See corresponding editorial on page 1257.

A high-fat meal induces low-grade endotoxemia: evidence of a novel mechanism of postprandial inflammation[1–3]

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ABSTRACT

Background: Bacterial endotoxin is a potently inflammatory antigen that is abundant in the human gut. Endotoxin circulates at low concentrations in the blood of all healthy individuals, although elevated concentrations are associated with an increased risk of atherosclerosis.

Objective: We sought to determine whether a high-fat meal or smoking increases plasma endotoxin concentrations and whether such concentrations are of physiologic relevance.

Design: Plasma endotoxin and endotoxin neutralization capacity were measured for 4 h in 12 healthy men after no meal, 3 cigarettes, a high-fat meal, or a high-fat meal with 3 cigarettes by using the limulus assay.

Results: Baseline endotoxin concentrations were 8.2 pg/mL (interquartile range: 3.4–13.5 pg/mL) but increased significantly ($P < 0.05$) by ≈50% after a high-fat meal or after a high-fat meal with cigarettes but not after no meal or cigarettes alone. These results were validated by the observations that a high-fat meal with or without cigarettes, but not no meal or smoking, also significantly ($P < 0.05$) reduced plasma endotoxin neutralization capacity, which is an indirect measure of endotoxin exposure. Human monocytes, but not aortic endothelial cells, were responsive to transient (30 s) or low-dose (10 pg/mL) exposure to endotoxin. However, plasma from whole blood treated with as little as 10 pg endotoxin/mL increased the endothelial cell expression of E-selectin, at least partly via tumor necrosis factor-α–induced cellular activation.

Conclusions: Low-grade endotoxemia may contribute to the postprandial inflammatory state and could represent a novel potential contributor to endothelial activation and the development of atherosclerosis. *Am J Clin Nutr* 2007;86:1286–92.

KEY WORDS Atherosclerosis, endotoxin, inflammation, diet, postprandial lipemia, smoking

INTRODUCTION

The agents responsible for initiating and maintaining the chronic inflammatory processes of atherosclerosis remain to be clearly identified (1). Recently, postprandial lipemia has emerged as a potential candidate following the discovery that ingestion of a high-fat meal causes systemic increases of a wide range of inflammatory mediators (2–5). For example, after a high-fat meal, circulating leukocytes express the activated form of nuclear transcription factor-κB (NF-κB; 2, 3), and up-regulate several markers of leukocyte activation, such as CD11A, CD11B, and CD62L (4). Plasma interleukin-8 and neutrophil counts also increase after a high-fat meal but not after a water challenge (5, 6), as do markers of endothelial cell activation, such as soluble intercellular adhesion molecule 1 and vascular adhesion molecule 1 and the atherogenic cytokine tumor necrosis factor-α (TNF-α), after a high-fat meal but not carbohydrate loading (7).

To date, the cause of these postprandial inflammatory events remains poorly understood. One potential candidate factor that has not been investigated previously in this context is bacterial endotoxin [lipopolysaccharide (LPS)], a potently inflammatory bacterial antigen that is present in large quantities in the human gut (8). Endotoxin circulates in the plasma of healthy human subjects at low concentrations (between 1 and 200 pg/mL; 9–13), yet it is increasingly considered to play a proatherogenic role (reviewed in reference 14). Elevated concentrations of circulating LPS correlate well with an increased atherosclerosis risk (9), whereas in vitro studies have shown LPS to potently up-regulate atherogenic gene expression (15), cholesterol retention, and foam cell formation (16). Moreover, LPS injection accelerates the formation of plaque in both mice (17) and rabbits (18), whereas genetic deletion of the LPS receptor Toll-like receptor 4 significantly reduces the development of plaque in apolipoprotein E–deficient mice (19).

Because the human gut is host to ≈100 trillion commensal organisms, which together contribute to an enteric reservoir of ≥1 g LPS (8), we hypothesized that most of the circulating endotoxin may derive from the gut and that a small amount of commensally derived LPS may cotransit with dietary fat from the gut after a high-fat meal, which thereby increases plasma endotoxin concentrations postprandially. As a secondary hypothesis, we considered that smoking may also contribute directly to circulating endotoxin via the absorption of smoke-derived LPS in the lung, because cigarettes contain up to 18 μg endotoxin, ≈1% of which survives combustion as inhaled bioactive LPS (11).

