

## Oil composition of high-fat diet affects metabolic inflammation differently in connection with endotoxin receptors in mice

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**Laugerette F, Furet J, Debard C, Daira P, Loizon E, Gélöën A, Soulage CO, Simonet C, Lefils-Lacourtablaise J, Bernoud-Hubac N, Bodenec J, Peretti N, Vidal H, Michalski MC.** Oil composition of high-fat diet affects metabolic inflammation differently in connection with endotoxin receptors in mice. *Am J Physiol Endocrinol Metab* 302: E374–E386, 2012. First published November 15, 2011; doi:10.1152/ajpendo.00314.2011.—Low-grade inflammation observed in obesity is a risk factor for cardiovascular disease. Recent studies revealed that this would be linked to gut-derived endotoxemia during fat digestion in high-fat diets, but nothing is known about the effect of lipid composition. The study was designed to test the impact of oil composition of high-fat diets on endotoxin metabolism and inflammation in mice. C57/B16 mice were fed for 8 wk with chow or isocaloric isolipidic diets enriched with oils differing in fatty acid composition: milk fat, palm oil, rapeseed oil, or sunflower oil. In vitro, adipocytes (3T3-L1) were stimulated or not with lipopolysaccharide (LPS; endotoxin) and incubated with different fatty acids. In mice, the palm group presented the highest level of IL-6 in plasma ( $P < 0.01$ ) together with the highest expression in adipose tissue of IL-1 $\beta$  and of LPS-sensing TLR4 and CD14 ( $P < 0.05$ ). The higher inflammation in the palm group was correlated with a greater ratio of LPS-binding protein (LBP)/sCD14 in plasma ( $P < 0.05$ ). The rapeseed group resulted in higher sCD14 than the palm group, which was associated with lower inflammation in both plasma and adipose tissue despite higher plasma endotoxemia. Taken together, our results reveal that the palm oil-based diet resulted in the most active transport of LPS toward tissues via high LBP and low sCD14 and the greatest inflammatory outcomes. In contrast, a rapeseed oil-based diet seemed to result in an endotoxin metabolism driven toward less inflammatory pathways. This shows that dietary fat composition can contribute to modulate the onset of low-grade inflammation through the quality of endotoxin receptors.

fatty acid; sCD14; lipopolysaccharide-binding protein; nutrition

OBESITY RESULTS FROM COMPLEX INTERACTIONS between environmental and genetic factors and is associated with a cluster of metabolic disorders such as hyperlipemia and diabetes and constitutes risk factors for cardiovascular diseases (34).

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Among environmental factors, a fat-enriched diet, excessive calorie intake, and a sedentary lifestyle increase this occurrence. Obesity and associated metabolic disorders are also characterized by a chronic or so-called “low-grade” inflammation that is a risk factor for metabolic diseases (32). Along with muscle and liver, the white adipose tissue (WAT) is an important tissue involved in several systemic processes such as glucose tolerance and inflammatory response via secretion of adipokines (73). Several distinct inflammatory pathways are involved in the secretion of proinflammatory cytokines and chemokines, such as interleukin-6 (IL-6), interleukin-1 $\beta$  (IL-1 $\beta$ ), and monocyte chemoattractant protein-1 (MCP-1), by WAT (57). However, whereas the effects of inflammatory reactions on lipid metabolism and insulin resistance are known, the mechanisms contributing to the generation of low-grade inflammation remain to be elucidated.

In this respect, recent studies have shown that rodents fed a high-fat diet display alterations of gut microbiota that increase intestinal permeability (10) and gut inflammation (16a). Previous data in humans and rodents revealed that ~90% of a normal gut microbiota is made of the Bacteroidetes and Firmicutes phyla, whereas obesity is linked to changes in their proportions (21, 31, 44, 80). More recently, a link was revealed between high-fat diet, inflammation, and the occurrence in plasma of proinflammatory products of Gram-negative bacteria of gut microbiota, namely lipopolysaccharides (LPS), so-called “endotoxins” (9, 10). The endotoxin metabolic pathway then includes the binding of LPS to LPS-binding protein (LBP) and its subsequent transfer to the receptor CD14. Two forms of CD14 have been characterized, a membrane-bound glycoprotein (mCD14) and a circulating soluble form (sCD14) (29). LBP and sCD14 levels can be considered as relevant markers of endotoxins in plasma (30). In this respect, given the long half-lives of sCD14 and LBP (24–48 h) compared with endotoxin (from <8 min in mice to a maximum of 3 h in humans), sCD14 and plasma LBP seem to reflect long-term exposure to endotoxin rather than the measurement of endotoxemia itself (49, 60), which is more reliable to measure transient kinetics of endotoxin absorption (18, 39). Regarding the metabolic impact of these LPS transporters and receptors, LBP-bound LPS initiates inflammation via Toll-like receptor-4 (TLR4) associated with mCD14 and induces the secretion of proinflamma-

