) and were analyzed with Statview 5.0. We calculated that 8 subjects per group would provide an 80% power at P < .05 to detect a 40% difference in postprandial endotoxemia according to the two fat doses taking into account a coefficient of variation of 40%. The incremental areas under the curve (iAUC; area above baseline fasting value) were calculated by the conventional trap- ezoid rule from data during the different postprandial periods. Data normality was checked using the Kolmogorov-Smirnov test. Endotoxemia and chylomicron data followed a normal distribution. IL-6 data of normal-weight subjects were not normally distributed, therefore a log-transformation was used. Intra-comparisons between meals were performed using paired Student’s t test and intercomparisons between subject groups with unpaired Student’s t test. Statistical effects of BMI (P_{BMI}), meal (P_{meal}), time (P_{time}) and their interactions were evaluated on postprandial data of CMRF LPS and plasma IL-6 using analysis of variance (ANOVA) followed by post hoc Fisher PLSD. Chylomicron sizes were compared by ANOVA for repeated measures followed by Fisher PLSD. Differences were considered significant at the P < .05 level. P < .1 were considered as trends due to the limited number of subjects and discussed if necessary.

### Results

#### Baseline characteristics of the subjects

The baseline characteristics of the 16 study subjects are shown in Table 1. As expected, weight, BMI and waist circumference were higher in the obese than in NW participants. Furthermore, fasting HDL cholesterol concentration was lower in the obese group. None of the subjects were insulin resistant (HOMA-IR < 2.6 in both groups) nor dyslipidemic (TG < 2.3 mmol/L; total cholesterol < 5.2 mmol/L in both groups) nor did they present significant systemic inflammation (CRP < 5 mg/L in both groups). Obese subjects presented higher plasma concentrations than NW subjects regarding IL-6 and LPS transporters, LBP and sCD14, despite similar endotoxemia. Moreover, fasting serum zonulin concentration shows that subjects did not present intestinal permeability and obese did not differ from NW.

### Postprandial accumulation of chylomicrons and plasma LPS

Figure 1A-B shows that in both groups, postprandial accumulation of CMRF-TAG was higher during the breakfast digestion period when breakfast contained 40g vs 10g fat (P < .05). However, after lunch, the effect of fat amount ingested at breakfast was observed in obese subjects only (P < .01 40g vs 10g; P < .05 vs NW for 40g). Altogether, cumulated chylomicronemia over 8h was higher in both groups when fat amount in breakfast increased from 10 to 40g (P < .01). Notably, CMRF sizes differed between subject groups. Up to 300 minutes, av-
Average CMRF size increased after 40g- vs 10g-breakfast in NW subjects (Pmeal<0.05, Ptime<0.0001, Pmeal×time<0.05), e.g., mean hydrodynamic diameter being of 216 ± 46 nm at 120 minutes after 40g fat vs 84 ± 24nm after 10g. In obese subjects, CMRF were of similar size after both meals.

Figure 1C shows that in NW subjects, the postprandial iAUC of endotoxemia was similar regardless of fat

<table>
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<th>Table 1. Clinical characteristics of study subjects.</th>
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<td>sCD14 (µg/mL)</td>
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<td>CRP (mg/liter)</td>
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Data are means ± SEM. Groups are compared using unpaired Student’s t test.

sCD14: soluble cluster of differentiation 14; BMI: body mass index; CRP: C-reactive protein; HDL: high density lipoprotein; HOMA-IR: homeostasis model assessment-estimated insulin resistance; IL-6: interleukin-6; LBP: lipopolysaccharide-binding protein; LPS: lipopolysaccharide.

Figure 1. Postprandial incremental area under the curve (AUC) (iAUC) of CMRF TAG and iAUC of plasma LPS in NW (A, C respectively) and obese (B, D) subjects after consumption of 10g (white bars) and 40g (black bars) of spread fat at breakfast. Data are means ± SEM; n = 8 per group. A, B: ** P < .01 for 40g compared with 10g of spread fat (paired Student’s t test). B, D: * P < .05 for 40g compared with 10g of spread fat (paired Student’s t test). B: §P < .05 for obese compared with NW subjects regarding 40g of spread fat iAUC300–480 (unpaired Student’s t test). CMRF, chylomicron-rich fraction; iAUC, incremental AUC; LPS: lipopolysaccharide; NW: normal-weight; TAG, triacylglycerol.
amount. Obese subjects presented higher iAUC of endotoxemia over 8h after ingestion of 40g- vs 10g fat ($P < .05$, Figure 1D; $P = ns$ vs NW).