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Using an interventional approach, we examined plasma endotoxin exposure both directly and indirectly for 4 h after treatment in 12 healthy men after no meal, 3 cigarettes, a high-fat meal, or a high-fat meal with 3 cigarettes. In vitro experiments were then performed to establish the potential mechanisms by which endothelial cells may be activated by exposure to low or transient doses of endotoxin.

SUBJECTS AND METHODS

Subjects

Twelve healthy men who were self-identified as occasional smokers were recruited by advertisement [mean age: 32 y (range: 20–58 y); mean body mass index (in kg/m^2) of 23 (range: 19–31)]. They were asked to abstain from eating or smoking overnight before the study and were excluded if evidence of an infection within the past month was identified [ie, C-reactive protein (CRP) concentrations >5 mg/dL]. Baseline measurements of differential blood count and CRP and triacylglycerol concentrations were made. On 4 separate visits in a randomized order, with ≈1 wk between visits, each individual received *1*) no meal, *2*) a high-fat meal, *3*) no meal and 3 cigarettes, or *4*) a high-fat meal and 3 cigarettes. The high-fat meal, which provided ≈900 kcal, consisted of a cup of tea and 3 slices of toast spread with a total of 50 g butter, which contained <3.5 ng endotoxin/g as measured by limulus assay. For measurement of plasma LPS, 4-mL venous blood samples were obtained from each subject at the following time points: baseline × 2 (−15 and −5 min) and 30, 60, 90, 120, 180, and 240 min after treatment. Additional blood samples were obtained at the 2- and 4-h time points to measure triacylglycerol and CRP. Plasma was collected by centrifugation (2000 × *g*, 5 min, 4 °C) and stored at −70 °C before batch analysis of plasma endotoxin content and endotoxin neutralization capacity (ENC). The protocol of the present study was approved by the Lothian Research Council Ethical Committee (LREC 2004/3/11), and written informed consent was obtained from all subjects.

Limulus amoebocyte lysate assays

Plasma endotoxin content was determined by using the limulus amoebocyte lysate (LAL) assay. Briefly, this assay uses an invertebrate-derived enzyme system that, in the presence of endotoxin, catalyzes reactions that have been adapted to form a microtiter plate–based chromogenic assay (9). Because contamination of the LAL assay with environmental endotoxin is a common cause of experimental error, exhaustive care was taken to avoid endotoxin contamination of any solution or vessel. Pyrogen-free pipette tips (Starlab, Milton Keynes, United Kingdom), water (Cambrex, East Rutherford, NJ), and pharmaceutical-grade heparin (Leo Laboratories, Aylesbury, United Kingdom) were used for all experiments. For direct measurement of plasma endotoxin concentration, defrosted plasma was diluted 1:10 in pyrogen-free water and then heated at 70 °C for 10 min to inactivate endotoxin-neutralizing agents that are present in human plasma and that otherwise inhibit the activity of endotoxin in the LAL assay. Fifty microliters of heat-inactivated plasma was combined with 50 μL LAL reagent (Pyrochrome; Quadratech, Epsom, United Kingdom) in duplicate in pyrogen-free plates (Greiner, Stonehouse, United Kingdom) and incubated at 37 °C for ≈1.5 h before the absorbance was read at 405 nm. Plasma endotoxin concentrations were estimated from a standard curve prepared from kit-supplied *Escherichia coli* O111 endotoxin standard in the same plate. Endotoxin units (EUs) were converted to picograms per milliliter equivalent *E. coli* R1 LPS by multiplying EUs/mL by 228, because *E. coli* R1 LPS was found to contain 228 pg/EU. This conversion allowed comparison of the present study with previous studies that also reported plasma endotoxin as *E. coli* LPS equivalent concentrations (9–13). The limit of sensitivity of the assay was 0.7 pg/mL.

Endotoxin neutralization capacity assays

Human plasma contains several substances that, if not heat-treated, neutralize the activity of endotoxin in the LAL assay. Numerous studies have shown that these substances are depleted after endotoxin exposure (12, 20, 21). Thus, measurement of ENC of the plasma samples may show prior exposure to endotoxin, which would be invisible to intermittent monitoring. Notably, an increase in recovered endotoxin in this assay reflects a reduction in the ability of plasma to neutralize endotoxin in the LAL assay (ENC) and, thereby, prior exposure to endotoxin. To measure the ENC of the plasma samples obtained at the −15-, 120-, and 240-min time points, 10 μL of a 200 ng/mL stock endotoxin solution was added to 90 μL non–heat-treated plasma and then incubated for 1 h at 37 °C. Nine-hundred microliters of 0.85% NaCl in pyrogen-free water was then added and vortexed briefly; 50 μL of this mixture was assessed in triplicate for the amount of recovered endotoxin by LAL assay as described above, except that the incubation time was reduced to 20 min. Values for the 120- and 240-min time point samples were normalized to a percentage of the absorbance (measured at 405 nm) of baseline plasma samples obtained at −15 min for each visit. All LAL and ENC assays were performed by a researcher blinded to the randomized treatment.