tory cytokines such as IL-6 that are involved in inflammation (67). In septic shock, high amounts of LBP inhibit LPS activity, but low amounts of LBP in the regular metabolic state are reported to increase LPS proinflammatory activity (38). Noticeably, sCD14 is reported to have suppressing effects on endotoxin activity by driving detoxification by HDL (70). Interestingly, however, fatty acids (FA) also bind TLR4 to induce proinflammatory cytokine expression in macrophages and adipocytes (66).

Recent studies, including ours, showed that metabolic endotoxemia could be generated by gut LPS absorption during digestion of a high lipid load (4, 9, 18, 27, 39). But none of these studies took into account the numerous dietary fat sources in Western diets and their FA profile that is suspected to affect the development of inflammatory response (5). Therefore, the impact of oil FA composition in Western-type diets on endotoxemia, LPS transporters and receptors, and the onset of inflammatory outcomes deserves to be elucidated. Moreover, up-to-date high-fat diet studies in rodents used a regimen with >60% of energy as lard and devoid of carbohydrates (9, 11); therefore, the mechanisms associating dietary fat with endotoxin metabolism and inflammation deserve to be studied in a more equilibrated fat-rich regimen.

Thus the aim of this study was to compare the effects of oils differing in FA composition on endotoxin metabolism and inflammation in plasma and adipose tissue in the dynamic phase of weight gain and inflammation onset. Therefore, we tested animal diets based on chow enriched with different fat sources, i.e., milk fat, palm oil, rapeseed oil, and sunflower oil. Because acute gavage studies had already been performed in previous studies (9, 18, 27, 39), and short-duration diets can be insufficient to observe inflammation, we chose to use a sufficient duration of 8 wk to be able to induce the onset of metabolic inflammation that we aimed to compare with LPS receptors and endotoxemia.

## MATERIALS AND METHODS

**Specific nonpyrogenic material.** Great care was taken to avoid contamination with exogenous LPS during experiments. Only single-use non pyrogenic material was used: PS Becton Dickinson tubes, Axygen tubes (VWR), and pyrogen-free pipette tips (Biogenic, Perols, France).

**Ethics statement.** Experiments were carried out according to the guidelines laid out by the French Ministère de l'Agriculture (no. 87-848 completed by no. 2001-464) and the European Union Council Directive for the Care and Use of Laboratory Animals (no. 86/609). C. Soulage (no. 69266257) and A. Géloën (no. 69266332) hold a license to experiment on living vertebrates issued by the French Ministry of Agriculture and Veterinary Service Department. However, French law on animal experimentation does not require for authorized people (with a license no.) the systematic review of animal research protocols by an ethics committee or institutional review board.

**Animals and diets.** Male C57Bl6/J mice (6 wk, 18 g) were purchased from Harlan (Gannat, France) and kept at  $24 \pm 1^\circ\text{C}$  on a 12:12-h light cycle (light on from 0600 to 1800) with free access to food and tap water. Animals were housed at four per cage and adapted to the laboratory conditions for 1 wk before the experiment began. Fat-rich diets were prepared by SAFE (Augy, France) and contained 22.4% lipids (Table 1). Mice were randomly divided into five groups ( $n = 8/\text{group}$ ) fed one of the five following diets for 8 wk: a milk fat-enriched diet (MF), a palm oil-enriched diet (P), a rapeseed oil-enriched diet (R), a sunflower oil-enriched diet (S), and a normal chow diet (Ch) that was used as a low-fat control (A04; SAFE).