**Correlation between endotoxemia and chylomicronemia**

In NW subjects, there was no association between postprandial chylomicronemia and endotoxemia (Figure 2A). In obese subjects, a significant correlation was observed between postprandial chylomicronemia and endotoxemia during the breakfast digestion ($P = .0085$ and $r = 0.62$; Figure 2B). Therefore, the dynamics of LPS handling by chylomicrons was explored. In NW subjects, chylomicron enrichment with LPS was similar along breakfast digestion (Figure 3A), with only a transient tendency for increased enrichment at 180 minutes after 40g fat ($P_{\text{time} \times \text{meal}} < 0.01$; $P = .05$ at 180 minutes for 40g vs 10g; tendency with $P < .1$ for 40g at 180 minutes vs 60 minutes and vs 300 minutes). In obese subjects, a dynamic enrichment of chylomicrons with LPS occurred during the postprandial phase ($P_{\text{time}} < 0.01$, Figure 3B), with a trend towards a higher enrichment with 40g vs 10g fat ($P_{\text{meal}} < 0.1$). CMRF of obese subjects got more enriched with LPS at 180 minutes after both fat amounts ($P < .05$) and were still be more enriched at 300 minutes after 40g fat ($P < .05$). Chylomicrons of obese subjects were more enriched with LPS than those of NW in the postprandial phase ($P_{\text{BMI}} < 0.01$;

![Figure 2](image-url)

**Figure 2.** Correlations between incremental AUC (iAUC) of plasma LPS and iAUC of CMRF TAG after consumption of 10g (white bars) and 40g (black bars) of spread fat in NW subjects (A) and obese subjects (B), during the 0–300 minutes postprandial period. For each group, individual iAUC (A, B) after both 10g and 40g spread fat breakfasts are represented. Insert in (B) indicates the regression coefficient ($r$) and significance ($P$) of the correlation. CMRF, chylomicron-rich fraction; iAUC, incremental AUC; LPS: lipopolysaccharide; NW: normal-weight; TAG, triacylglycerol.

![Figure 3](image-url)

**Figure 3.** Postprandial variations of LPS in the CMRF (fold-change vs 60 minutes) in NW (A) and obese (B) subjects after consumption of 10g (white bars) and 40g (black bars) of fat at breakfast. A, *$P < .1$ for 40g vs 10g of spread fat at 180 minutes, $P_{\text{meal}} = ns$, $P_{\text{time}} < 0.1$ and $P_{\text{meal} \times \text{time}} < 0.01$ (ANOVA followed by post hoc Fisher PLSD). B, *$P < .05$ for time 180 minutes vs 60 minutes regarding 10g and 40g of spread fat and *$P < .05$ for time 300 minutes vs 60 minutes regarding 40g of spread fat (paired Student’s t test); *$P < .05$ for obese subjects compared with NW regarding (i) 10g of spread fat at 180 minutes and (ii) 40g of spread fat at 300 minutes (unpaired Student’s t test); $P_{\text{meal}} < 0.1$, $P_{\text{time}} < 0.01$ and $P_{\text{meal} \times \text{time}} = ns$ (ANOVA followed by post hoc Fisher PLSD). A–B, $P_{\text{BMI}} < 0.01$ (ANOVA followed by post hoc Fisher PLSD). CMRF, chylomicron-rich fraction; LPS: lipopolysaccharide; NW: normal-weight.
obese vs NW: \( P < .05 \) at 180 minutes after 10g and at 300 minutes after 40g).

**Postprandial inflammation and LPS-binding protein**

Figure 4A-B shows that the postprandial accumulation of plasma IL-6 was similar after 10g- and 40g-fat in both groups. However, regardless of fat amount, the postprandial accumulation of IL-6 was significantly higher in obese vs NW subjects \( (P_{BMI < 0.0001}) \). No correlation was observed between plasma endotoxemia or chylomicron-bound LPS and postprandial plasma IL-6. No translocation of nuclear NF-\( \kappa \)B was observed in extracted leukocytes after breakfasts in both groups (data not shown). IL-6 gene was only weakly expressed in circulating leukocytes regardless of meal and group (data not shown). A significant correlation was observed between postprandial accumulation of IL-6 during breakfast digestion and fasting concentration of LBP \( (P < .01, \text{Figure} \ 4C) \), which transports LPS in plasma and was higher in obese subjects \( (P = .01) \).

