Postprandial Endotoxemia Linked With Chylomicrons and Lipopolysaccharides Handling in Obese Versus 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 lipopolysaccharides (LPS) using nonphysiological high-fat tests.

Objective: This study aimed to determine whether different realistic fat amounts can modulate postprandial dynamics and handling of LPS by varying postprandial lipidemia in humans of different body mass indices.

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

Main outcome: The postprandial fatty acid handling after ingesting 40 g 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 10 or 40 g fat.

Results: Chylomicronemia increased in all subjects according to ingested fat amount ($P < .01$), but only obese had higher postprandial endotoxemia after 40 g ($P < .05$). Obese subject chylomicrons were more enriched with LPS compared with NW ($P_{BMI} < .01$). We observed neither NF-κB translocation, nor variation of IL-6 expression in leukocytes. In both groups, fat amount did not modify postprandial response of plasma IL-6. However, the area under the curve (AUC) of IL-6 in obese was higher than in NW ($P < .05$) parallel to higher fasting LPS-binding protein (LBP; $P < .05$). AUC of IL-6 was correlated with LBP ($P < .01$).

Conclusion: Postprandial endotoxemia is modulated by ingested fat amount in obese men. LPS handling in plasma through chylomicrons and LBP seems 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. (***J Clin Endocrinol Metab*** **100: 3427–3435, 2015)**

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Abbreviations: BMI, body mass index; CMRF, chylomicron-rich fraction; HDL, high-density lipoprotein; HOMA-IR, homeostasis model of assessment–insulin resistance; iAUC, incremental areas under the curve; LBP, lipopolysaccharide transporters; LPS, lipopolysaccharides; NF-κB, nuclear factor κB; NW, normal weight; PLSD, Protected Least Significant Difference; sCD14, Soluble Cluster of Differentiation 14; TAG, triacylglycerol.

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 with 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 seems 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 1) postprandial endotoxemia can be modified by the fat amount in the meal, namely 10 vs 40 g, in subjects of different body mass index (BMI) due to chylomicron transport; 2) differences in acute endotoxin absorption and handling would contribute to modulate postprandial systemic inflammation, notably proinflammatory cytokine IL-6 and translocation of nuclear factor κB (NF-κB) in plasma immune cells; and 3) 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 ClinicalTrials.gov (No. 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, eight of normal weight (NW) ($20 < \text{BMI} < 25$ kg/m^2) and eight obese ($30 < \text{BMI} < 35$ kg/m^2 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 NW and noninsulin-resistant obese subjects the postprandial responses to two mixed meals differing only by fat amount, 10 vs 40 g, using a cross-over design. The first dose of 10 g 1) could be of interest for dietary advice purpose, and 2) consistent with the mean lipid amount usually consumed at breakfast by the studied subjects in their daily life. The second dose of 40 g 1) is known to induce a significant postprandial lipid response while remaining within a nutritional range, and 2) represents the maximum fat amount ingested at breakfast by some of the subjects.

After 48 hours of specific dietary recommendations and an overnight fast, the subjects came to the Human Nutrition Research Center Rhône-Alpes (Lyon, France) and ingested 1) a test breakfast containing 10 or 40 g of anhydrous milk fat with bread and a glass of skim milk (282 kcal and 551 kcal, respectively) and 2) a standardized lunch 5 hours later providing 713 kcal (15). Metabolic explorations were divided in postprandial phases including a first period of 5 hours (0–300 min) post-breakfast, a second period of 3 hours post-lunch (300–480 min), and the entire exploration day (0–480 min). 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 suggested 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 isolation and characterization

The chylomicron-rich fraction (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. Triacylglycerol (TAG) concentrations of CMRF were measured using a lipase glycerokinase method. Hydrodynamic diameter of CMRF was measured by dynamic light scattering (ZetaSizer-NanoS; coefficient of variation $< 10\%$).

Endotoxemia analysis

Endotoxemia in plasma and CMRF was determined using the Limulus Amoebocyte Lysate assay in kinetic chromogenic conditions (Biogénic) (16). Extreme care was taken to avoid contamination with exogenous LPS by using single-use nonpyrogenic supplies, ie, PS tubes (Becton Dickinson), Maxymum Recovery tubes (Axygen, VWR) and pyrogen-free pipet tips (Eppendorf, VWR). 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 $132 \pm 2\%$ and $115 \pm 4\%$ 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) following the manufacturer's instructions. Plasma hsIL-6 levels were measured using a sandwich Ultrasensitive ELISA kit (Invitrogen). Serum CRP concentrations were measured using an immunoturbidimetric method (AU2700 Beckman Coulter). 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) 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) 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.