Fat-rich diets were thus designed especially to differ in oil composition only (76), and oils were chosen among the major dietary fats consumed worldwide (17). Importantly, diets were manufactured using fats and oils for human consumption. Rapeseed and sunflower oil were from Lesieur, palm oil was from SIO (Saint Laurent Blangy, France), and anhydrous milk fat was from Lactalis R & D (Retiers, France). Oils were therefore certified to be devoid of Gram-negative bacteria; they were found to be nonpyrogenic using the limulus amoebocyte lysate (LAL) assay (no detectable endotoxemia). Moreover, diets produced by manufacturer (SAFE) passed all microbiological quality checks.

Food intake was measured twice/wk for 8 wk, and average food intake per day was subsequently calculated. After 8 wk under diet, mice were randomly euthanized after being nonfasted for 4 days ( $2 \text{ mice-group}^{-1} \cdot \text{day}^{-1}$ ) between 0900 and 1200 by intraperitoneal injection of pentobarbital sodium. Blood was collected by cardiac puncture under pyrogen-free conditions in heparin-containing tubes. Plasma was obtained by centrifugation (8,000 g, 10 min), frozen in liquid nitrogen, and stored at  $-80^\circ\text{C}$  until analysis. Small intestine mucosa, caecum contents, liver, and WAT corresponding to epididymal tissue were collected, frozen in liquid nitrogen, and stored at  $-80^\circ\text{C}$ .

**3T3-L1 cell culture and treatment.** 3T3-L1 cells were obtained from American Type Culture Collection (Manassas, VA) and cultured at  $37^\circ\text{C}$  under 5%  $\text{CO}_2$  in Dulbecco's modified Eagle's medium (DMEM; Sigma), to which 10% fetal calf serum (BioWest), 4 mM L-glutamine, and antibiotics (Sigma) were added. Cell differentiation was performed in DMEM containing 5  $\mu\text{g/ml}$  insulin (Actrapid 100 UI/ml; Novo-Nordisk), 0.5 mM 3-isobutyl-1-methylxanthine (Sigma), 0.25  $\mu\text{mol/l}$  dexamethasone (Sigma), and 10  $\mu\text{mol/l}$  rosiglitazone (Molekula) for 48 h. On the 10th day after differentiation, cells were harvested 4 h before treatment. In our experiment, the first set of cells ( $n = 6/\text{treatment}$ ) was treated with DMEM containing 5  $\mu\text{g/ml}$  insulin and one of the following FAs: myristic, palmitic, linoleic, and linolenic acids (Sigma) at 100  $\mu\text{mol/l}$  conjugated to 50  $\mu\text{mol}$  of bovine serum albumin (Sigma). The second set of cells ( $n = 6/\text{treatment}$ ) received the same FAs with 0.1  $\mu\text{g/ml}$  of LPS from *Escherichia coli* (*E. coli*) O55:B5 (Sigma) (24). Control cells were incubated with DMEM with or without 0.1  $\mu\text{g/ml}$  LPS. Treatments lasted 24 h. At the end of the experiment, cells and medium were taken and stored at  $-80^\circ\text{C}$ .

**LAL assays.** Plasma endotoxemia [endotoxin unit per milliliter (EU/ml)] was determined by using the LAL assay in kinetic chromogenic conditions (Biogenic, Pérols, France), as described previously (39). Briefly, plasma sample was thawed and diluted 1:40 in pyrogen-free water (Biogenic), heated at  $70^\circ\text{C}$  for 10 min, and subjected to an ultrasonic bath for 5 min and to a 1-min vortex. One hundred microliters of sample was combined with 100  $\mu\text{l}$  of LAL reagent (Chromolal; Biogenic) in triplicate in pyrogen-free 96-well plates (Biogenic). For each sample, a spiked control at 0.45 EU/ml was performed to check that no significant inhibition or activation occurred. According to the FDA procedure, endotoxemia measurement is considered valid in these conditions if spike recovery is in the range of 50–200% (75), that is, 0.225–0.9 EU/ml greater than sample endotoxemia. In this study, spike recovery was in the range of 95–124% (no significant differences among groups). Moreover, standard curve always presented regression coefficient of  $\sim 0.99$ , and the nonpyrogenicity of water was checked systematically. Data were analyzed for endotoxemia calculation using the Softmax Pro software (Biogenic).

**sCD14, LBP, IL-6, and MCP-1 measurements.** IL-6, MCP-1 (Clinisciences), sCD14, and LBP (Enzo Life Sciences) were assayed by ELISA kit according to the manufacturer's instructions. For 3T3-L1, IL-6 concentration of media was measured using the mouse-specific ELISA kit (Pierce Endogen).