**Discussion**

This study is the first to examine the differential changes in postprandial endotoxemia according to both BMI and fat amount using realistic doses of 10 g and 40 g in the meal, similar to the subject usual consumption, rather than exaggerated fat loads. We reveal that in nonmorbid obese subjects \( (BMI = 32 \text{ kg.m}^{-2}) \), compared to NW, postprandial endotoxemia increases with fat amount in the meal proportionally to chylomicronemia. Up to date, only one study tested different fat amounts of 34 to 102 g fat (in 500 to 1500-kcal meals) and showed no difference in the dose-response of postprandial endotoxemia between normal-weight and insulin-resistant obese subjects \( (BMI = 39 \text{ kg.m}^{-2}) \) (17). We thus suggest that differences in LPS absorption may only be revealed in a physiological range of fat doses, as tested in the present study, which may not further be possible at higher doses. In altered metabolic conditions, ie, in insulin-resistant obese men (17), in morbidly obese patients (18) and in type 2 persons with diabetes (19), and using exaggerated high-fat loads (50 to 102 g), a correlation was observed between postprandial hypertriglyceridemia and endotoxemia. Our results ob-

![Figure 4](https://example.com/figure4.png)

**Figure 4.** Postprandial accumulation of plasma IL-6 (AUC) during the different postprandial periods (0–300 minutes; 300–480 minutes; total: 0–480 minutes; A, B) in normal-weight (A) and obese (B) subjects after 10g (white bars) vs 40g (black bars) fat-breakfast and postprandial IL-6 AUC correlation with fasting LBP (C). B, \( P_{BMI < 0.0001} \) regarding obese compared with normal-weight subjects after 10g of fat (unpaired Student’s \( t \) test). A–B, \( P_{BMI < 0.0001} \) (ANOVA followed by post hoc Fisher PLSD). Insert in (C) indicates the regression coefficient (\( r \)) and significance (\( P \)) of the correlation.
tained in insulin sensitive obese show that this phenomenon is an early one that can occur (i) before the onset of metabolic disorders and (ii) using realistic ingested fat amounts consistent with the subject dietary habits.

The presence of LPS and chylomicrons in plasma result from the difference between their intestinal absorption and their clearance. Here, we ruled out an intestinal permeability-induced paracellular passage of LPS because of the lack of differences in fasting endotoxemia and circulating zonulin between NW and obese subjects. Our results support the contribution of a greater transcellular intestinal absorption and/or decreased clearance in obese subjects. Indeed, cumulative endotoxemia in obese subjects was found correlated with cumulative chylomicronemia according to ingested fat amount. Moreover, the relative enrichment of chylomicrons with LPS along the postprandial phase was higher in obese that in NW subjects and tended to increase with fat amount. This might be explained by higher LPS content in gut lumen, considering that high-fat fed mice present increased fecal LPS content (20). In NW subjects, the absence of correlation between endotoxemia and chylomicronemia can partly be explained by the lack of enrichment of chylomicrons with LPS during the peak of lipidemia compared with early postprandial phase. It can also reveal a more efficient clearance in NW than obese subjects.

When LPS appear in plasma, they can be handled by different transporters to be cleared either (i) towards detoxification process, notably through LPS exchange with HDL mediated by sCD14 (21, 22) or (ii) towards inflammatory pathways through handling and transport by LBP and activation of macrophages (4, 22). Moreover, triglyceride-rich lipoproteins are reported to contribute to LPS detoxification by the liver (22, 23). In this respect, large chylomicrons are cleared more efficiently than small chylomicrons (24). Therefore, we cannot rule out that the larger chylomicrons after 40g vs 10g in NW subjects could contribute to an efficient/rapid clearance of chylomicrons and their LPS by the liver, contributing to a similar plasma endotoxemia regardless of ingested fat amount. In obese subjects, because chylomicrons did not vary in size, we propose that they could be less efficiently cleared; thus the chylomicron-bound LPS would remain longer in the bloodstream.