Circulating zonulin measurement

Serum zonulin concentrations were measured at baseline by zonulin ELISA Kit (K5600, Immundiagnostik AG), 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 eight 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 trapezoid 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 *t* test and inter-comparisons between subject groups with unpaired Student *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 ANOVA followed by post-hoc Fisher Protected Least Significant Difference (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 (homeostasis model of assessment–insulin resistance [HOMA-IR] $<$ 2.6 in both groups) nor dyslipidemic (TAG < 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 1, A and B show that in both groups, postprandial accumulation of CMRF-TAG was higher during the breakfast digestion period when breakfast contained 40 vs 10 g fat ($P <$ .05). However, after lunch, the effect of fat amount ingested at breakfast was observed in obese subjects only ($P < .01$, 40 g vs 10 g; $P < .05$ vs NW for 40 g). Altogether, cumulated chylomicronemia over 8 hours was higher in both groups when fat

Table 1. Clinical Characteristics of Study Subjects

	Normal Weight	Obese	*P* Value
n	8	8	
Age, y	29 ± 1	31 ± 2	.426
Anthropometric parameters			
Body weight, kg	72.5 ± 2.1	101.1 ± 2.1	<.001
BMI, kg · m^{-2}	22.4 ± 0.5	31.8 ± 0.3	<.001
Waist circumference, cm	83.6 ± 1.7	105.6 ± 0.8	<.001
Fasting metabolic parameters			
HOMA-IR	0.90 ± 0.14	1.75 ± 0.25	.015
Triglycerides, mmol/L	0.86 ± 0.06	1.46 ± 0.18	.015
Total cholesterol, mmol/L	4.90 ± 0.23	5.04 ± 0.21	.688
HDL cholesterol, mmol/L	1.53 ± 0.11	1.09 ± 0.06	.007
LPS, EU/mL	0.19 ± 0.05	0.18 ± 0.04	.729
LBP, μg/mL	9.24 ± 1.61	13.94 ± 1.75	.001
sCD14, μg/mL	1.19 ± 0.12	1.48 ± 0.20	.032
IL-6, pg/mL	0.18 ± 0.04	0.45 ± 0.16	.006
CRP, mg/L	1.96 ± 0.01	2.98 ± 0.47	.036
Zonulin, ng/mL	18.3 ± 0.16	14.8 ± 1.6	.189

Data are means ± SEM. Groups are compared using unpaired Student *t* test.

amount in breakfast increased from 10 to 40 g ($P < .01$). Notably, CMRF sizes differed between subject groups. Up to 300 minutes, average CMRF size increased after 40- vs 10-g breakfast in NW subjects ($P_{meal} < .05$; $P_{time} < .0001$; $P_{meal \times time} < .05$), eg, mean hydrodynamic diameter being of 216 ± 46 nm at 120 minutes after 40 g fat vs 84 ± 24 nm after 10 g. 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 amount. Obese subjects presented higher iAUC of endotoxemia over 8 hours after ingestion of 40 vs 10 g 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