**Plasma triacylglycerol and nonesterified FA measurements.** Plasma triacylglycerols (TAG) were measured with the triglyceride PAP kit

Table 1. Diet composition

High Fat Based On:	MF	P	R	S	Ch
Ingredient, g/100 g					
A04 SAFE	80	80	80	80	100
Among which protein	12.8	12.8	12.8	12.8	16
Lipid	2.4	2.4	2.4	2.4	3
CH*	37.6	37.6	37.6	37.6	47
Among which starch	35.2	35.2	35.2	35.2	44
Sugars	2.4	2.4	2.4	2.4	3
Cellulose	3.2	3.2	3.2	3.2	4
Anhydrous milk fat	20				
Palm oil		20			
Rapeseed oil			20		
Sunflower oil				20	
Total lipids	22.4	22.4	22.4	22.4	3
Energy, kcal/g	4.12	4.12	4.12	4.12	2.82
Energy, %					
CH	47.7	47.7	47.7	47.7	67.7
Protein	12.6	12.6	12.6	12.6	22.7
Lipid	37.7	37.7	37.7	37.7	9.6
FA mol/100 mol total FA in the diet					
10:0	0.5	ND	ND	ND	ND
12:0	2.2	Tr	ND	ND	ND
14:0	12.1	0.5	Tr	Tr	0.5
15:0	1.1	Tr	ND	ND	Tr
16:0	32.3	45.0	6.6	6.8	17.0
18:0	14.0	4.4	1.7	3.4	2.7
18:1 n-9 <i>cis</i>	29.0	36.6	58.5	42.9	20.3
18:2 n-6 <i>cis</i>	7.1	12.7	23.1	46.1	45.1
18:3 n-3	1.2	0.5	9.7	0.4	3.5
20:4 n-6	Tr	Tr	Tr	Tr	0.7
20:5 n-3	Tr	Tr	Tr	Tr	0.8
22:6 n-3	Tr	Tr	Tr	Tr	1.7
SFA					
Subtotal 10:0-14:0	14.8	0.5	ND	ND	0.5
Total	62.2	50.1	8.5	10.4	22.5
Ratio n-6/ n-3 FA	4.8	21.1	2.4	91.4	7.0

MF, milk fat; P, palm oil; R, rapeseed oil; S, sunflower oil; Ch, normal low-fat chow; Tr, trace (<0.2%); ND, not detected. \*Major sources of carbohydrates (CH) in this diet are nonprocessed cereals: wheat, corn, barley, and wheat bran.

(Biomérieux, France), using culture plates (Corning) as described previously (52). Real plasma TAG concentration was calculated by subtracting the free glycerol in plasma measured with the glycerol UV method (R-Biopharm/Boehringer, Mannheim, Germany) (52). Plasma nonesterified FAs (NEFA) were measured using NEFA-HR R2 Set kit (Wako Chemicals, Neuss, Germany).

**Western blots.** WATs were lysed in RIPA buffer (1% Triton X-100, 50 mM Tris, pH 7.5, 150 mM NaCl, 5 mM EDTA, 0.5 mM Na<sub>3</sub>VO<sub>4</sub>, 50 mM NaF, 10 mg/l aprotinin, 10 mg/l leupeptin, 10 mg/l pepstatin, and 1 mM PMSF). Fifty micrograms of proteins (8) was subjected to Western blotting by separation using SDS-PAGE (10%) and transfer to polyvinylidene fluoride membrane. Membranes were blotted for TLR4 and tubulin (Santa Cruz Biotechnology) using specific antibodies combined with horseradish peroxidase-conjugated anti-rabbit IgG antibody. Blots were developed using the enhanced chemiluminescence method (GE Healthcare, Buckinghamshire, UK). Quantification was performed by densitometric analysis of specific bands on immunoblots using Quantity One software (Bio-Rad).

**Quantitative PCR analysis of cecal microbiota.** Total bacteria DNA was extracted from 0.2 g of animal cecal material ( $n = 3/\text{group}$ ) using the G'NOME kit (BIO 101, La Jolla, CA) with modifications, as described previously (22). After the final precipitation, DNA was resuspended in 150 ml of TE buffer and stored at  $-20^{\circ}\text{C}$  prior to further analysis.