Human primary aortic endothelial cell, monocyte, and whole-blood in vitro experiments

Human primary aortic endothelial cells (HAECs; Cascade Biologics, Portland, OR) were cultured between passages 3 and 7 in M200 medium according to the supplier's recommendations. To determine the responsiveness of the HAECs to endotoxin, cells were plated in 96-well plates at 10 000 cells/well 24 h before incubation with 10–10 000 pg standard *E. coli* R1 endotoxin/mL (strain NCTC 13114) for 18 h or with 2 ng endotoxin/mL for indicated times before replacement with medium alone for the remainder of the 18 h for the time course studies. Supernatant interleukin-8 (IL-8) was measured by use of enzyme-linked immunosorbent assay (ELISA; R&D Systems, Minneapolis, MN). Human monocytes were separated and plated as described previously (22) and incubated with LPS as for HAECs, except that supernatant TNF-α was measured at 3.5 h. Whole heparinized blood (1 mL), untreated or adjusted to 10–1000 pg endotoxin/mL, was incubated at 37 °C for 6 h before plasma was harvested by use of centrifugation (13 000 × *g*, 10 min, room temperature). Confluent HAEC monolayers in 96-well plates were then exposed to 50 μL harvested plasma alone or supplemented with 10 μg anti-TNF-α (MAb1; eBioscience, San Diego, CA) or to isotype-matched control antibody (TLR3.7; Hycult Biotechnology, Uden, Netherlands) or to 100 pg LPS/mL for 4 h before measurement of cell-surface expression of E-selectin by ELISA as described previously (23).

Statistical analyses

Data were analyzed by use of GRAPHPAD PRISM software (version 4.02; GraphPad Software Inc, San Diego, CA) or MINITAB software (version 14; Minitab Ltd, Coventry, United Kingdom). ENC and inflammatory mediators were compared by use of analysis of variance (ANOVA) followed by Tukey's or Dunnett's test. Direct measurements of plasma endotoxin concentrations were subjected to log transformation before comparison with parametric ANOVA (Tukey's test). For ENC and transformed endotoxin values, 2-factor ANOVA was also performed to examine the contribution of different factors to the endotoxin response. Differences were significant at $P < 0.05$.

RESULTS

Limulus amoebocyte lysate assay measurements of plasma endotoxin concentrations

Baseline endotoxin concentrations in all the volunteers were very low, ranging between ≈1 and 9 pg/mL. However, transient increases in plasma endotoxin concentrations were observed in all individuals. These increases were not due to plate contamination or to experimental error at the stage of assay, because repeat measurements of the same plasma samples were made on separate occasions, which confirmed the accuracy of these measurements (**Figure 1**A). Elevations from baseline were typically modest, only rarely exceeding 80 pg/mL (Figure 1B), and were transient, occasionally returning to baseline in as little as 10 min.