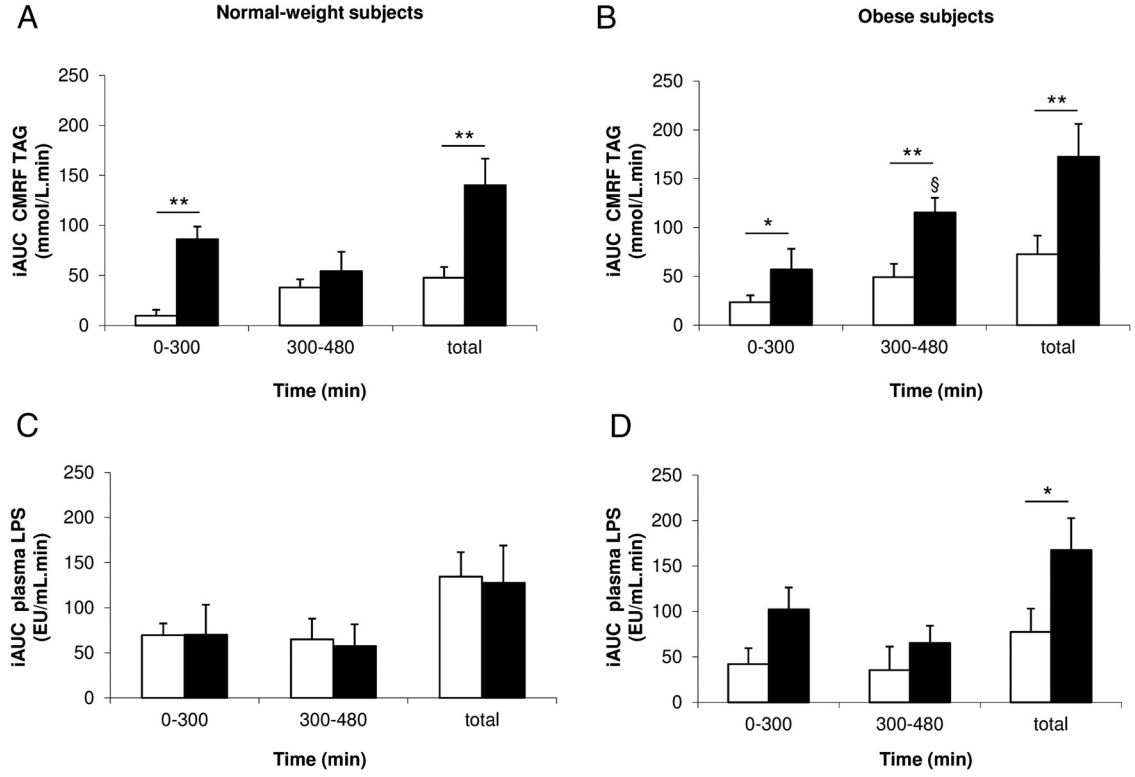

Figure 1. Postprandial iAUC of CMRF TAG and iAUC of plasma LPS in NW (A and C, respectively) and obese (B and D) subjects after consumption of 10 g (white bars) and 40 g (black bars) of spread fat at breakfast. Data are means ± SEM; n = 8 per group. A and B, ** , $P < .01$ for 40 g compared with 10 g of spread fat (paired Student *t* test). B and D, *, $P < .05$ for 40 g compared with 10 g of spread fat (paired Student *t* test). B, §, $P < .05$ for obese compared with NW subjects regarding 40 g of spread fat iAUC, 300–480 min (unpaired Student *t* test).

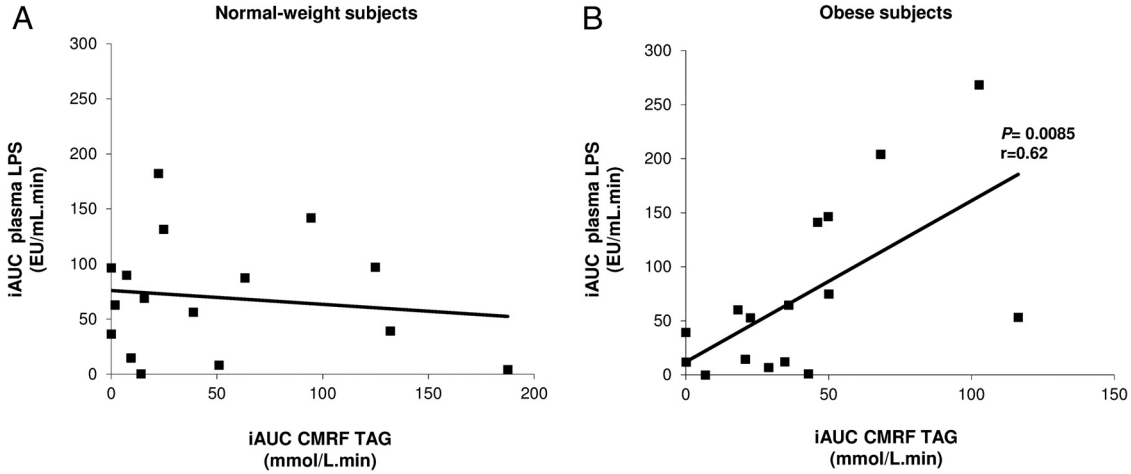

Figure 2. Correlations between iAUC of plasma LPS and iAUC of CMRF TAG after consumption of 10 g (white bars) and 40 g (black bars) of spread fat in NW subjects (A) and obese subjects (B), during the 0–300-min postprandial period. For each group, individual iAUC (A and B) after both 10-g and 40-g spread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

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 40 g fat ($P_{time \times meal} < .01$; $P = .05$ at 180 min for 40 vs 10 g; tendency with $P < .1$ for 40 g at 180 vs 60 min and vs 300 min). In obese subjects, a dynamic enrichment of chylomicrons with LPS occurred during the postprandial phase ($P_{time} < .01$, Figure 3B), with a trend toward a higher enrichment with 40 vs 10 g fat ($P_{meal} < .1$). 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 40 g fat ($P < .05$). Chylomicrons of obese subjects were more enriched with LPS than those of NW in the postprandial phase ($P_{BMI} < .01$; obese vs NW: $P < .05$ at 180 min after 10 g and at 300 min after 40 g).