The primers and probes used in this study, performed using an ABI 7000 Sequence Detection System with software version 1.2.3 (Applied Biosystems), were described previously (22). TaqMan quantitative PCR (qPCR) was adapted to quantify the total bacteria popu-

lation in addition to the dominant (>1% of fecal bacteria) bacterial group: *Clostridium leptum* (*C. leptum*), *Blautia* (*Clostridium*) *coccoides* (*Bl. coccoides*), *Bacteroides/Prevotella*, and *Bifidobacterium* *genus*. Real-time qPCR using SYBR Green was performed for the subdominant bacterial group *Lactobacillus/Leuconostoc/Pediococcus* and for the species *E. coli*. The TaqMan probes were synthesized by Applied Biosystems Applera-France (Courtaboeuf, France), and primers were purchased from MWG-Biotech (Ebersberg, Germany).

Real-time qPCR was performed using an ABI 7000 Sequence Detection System with software version 1.2.3 (Applied Biosystems, Foster City, CA). Amplification and detection were carried out in 96-well plates with TaqMan Universal PCR 2× MasterMix (Applied Biosystems) or with SYBR Green PCR 2× Master Mix (Applied Biosystems). Each reaction was run in duplicate in a final volume of 25  $\mu\text{l}$  with a 0.20  $\mu\text{mol/l}$  final concentration of each primer, a 0.25  $\mu\text{mol/l}$  final concentration of each probe, and 10  $\mu\text{l}$  of appropriately diluted DNA samples. Amplifications were carried out using the following ramping profile: one cycle at  $95^{\circ}\text{C}$  for 10 min, followed by 40 cycles of  $95^{\circ}\text{C}$  for 30 s and  $60^{\circ}\text{C}$  for 1 min. The total numbers of bacteria were inferred from averaged standard curves as described (46).

**qPCR analysis of WAT.** Total RNA were extracted from 50 mg of WAT with TRIzol (Invitrogen, Eragny, France). First-strand cDNAs were synthesized from 1  $\mu\text{g}$  of total RNA in the presence of 100 units of Superscript II (Invitrogen) using a mixture of random hexamers and oligo(dT) primers (Promega, Charbonnières, France). Real-time PCR assays were performed using a Rotor-Gene Q (Qiagen, France). TATA box-binding protein mRNA level was used to normalize the data. The

PCR primers were as follows: TLR4 (forward: AGTGGCTGGATT-TATCCAGG; reverse: TCCACAGCCACCAGATTCTC), IL-6 (forward: AGTTGCCTTCTGGGACTGAT; reverse: TCCACGATTC-CCAGAGAAC), CD14 (forward: TCAGCTAAACTCGCTCAATC; reverse: TCCAGCCTGTTGTAAGTACTGAG), CD68 (forward: AGC-ATAGTTCTTTCTCCAGC; reverse: ATGATGAGAGGCAGCAA-GAG), IL-1 $\beta$  (forward: ACTGTTCCCTGAACTCAACTG; reverse: CTT-GTTGATGTGCTGCTGCG), and TATA box-binding protein (forward: TGGTGTGCACAGGAGCCAAG; reverse: TTCACATCACAGCTC-CCCAC).

**Immunogold labeling for transmission electron microscopy.** A fraction of jejunum was placed against freshly ionized formvar-carbon-coated 200- $\mu$ m mesh nickel grid suspension for 2 min. To avoid false positives, nonspecific sites were blocked with 1% BSA in 50 mM Tris-HCl, pH 7.4, for 10 min at RT. The jejunal pieces were then incubated with a 1:30 dilution of mouse monoclonal antibody anti-LPS core (*E. coli* and *Enterobacteria*; Hycult Biotechnology) in a wet chamber for 2 h at 4°C. Grids with suspension were successively washed once in 50 mM Tris-HCl, pH 7.4 and pH 8.2, at room temperature (RT). Then they were incubated in a wet chamber for 45 min at RT in 1% BSA and 50 mM Tris-HCl, pH 8.2, for 20 min at RT and labeled with 20 nm of gold-conjugated goat anti-mouse IgG (Tebu-Bio) diluted 1:80 in 1% BSA and 50 mM Tris-HCl, pH 8.2. They were successively washed once in 50 mM Tris-HCl, pH 8.2 and 7.4, and in filtrated distilled water. Finally, immunocomplexes were fixed in 4% glutaraldehyde and negatively stained with 1% neutral phosphotungstic acid. Grids were observed on a transmission electron microscope (Jeol 1400JEM, Tokyo, Japan) operating at 80 Kv equipped with a camera Orius 1000 and digital Micrograph (CeCIL; Faculty of Medecine Laënnec, Lyon, France).