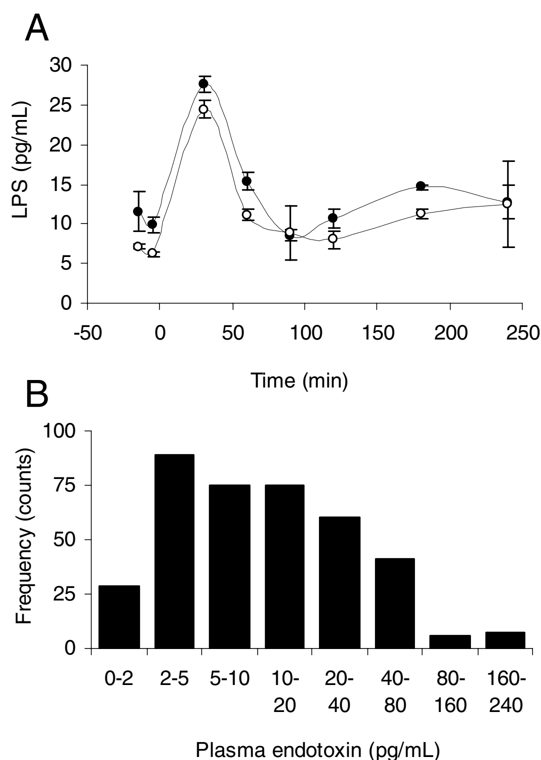

FIGURE 1. Reproducibility and distribution of plasma endotoxin measurements. A: Mean (±SD) lipopolysaccharide (LPS) concentrations in plasma samples obtained from a single volunteer at time points ranging from 15 min before to 240 min after a high-fat meal and cigarette smoking determined in 2 independent limulus amoebocyte lysate assays (○ and ●; results of triplicate wells). The data represent 3 similar experiments. B: Distribution of endotoxin concentrations measured in all 384 plasma samples tested during the study; $n = 12$ (32 measurements per subject).

TABLE 1
Plasma endotoxin concentrations before and after the test meals[1]

	Plasma endotoxin
	pg/mL
Before meal	8.2 (3.4–13.5)
After no meal	8.2 (3.3–20.7)
After high-fat meal	12.3 (4.7–26.3)[2]
After cigarettes	10.3 (3.4–26.4)
After high-fat meal and cigarettes	12.6 (5.7–24.5)[2]

[1] All values are medians; interquartile ranges in parentheses; $n = 12$ healthy men. Plasma endotoxin concentrations were measured by use of direct limulus amoebocyte lysate assay before (−15 and −5 min) and after (30, 60, 90, 120, 180, and 240 min) no meal, 3 cigarettes, a high-fat meal, or a high-fat meal and 3 cigarettes. Two-factor ANOVA of $\log_{10}$-transformed endotoxin concentrations was performed: effect of fat, $P = 0.014$; effect of cigarettes, $P = 0.424$; cigarettes × fat interaction, $P = 0.742$.

[2] Significantly different from before meal, $P < 0.05$ (ANOVA followed by Tukey's test by using $\log_{10}$-transformed endotoxin concentrations).

The fluctuating nature of plasma endotoxin concentrations, together with the variability of baseline between individuals, interfered with a robust analysis of mean plasma endotoxin values at individual time points. However, when endotoxin concentrations measured at all time points before meals (median: 8.2 pg/mL) were compared with all time points after meals, plasma endotoxin was significantly higher after a high-fat meal (12.3 pg/mL) or after a high-fat meal with cigarettes (12.6 pg/mL) but was not significantly increased after cigarettes alone (10.3 pg/mL; **Table 1**). Two-factor ANOVA confirmed a significant effect of fat, but not of cigarettes, on log-transformed endotoxin concentrations (Table 1).

Measurements of endotoxin neutralization capacity

Given the fluctuating nature of plasma endotoxin, it was realized that direct measurement at distinct time points was not likely to provide an accurate measure of total endotoxin exposure. For this reason, we aimed to validate the results of the direct LAL assays by using a more robust measure of prior endotoxin exposure, the ENC assay (12, 20, 21). Overall, recovered endotoxin increased on average 18% after the high-fat meal and 12% after the high-fat meal with cigarettes ($P < 0.05$ for both), which indicates a significant reduction in ENC (**Figure 2**). In contrast, mean ENC at 240 min was not significantly different from ENC at −15 min after no meal or cigarettes only. A factorial analysis also showed a significant effect of meal ($P < 0.001$) and meal-by-time interaction ($P < 0.01$) but not of cigarettes or time alone, on ENC (Figure 2). Mean triacylglycerol concentrations did not increase after smoking or no meal but increased from 1.0 to 1.8-1.9 mmol/L 4 h after a high-fat meal or a high-fat meal with smoking, respectively (**Table 2**). Consistent with our hypothesis that gut bacteria–derived endotoxin may cotransit with dietary fat, recovered endotoxin in the ENC assay correlated positively with postprandial triacylglycerol concentrations ($P < 0.05$).