Postprandial inflammation and LPS-binding protein

Figure 4, A and B shows that the postprandial accumulation of plasma IL-6 was similar after 10 and 40 g fat in both groups. However, regardless of fat amount, the postprandial accumulation of IL-6 was significantly higher in obese vs NW subjects ($P_{BMI} < .0001$). No correlation was observed between plasma endotoxemia or chylomicron-bound LPS and postprandial plasma IL-6. No translocation of nuclear NF-κB 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 sig-

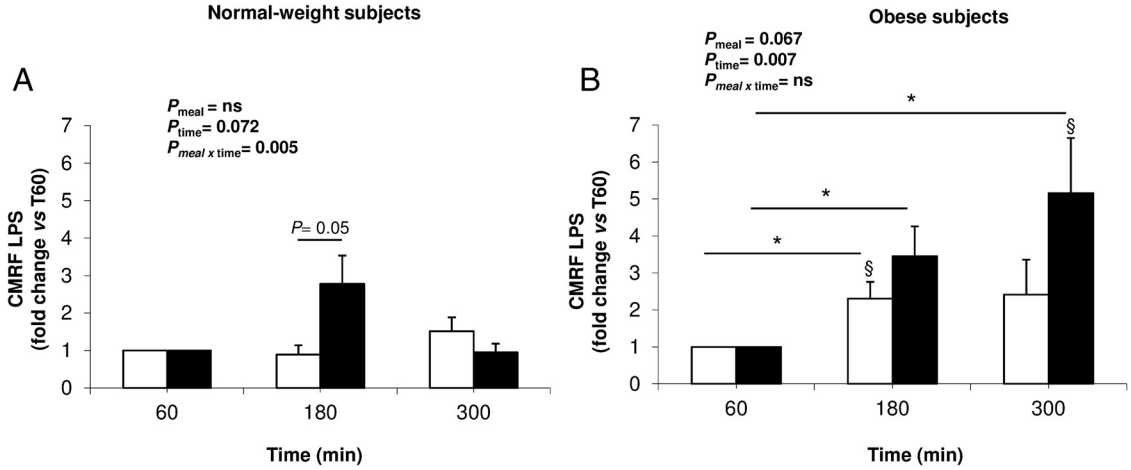

Figure 3. Postprandial variations of LPS in the CMRF (fold-change vs 60 minutes) in NW (A) and obese (B) subjects after consumption of 10 g (white bars) and 40 g (black bars) of fat at breakfast. A, $P = .05$ for 40 g vs 10 g of spread fat at 180 minutes; P_{meal} = ns, $P_{time} < .1$, and $P_{meal \times time} < .01$ (ANOVA followed by post-hoc Fisher PLSD). B, *, $P < .05$ for time 180 vs 60 min regarding 10 and 40 g of spread fat; and *, $P < .05$ for time 300 vs 60 min regarding 40 g of spread fat (paired Student *t* test); §, $P < .05$ for obese subjects compared with NW regarding 1) 10 g of spread fat at 180 min, and 2) 40 g of spread fat at 300 min (unpaired Student *t* test); $P_{meal} < .1$, $P_{time} < .01$, and $P_{meal \times time}$ = ns (ANOVA followed by post-hoc Fisher PLSD). A and B, $P_{BMI} < .01$ (ANOVA followed by post-hoc Fisher PLSD).