**Lipid analysis.** For total FA profile of plasma and WAT, total lipids were extracted from ~25 mg of epididymal adipose tissue or 50  $\mu$ l of plasma using ethanol-chloroform (1:2 vol/vol) (41). The organic phase was dried under N<sub>2</sub>, and total FAs were transmethylated using boron trifluoride in methanol (41). The FA methyl esters were then analyzed by GC using a DELSI instrument model DI 200 equipped

with a fused silica capillary SP-2380 column (60  $\times$  0.22 mm) (41). For estimation of the long-chain n-3 fatty acid status in phospholipids (PLs) of adipose tissue, PLs were separated from neutral lipids by thin-layer chromatography using hexane-diethylether-acetic acid (80:20:1 vol/vol/vol). PL spot on silica gel was scrapped off and further transmethylated and analyzed by GC, as described above.

**Statistical analysis.** All data are presented as means  $\pm$  SE and were analyzed with Statview 5.0 software (Abacus Concepts, Berkeley, CA). Results of the five groups were compared by analysis of variance (ANOVA), followed by post hoc test (Fisher protected least significant difference). Simple comparisons were performed using Student's *t*-test. Differences were considered significant at the *P* < 0.05 level. Principal component analysis was performed using JMP Software (version 9; SAS).

## RESULTS

**Lipid markers related to dietary fat composition in vivo.** Considering the reported importance of FA composition in food on metabolic inflammation, we studied different oils in high-fat diets (Table 1). The daily caloric intake of mice was similar among the MF, P, R, and S groups (data not shown). The body weight, liver, and WAT weights of the MF group were significantly higher than in mice of the other groups (*P* < 0.001 or *P* < 0.05; Table 2); MF and R gained more weight than Ch. The profile of total FAs in WAT (Table 2) reflected the composition of ingested dietary fats (Table 1). Arachidonic acid (20:4, n-6), an n-6 FA precursor of proinflammatory derivatives in biological membranes, was similar in the PLs of WAT in all high-fat groups (Table 2). The global n-6/n-3 ratio, reflecting increased risk of inflammation derived from PUFA metabolites, was lower in WAT-PL of the R group than other high-fat groups (not different from Ch group) and higher in WAT-PL of S group. However, n-6/n-3 ratio was not different

Table 2. Morphological parameters, profile of FA of the epididymal WAT, and plasma palmitate content in mice fed diet with MF, P, R, S, or Ch