Endothelial cell and monocyte activation assays

Plasma CRP ($\bar{x}$: 0.6 mg/dL; range: 0.1–2.0 mg/dL at baseline) did not increase significantly within 4 h after any of the treatments (data not shown). Because the inflammatory marker

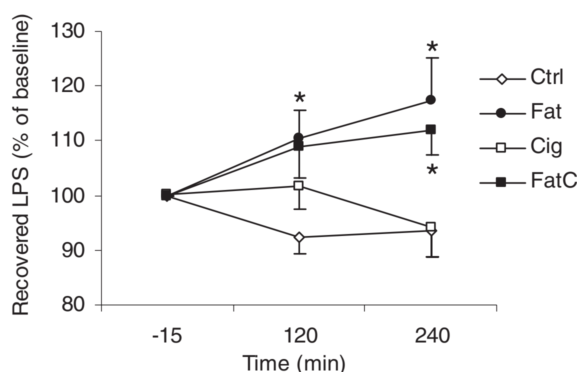

FIGURE 2. Mean (± SEM) postprandial endotoxin neutralization capacity measured as recovered lipopolysaccharide (LPS). Plasma samples were obtained from 12 healthy men at −15-, 120-, and 240-min time points after no treatment [control (Ctrl)], a high-fat meal (Fat), 3 cigarettes (Cig), or a high-fat meal and cigarettes together (FatC). Samples were then assessed for their ability to neutralize endotoxin activity in the limulus amoebocyte lysate (LAL) assay as a measurement of prior exposure to endotoxin. Non–heat-treated plasma was incubated with 200 ng endotoxin/mL for 1 h before measurement of the amount of recovered endotoxin by LAL assay. Absorbances were normalized to a percentage of the absorbance (at 405 nm) of the baseline plasma samples obtained before the test meals. An increase in recovered endotoxin in this assay reflects a reduction in endotoxin neutralization capacity, which indicates increased prior endotoxin exposure. Balanced ANOVA: effect of fat, $P < 0.001$; effect of cigarettes, $P = 0.848$; effect of time, $P = 0.319$; meal × time interaction, $P = 0.003$. *Significantly different from baseline, $P < 0.05$ (Tukey's test).

TNF-α was also below the limit of detection in the plasma samples (data not shown), we sought in vitro evidence of mechanisms by which low concentrations of endotoxin may stimulate monocyte or endothelial cell activation over a longer time course. HAECs were cultured with 10–10 000 pg/mL *E. coli* LPS, and IL-8 release was measured as an indicator of HAEC activation. HAECs were responsive to endotoxin concentrations ≥100 pg/mL (**Figure 3**A). However, because we found that endotoxin in this concentration range occurs infrequently and transiently, we investigated whether brief exposure to endotoxin could activate HAECs. Even incubation of cells with 2 ng LPS/mL for up to 30 min was not sufficient to activate HAECs, whereas overnight incubation led to a 4-fold increase in IL-8 production (Figure 3B). In contrast, human monocytes were found to be responsive to as little as 10 pg LPS/mL (Figure 3C). Moreover, transient treatment of monocytes with LPS led to the significant release of TNF-α, which was observed after as little as 2 s LPS exposure before being washed (Figure 3D).

Whole-blood stimulation assays

To determine whether activation of whole blood could lead to endothelial cell activation indirectly, HAECs were cultured in conditioned plasma collected from human whole blood exposed to very low concentrations of endotoxin for 6 h. Although the plasma samples from the clinical study collected at various time points did not induce HAEC E-selectin expression (data not shown), E-selectin expression increased significantly in response to plasma from whole blood incubated with as little as 10 pg/mL LPS for 6 h (**Figure 4**A). Induction of E-selectin was found to at least partly depend on the presence of TNF-α in the conditioned plasma, because the antibody-mediated blockade of TNF-α reduced the expression of E-selectin by ≈35% (Figure 4B).

DISCUSSION

Bacterial endotoxin is increasingly being considered as a potential inflammatory mediator of atherosclerosis (14, 17, 18) and has emerged as an independent predictor of atherosclerosis risk (9), although the mechanisms for increased endotoxin in the plasma of some healthy individuals remain unknown. We found that plasma endotoxin concentrations fluctuate rapidly in healthy subjects, from a very low concentration at baseline (≈1–9 pg/mL) to concentrations that in vitro experiments suggest may be sufficient to induce some degree of cellular activation (Figures 3 and 4). The present study appears to be the first to examine the kinetics of baseline endotoxin concentrations in healthy human subjects, although our observation that endotoxin is detectable in the blood of all healthy subjects is consistent with many previous studies that also reported endotoxin concentrations in the range of 1–200 pg/mL (9–13). Moreover, our observation of transient increases in plasma endotoxin is also consistent with the long-held understanding that endotoxemia in septic patients is transient in nature and is easily missed by intermittent sampling protocols (a problem that led to the development of assays such as ENC; 24). The equally rapid clearance of elevated endotoxin concentrations that we observed in the present study is also consistent with the very short half-life of circulating endotoxin (≈5 min) determined experimentally in human subjects (25).