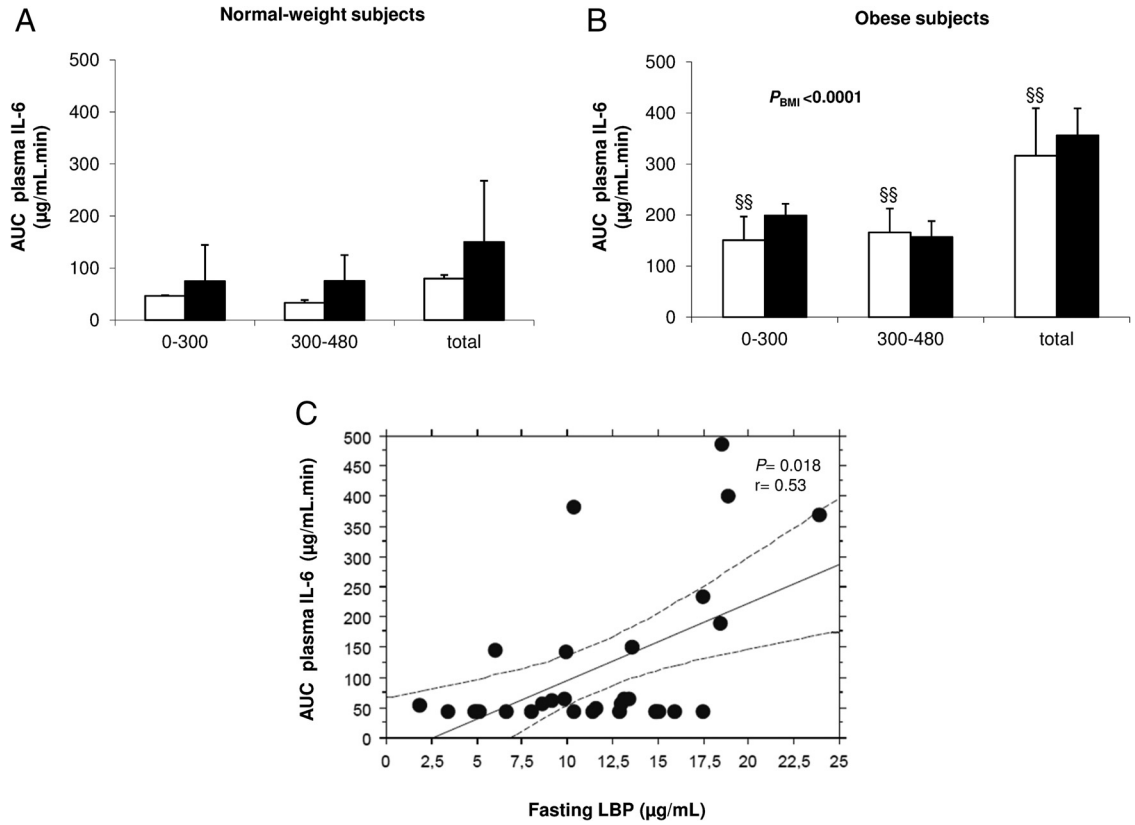

Figure 4. Postprandial accumulation of plasma IL-6 (AUC) during the different postprandial periods (0–300 min, 300–480 min, and total: 0–480 min; A, B) in normal-weight (A) and obese (B) subjects after 10 g (white bars) vs 40 g (black bars) fat-breakfast and postprandial IL-6 AUC correlation with fasting LBP (C). B, §§, $P < .01$ regarding obese compared with normal-weight subjects after 10 g of fat (unpaired Student *t* test). A and B, $P_{BMI} < .0001$ (ANOVA followed by post-hoc Fisher PLSD). Insert in (C) indicates the regression coefficient (*r*) and significance (*P*) of the correlation.

nificant correlation was observed between postprandial accumulation of IL-6 during breakfast digestion and fasting concentration of LBP ($P < .01$, 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 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 kg·m^{-2}), compared with NW, postprandial endotoxemia increases with fat amount in the meal proportionally to chylomicronemia. Up to date, only one study tested different fat amounts of 34–102 g fat (in 500–1500-kcal meals) and showed no difference in the dose-response of postprandial endotoxemia between normal-weight and insulin-resistant obese subjects (BMI = 39 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 persons with type 2 diabetes (19), and using exaggerated high-fat loads (50–102 g), a correlation was observed between postprandial hypertriglyceridemia and endotoxemia. Our results obtained in insulin sensitive obese show that this phenomenon is an early one that can occur 1) before the onset of metabolic disorders, and 2) 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 lipidmia compared with early postprandial phase. It can also reveal a more efficient clearance in NW than obese subjects.

When LPS seem in plasma, they can be handled by different transporters to be cleared either 1) toward detoxification process, notably through LPS exchange with HDL mediated by sCD14 (21, 22); or 2) toward 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 40 vs 10 g 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 1) stimulate macrophages, and endothelial cells due to a combined action with sCD14, thereby producing numerous soluble mediators of inflammation; and 2) 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 seems thus to be crucial for their final catabolic pathway and was recently showed to be altered in patients with type 2 diabetes (26). Here obese subjects presented both 1) lower HDL reverse transport illustrated by lower HDL-cholesterol concentration, and 2) 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 toward 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 (> 50 g) 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 seems to be a major factor predicting the postprandial inflammatory response, strongly influenced by 1) excess weight (35), 2) associated metabolic disorders including insulin resistance (27), and, 3) 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 1) no effect of fat amount on postprandial response of plasma IL-6 in both groups, and 2) 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 suspected 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 1) 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 2) 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 1) learn more about postprandial endotoxemia, and 2) 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 onset of low-grade inflammation, especially for high cardio-metabolic risk individuals.

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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