	MF	P	R	S	Ch
Morphological parameters					
Initial body weight, g	18.0 $\pm$ 0.6	18.1 $\pm$ 0.6	17.9 $\pm$ 0.5	18.0 $\pm$ 0.3	18.2 $\pm$ 0.5
Body weight gain, g	16.6 $\pm$ 3.0	9.8 $\pm$ 0.9\$	11.3 $\pm$ 0.8#	9.8 $\pm$ 1.6\$	8.7 $\pm$ 0.4\$
Food intake, g $\cdot$ mouse <sup>-1</sup> $\cdot$ day <sup>-1</sup>	4.73 $\pm$ 0.23	4.84 $\pm$ 0.21	6.12 $\pm$ 0.48	5.79 $\pm$ 0.38	4.74 $\pm$ 0.29
Liver weight, g	1.36 $\pm$ 0.08	1.04 $\pm$ 0.04	1.09 $\pm$ 0.05	1.12 $\pm$ 0.07	1.08 $\pm$ 0.04
WAT weight, g	1.23 $\pm$ 0.23	0.58 $\pm$ 0.07\$	0.69 $\pm$ 0.06\$	0.73 $\pm$ 0.07\$	0.38 $\pm$ 0.04\$
Major FA in WAT total FA, mol/100 mol FA					
12:0	0.9 $\pm$ 0.2	Tr	Tr	Tr	Tr
14:0	6.2 $\pm$ 0.2	1.1 $\pm$ 0.0	Tr	Tr	2.0 $\pm$ 0.2
15:0	0.8 $\pm$ 0.0	Tr	Tr	Tr	Tr
16:0	25.5 $\pm$ 1.2 <sup>a</sup>	29.3 $\pm$ 0.9 <sup>a</sup>	12.3 $\pm$ 0.2 <sup>b</sup>	13.3 $\pm$ 0.3 <sup>b</sup>	31.2 $\pm$ 3.7 <sup>a</sup>
16:1 n-7t	8.1 $\pm$ 0.4 <sup>a</sup>	7.2 $\pm$ 0.5 <sup>a</sup>	0.9 $\pm$ 0.5 <sup>b</sup>	2.2 $\pm$ 0.4 <sup>b</sup>	11.1 $\pm$ 1.2 <sup>a</sup>
18:0	4.8 $\pm$ 0.3 <sup>a</sup>	4.6 $\pm$ 0.3 <sup>a</sup>	2.2 $\pm$ 0.7 <sup>c</sup>	6.0 $\pm$ 0.3 <sup>b</sup>	6.1 $\pm$ 0.7 <sup>a,b</sup>
18:1 n-9c	37.5 $\pm$ 1.7 <sup>a</sup>	41.5 $\pm$ 0.4 <sup>a</sup>	55.0 $\pm$ 1.6 <sup>b</sup>	38.3 $\pm$ 0.4 <sup>a</sup>	23.2 $\pm$ 6.3 <sup>a</sup>
18:2 n-6c	8.2 $\pm$ 0.6 <sup>a</sup>	12.2 $\pm$ 0.3 <sup>a</sup>	21.7 $\pm$ 0.9 <sup>b</sup>	30.2 $\pm$ 1.4 <sup>c</sup>	18.2 $\pm$ 1.3 <sup>a,b</sup>
18:3 n-3	0.7 $\pm$ 0.1 <sup>a</sup>	0.3 $\pm$ 0.0 <sup>a</sup>	2.9 $\pm$ 0.2 <sup>b</sup>	0.3 $\pm$ 0.0 <sup>a</sup>	1.0 $\pm$ 0.2 <sup>a</sup>
20:4 n-6	Tr	Tr	Tr	Tr	Tr
20:5 n-3	Tr	Tr	Tr	Tr	Tr
22:6 n-3	Tr	Tr	Tr	Tr	Tr
Status of WAT PL in n-6/n-3 FA					
20:4 n-6, mol/100 mol FA in PL	3.0 $\pm$ 1.0 <sup>a,b</sup>	2.9 $\pm$ 0.4 <sup>a</sup>	2.9 $\pm$ 1.0 <sup>a,b</sup>	2.8 $\pm$ 0.5 <sup>a,b</sup>	8.0 $\pm$ 1.9 <sup>b</sup>
22:6 n-3, mol/100 mol FA in PL	1.1 $\pm$ 0.3 <sup>a</sup>	0.9 $\pm$ 0.1 <sup>a</sup>	1.1 $\pm$ 0.3 <sup>a</sup>	0.6 $\pm$ 0.1 <sup>a</sup>	2.8 $\pm$ 0.8 <sup>a</sup>
n-6/n-3 Ratio	13.9 $\pm$ 2.2 <sup>a</sup>	14.4 $\pm$ 4.0 <sup>a</sup>	3.3 $\pm$ 1.2 <sup>b</sup>	45.2 $\pm$ 5.1 <sup>c</sup>	6.6 $\pm$ 2.8 <sup>a,b</sup>
Palmitate in plasma total FA, $\mu$ g 16:0/ml plasma	227 $\pm$ 32 <sup>a</sup>	231 $\pm$ 28 <sup>a</sup>	144 $\pm$ 15 <sup>b</sup>	133 $\pm$ 20 <sup>b</sup>	220 $\pm$ 19 <sup>a</sup>

Data are means  $\pm$  SE; *n* = 8/group. FA, fatty acids; WAT, white adipose tissue; PL, phospholipids. For morphometric parameters: \$*P* < 0.001, MF vs. other groups; #*P* < 0.01, MF vs. other groups; #*P* < 0.01, R vs. Ch. ANOVA followed by Fisher test.<sup>a-c</sup>Means in a row that do not share a common letter are significantly different (*P* < 0.05); *n* = 4/group for WAT-PL and *n* = 7/group for plasma palmitate.

in WAT-PL of MF, P, or Ch groups. Moreover, palmitate content in plasma lipids was similar in the Ch, MF, and P groups on the one hand and in the S and R groups on the other hand (Table 2). Figure 1A shows that the plasma TAG concentration was higher in the MF group than in the P and S groups. Moreover, the P, R, and S groups presented lower plasma TAG than the Ch group. However, plasma NEFA were similar among all groups (Fig. 1B).