We found no evidence of systemic absorption of endotoxin from cigarette smoke, which suggests that the lungs are an efficient barrier to the absorption of environmental LPS into the circulation. This finding confirms the results observed in guinea pigs that were exposed experimentally to endotoxin inhalation, which also absorb very little LPS systemically (26). In contrast, we found both direct and indirect evidence that plasma endotoxin increases significantly after a high-fat meal. Notably, because the ENC assay uses very high concentrations of endotoxin (200 ng/mL), it is much less likely than direct LAL assay to be affected by accidental contamination and therefore provides much more robust evidence of endotoxin exposure.

To our knowledge, this is the first time that these observations have been made in human subjects, although increased plasma endotoxin exposure, as measured both directly and by ENC, has been observed after colonoscopy (12), major surgery (20), extreme physical exertion (27), and acute exposure to large amounts of alcohol (which disrupts intestinal barrier function) (20, 28). Thus, the notion of endotoxin release from the gut into

TABLE 2
Triacylglycerol concentrations before and after the test meals[1]

	Triacylglycerol		
	Before meal	2 h after meal	4 h after meal
		mmol/L	
No meal	1.02 ± 0.15	0.90 ± 0.15	0.99 ± 0.16
Cigarettes only	1.01 ± 0.21	0.98 ± 0.20	0.97 ± 0.20
High-fat meal	0.92 ± 0.19	1.51 ± 0.22[2]	1.77 ± 0.29[2]
High-fat meal and cigarettes	0.97 ± 0.12	1.66 ± 0.24[2]	1.91 ± 0.28[2]

[1] All values are $\bar{x}$ ± SEM; $n = 12$. Balanced ANOVA: effect of fat, $P < 0.001$; effect of cigarettes, $P = 0.663$; effect of time, $P = 0.011$; meal × time interaction, $P = 0.006$.

[2] Significantly different from before meal, $P < 0.05$ (ANOVA followed by Tukey's test).