Vascular lipolysis of chylomicrons induces the release of polar lipids located at chylomicron surface and their exchange with other lipoproteins (4, 22). Chylomicron-bound LPS are located with these polar lipids due to their amphiphilic structure. Consequently, chylomicron lipolysis may also induce some LPS release contributing to the total plasma endotoxemia. Such free LPS are known to be rapidly handled by their specific transporter LBP. LBP-bound LPS can then (i) stimulate macrophages, and endothelial cells due to a combined action with sCD14, thereby producing numerous soluble mediators of inflammation, and (ii) be shuttled to HDL thanks to a facilitated transfer via sCD14-LBP complex (25), leading to a neutralization and clearance of LPS. The plasma LPS distribution appears thus to be crucial for their final catabolic pathway and was recently showed to be altered in type 2 diabetic patients (26). Here obese subjects presented both (i) lower HDL reverse transport illustrated by lower HDL-cholesterol concentration and (ii) higher LBP and sCD14 concentrations, thereby promoting an inflammatory fate for postprandial LPS.

Regardless of fat amount, the IL-6 postprandial response was correlated with LBP which is known to handle endotoxins towards inflammatory pathways. The relevance of plasma levels of proinflammatory cytokines and leukocyte markers is still under debate in the study of high-fat induced postprandial inflammation. Some human studies showed an increase of plasma IL-6 after consumption of lipid-rich mixed meals (17, 27–30). Importantly, such meals contained exaggerated fat loads (>50g) and these results remain controversial because no increase of plasma IL-6 was reported elsewhere despite a high ingested fat load (31–34). Therefore, more than meal lipid content, the subject metabolic status appears to be a major factor predicting the postprandial inflammatory response, strongly influenced by (i) excess weight (35), (ii) associated metabolic disorders including insulin resistance (27) and, (iii) in obese subjects, the low-grade inflammation state (36). Such baseline inflammation would be able to increase the amount of cytokines secreted in the postprandial phase (36). Our present results are consistent with the latter evidence regarding (i) no effect of fat amount on postprandial response of plasma IL-6 in both groups and (ii) BMI effect with increased postprandial accumulation of IL-6 in obese vs NW subjects.

In the present study, we observed neither activation of leukocytes through translocation of NF-κB transcription factor, nor modification of IL-6 gene expression in plasma leukocytes, regardless of BMI. In line with this, recent data suggest that sequential fat loads were unable to stimulate cytokine secretion from polymorphonuclear cells (37), which also represent the main population among leukocytes in our study. The higher circulating IL-6 in the present obese subjects should thus be mostly of adipose tissue origin. Consistently, individuals with metabolic syndrome exhibit exacerbated adipose tissue postprandial inflammatory responses, seemingly independent of the quality and quantity of dietary fat (38). Moreover, the high IL-6 of obese subjects can be driven by LBP, which has been recently shown to be secreted by adipose tissue and sus-
pected to have an essential role in inflammation- and obesity-associated adipose tissue dysfunction (39). LBP was also highlighted as an important contributor in the inflammatory response to overfeeding in NW and overweight men (40). Here we even observed a significant correlation between postprandial IL-6 and fasting LBP.

Of note, our observations are limited by the restricted number of subjects due to the cumbersome nature of postprandial explorations. However, the choice of a crossover design strengthened data validity. Besides, the practical importance of the present results must not be underrated because we used moderate fat amounts that were observed in subject habits. In previous studies, authors (i) recognized that very high-fat meals were used, roughly equivalent to the subject total daily intake of fat that were no physiological amounts of fat (19) or (ii) used such high-fat doses for a caloric challenge purpose (17). Moreover, the choice of studying nonmorbid and asymptomatic obese without metabolic endotoxemia constitutes a relevant model to (i) learn more about postprandial endotoxemia and (ii) better understand its role in the early events of the human visceral obesity pathophysiology.

Importantly, even if the present subjects showed postprandial endotoxemia, there was no fat meal-induced inflammation. However, the postprandial accumulation of IL-6 was significantly correlated with fasting LBP, leading to suspect the critical role of such LPS transporter in the pathophysiological importance of chronic postprandial inflammation. Moreover, the choice of studying nonmorbid and asymptomatic obese without metabolic endotoxemia constitutes a relevant model to (i) learn more about postprandial endotoxemia and (ii) better understand its role in the early events of the human visceral obesity pathophysiology.

Altogether, this study supports a deeper exploration of the pathophysiological importance of chronic postprandial endotoxemia. We showed specific postprandial dynamics and handling of LPS according to ingested fat amount, especially in obese subjects, which altogether can drive the inflammatory outcome in such individuals. This raises the question of whether specific nutritional strategies targeting the modulation of postprandial endotoxemia would be efficient in contributing to prevent metabolic inflammation and associated disorders in obese subjects.

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