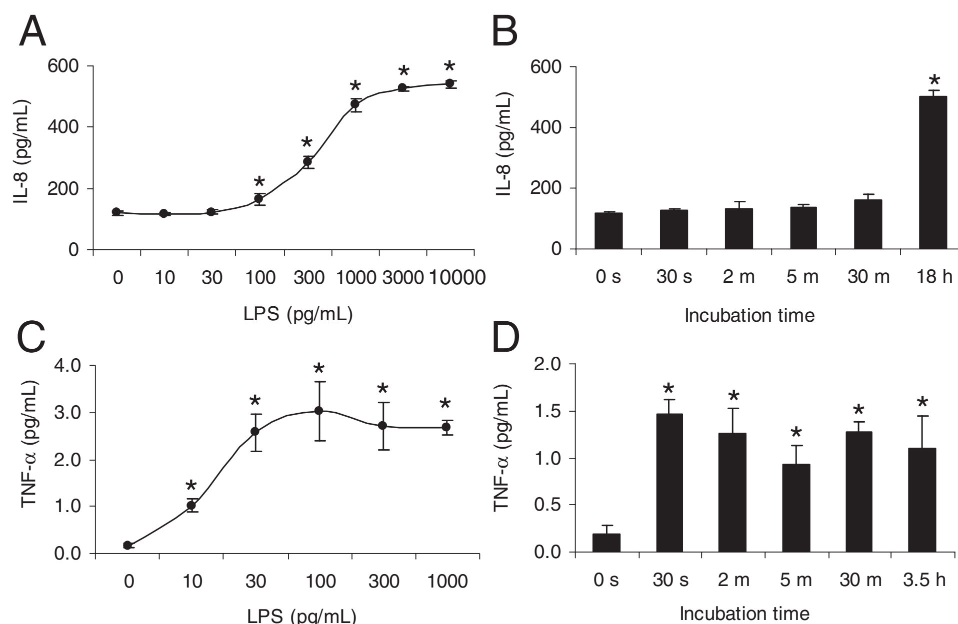

FIGURE 3. Results of endotoxin-induced monocyte and human aortic endothelial cell (HAEC) stimulation assays expressed as the mean ($\pm$SD) of triplicate cultures from $\geq$3 experiments in blood samples from healthy subjects. A: HAECs were cultured in vitro with 0–10 000 pg/mL of the same *Escherichia coli* endotoxin [lipopolysaccharide (LPS)] that was used to calibrate the plasma limulus amoebocyte lysate (LAL) assays. Interleukin-8 (IL-8) release was measured at 18 h by enzyme-linked immunosorbent assay (ELISA) as a measure of cellular activation. $n = 3$. B: To mimic transient exposure to endotoxin, HAEC IL-8 release was measured after incubation with medium alone (indicated by 0 s) or with 2 ng endotoxin/mL for the indicated times before replacement of the supernatant fluid with medium alone for the remainder of the 18-h incubation. $n = 3$. C: Freshly collected human monocytes were challenged with indicated concentrations of *E. coli* LPS, and supernatant tumor necrosis factor-α (TNF-α) was measured by ELISA at 3.5 h. $n = 3$. D: Human monocytes were treated with medium alone (indicated by 0 s) or 2 ng endotoxin/mL for the indicated times before replacement of the supernatant fluid with medium alone for the remainder of 3.5 h. $n = 3$. *Significantly different from cells cultured in medium alone, $P < 0.05$ (ANOVA followed by Dunnett's test).

the circulation in relatively healthy individuals is not entirely without precedent. It is also interesting to note that hyperphagic leptin-deficient (*ob*/*ob*) and hyperleptinemic (*db*/*db*) mice have both been shown to develop circulating endotoxin concentrations up to 3-fold those of wild-type mice (29) and that apolipoprotein E–deficient mice also develop portal endotoxemia when fed a high-fat diet but not when fed normal chow (30); these findings indicate that diet-induced endotoxin translocation may also occur in other species.

We also investigated potential mechanisms of endothelial cell activation in response to low concentrations of endotoxin. The observation that HAECs are responsive to as little as 100 pg/mL *E. coli* endotoxin (Figure 3A) is consistent with the findings of earlier in vitro studies (31). However, it was found that the presence of whole blood potentiated the activation of cultured HAECs, which allowed responsiveness to concentrations of endotoxin $\approx$10-fold lower than previously thought possible (31), in a manner at least partly dependent on TNF-α (Figure 4B). The data presented in Figures 3 and 4 also suggest that when LPS exposure is transient or is of low concentrations, endothelial cell activation is more likely to occur via the release of soluble inflammatory mediators, such as TNF-α, from monocytes or other LPS-responsive cell types present in blood. We note, however, that because postprandial plasma did not induce detectable HAEC activation in this assay, it is possible that naturally occurring LPS may not be as potent as the test *E. coli* endotoxin or that HAECs cultured in vitro are not as sensitive to inflammatory stimuli as are HAECs in vivo.

Thus, the present model predicts that, in the postprandial phase, increases in endotoxin released from the gut may contribute to increased leukocyte activation, to the release of cytokines such as TNF-α, and, indirectly, to endothelial cell activation. Accordingly, much evidence from other recent studies suggests that inflammatory indicators are indeed increased after a high-fat meal. Circulating leukocytes express the activated form of the proinflammatory transcription factor NF-κB (2, 3) and upregulate several markers of leukocyte activation, such as CD11A, CD11B, and CD62L, after a high-fat meal (4). Plasma IL-8 and neutrophil counts also increase after a high-fat meal but not after water challenge (5, 6), as do markers of endothelial cell activation, such as soluble intercellular adhesion molecule 1 and vascular adhesion molecule 1 (7). A role for TNF-α in postprandial inflammation has also been implicated, because baseline TNF-α correlates with the degree of alimentary lipemia in healthy men (32) and increases significantly after a high-fat meal but not after carbohydrate loading (7). In the present study, we were unable to detect TNF-α in the plasma samples, and CRP did not increase over the duration of the study, although it is possible that, because many of the previous studies of postprandial inflammation reported maximal increases in inflammatory markers between 6 and 9 h after treatment (3, 6, 33), the 4-h duration of the present study may have been insufficient to witness increases in these markers.

To date, the cause of postprandial inflammatory events remains poorly understood. Our results provide a possible explanation for these effects. It is possible that the brief and modest increases in plasma endotoxin that occur after a high-fat meal may be sufficient to increase the expression of these mediators and thereby contribute to the postprandial inflammatory state and endothelial cell activation, a primary step in atherogenesis. Interestingly, it has been shown that the induction of peripheral blood mononuclear cell NF-κB activation by a high-fat meal can

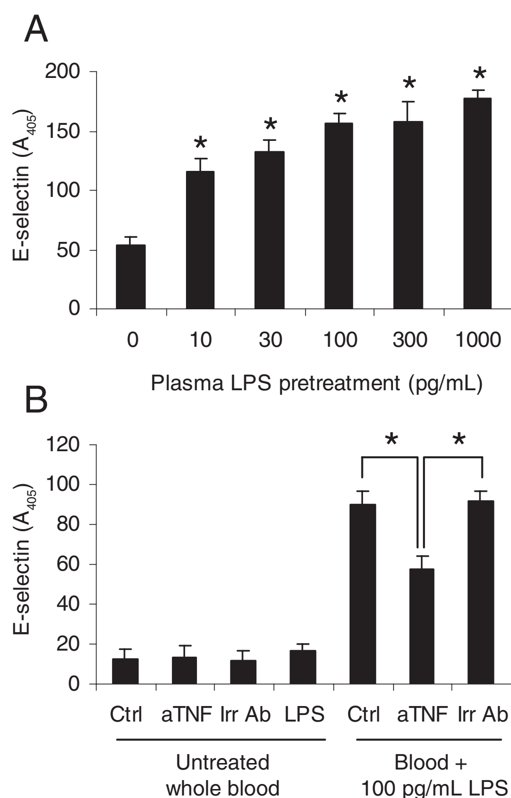

FIGURE 4. Results of endotoxin-induced whole-blood and human aortic endothelial cell (HAEC) stimulation assays expressed as the mean (± SD) of triplicate wells from 3 experiments. A: To investigate the mechanisms of whole-blood potentiation of endothelial cell responses to lipopolysaccharide (LPS), plasma was collected from whole heparinized blood incubated for 6 h with 0–1000 pg/mL of the same *E. coli* endotoxin that was used to calibrate the plasma limulus amoebocyte lysate (LAL) assays. HAECs were then exposed to LPS-conditioned plasma for 4 h before measurement of E-selectin expression by cell-surface enzyme-linked immunosorbent assay [ELISA; absorbance measured to 405 nm (A_{405})]. $n = 3$. B: Plasma from untreated blood or plasma from whole blood that had been incubated with 100 pg endotoxin/mL for 6 h was added to HAECs with medium [control (Ctrl)], or 10 μg/mL tumor necrosis factor-α–neutralizing antibody (aTNF), or irrelevant isotype control antibody (Irr Ab), or 100 pg LPS/mL (LPS). $n = 3$. *Significantly different from nonconditioned plasma or as indicated in B, $P < 0.05$ (ANOVA followed by Tukey's test).

be reduced by the concurrent ingestion of red wine or olive oil (3, 33). An antioxidant mechanism has been put forward to explain this effect, although it is interesting to note that both olive oil and resveratrol, a phytoalexin present in red wine, are potent inhibitors of LPS signaling and the detrimental effects of experimental endotoxin challenge (34, 35). Moreover, it has been shown very recently in mice that high-fat diet–induced vascular inflammation and insulin resistance are reversed completely by genetic deletion of the endotoxin receptor Toll-like receptor 4 (36).

Finally, although the present study shows that circulating endotoxin increases after a high-fat meal, but not after smoking or no meal, which dietary components in particular contribute to this process, and their exact roles, remain to be established. Other experimental meals, including glucose, complex carbohydrate, protein, and low-fat–based meals, will need to be examined to determine whether the effect we observed was due to fat translocation rather than to events common to the digestive process in general. We note, however, that most of the previous studies in this area have shown postprandial inflammation after a high-fat meal (2–6, 33), and it was shown very recently that circulating endotoxin increases ≈2- to 3-fold in mice fed a high-fat diet (37).

In conclusion, we presented both direct and indirect evidence of increased circulating plasma endotoxin after a high-fat meal in healthy subjects. Increased postprandial LPS may contribute to the development of the postprandial inflammatory state, endothelial cell activation, and early events of atherosclerosis. As such, therapeutic approaches aimed at reducing the translocation of endotoxin from the gut to the circulation may prove of worth with regard to possible treatments in the prevention of atherosclerosis.

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