**Markers of inflammation and of metabolic endotoxemia in plasma are modified by dietary fat composition.** Regarding inflammation, the P diet led to a higher concentration in plasma of the inflammatory cytokine IL-6 than in other groups (Fig. 2A). The P diet was also the only high-fat diet to result in higher concentration in plasma of MCP-1 than Ch ( $194.4 \pm 39.9$  vs.  $101.4 \pm 40.1$  pg/ml,  $P < 0.05$ ). Overall, the P group was thus the only high-fat group to induce significantly higher inflammation in plasma than the Ch group after 8 wk ( $P < 0.05$ ).

We then measured different circulating transporters and receptors in plasma that are involved in the proinflammatory potential of endotoxins, namely LBP and sCD14. Among high-fat groups, only the P group presented higher LBP than the Ch group ( $P < 0.01$ ; Fig. 2B). LBP was also higher in the P group than in other high-fat groups. Strikingly, in mice of the P group, plasma IL-6 levels were even highly correlated with plasma LBP levels ( $P < 0.003$ ; Fig. 2C). The highest concentration of sCD14 was observed in the R group (Fig. 2D) compared with the MF, P, and S groups ( $P < 0.05$ ). Moreover, Fig. 2E shows that the P group had the highest LBP/sCD14 ratio compared with all other groups, including Ch ( $P < 0.01$ ); this P group also exhibited the greatest proinflammatory pro-

file. This higher LBP/sCD14 ratio indicates a higher ability for plasma LPS to be vehicled to target tissues in the P group than in other groups. In contrast, the MF, R, S, and Ch groups presented both similar LBP/sCD14 ratios and similar IL-6 levels in plasma, which were lower than the P group.

Parallel to these differences in LPS transport ability, we also measured plasma endotoxemia by the LAL assay. As shown in Fig. 2F, mice fed the unsaturated FA diets tended to present a higher plasma endotoxemia than mice fed the saturated FA diets. The highest plasma endotoxemia measured by LAL assay was actually detected in the R group ( $P < 0.01$  vs. Ch, MF, and P groups).

**Markers of inflammation and endotoxin receptors in epididymal adipose tissue are modified by dietary fat composition.** In WAT, expression of proinflammatory cytokine IL-1 $\beta$  that is produced by both adipocytes and macrophages was significantly higher in P group compared with all other groups ( $P < 0.05$ ; Fig. 3A). Interestingly, expression of LPS receptor CD14 was higher in the WAT of the P group compared with all other groups, including Ch ( $P < 0.01$ ; Fig. 3B). Moreover, the LPS receptor TLR4 was enhanced significantly in the WAT of the P group compared with other groups, as revealed by both Western blotting (Fig. 3C) and mRNA expression (Fig. 3F). IL-6 that is expressed in macrophages was on the whole little expressed in WAT (Fig. 3D). The global marker of macrophage infiltration CD68 tended to be more expressed in several high-fat groups, namely P, R, and S (Fig. 3E).

These results show that the high-fat diet containing palm oil induced the highest amounts of inflammatory markers in plasma and highest expression of IL-1 $\beta$ , TLR4, and CD14 in WAT. The milk fat diet resulted in a lower inflammatory profile than the palm oil diet, albeit similarly rich in saturated FA but containing medium-chain FA (Table 1). Moreover, MF, R, and S diets resulted in similar plasma levels of MCP-1 and IL-6 and of IL-1 $\beta$ , TLR4, and CD14 expression in WAT, which was not significantly different from Ch.

Altogether, the results suggest that mice fed a high-fat diet enriched with palm oil for 8 wk presented a more active gut-derived endotoxin transport oriented toward inflammation than diets enriched with milk fat, rapeseed oil, or sunflower oil. This could explain the higher circulating proinflammatory cytokine with the palm oil diet and higher WAT inflammation.

**Endotoxemia could originate from LPS absorption from the gut microbiota in jejunum.** In gut microbiota, total bacteria, *Bacteroides/Prevotella C. leptum*, and *Bl. coccoides* groups did not appear to be modified by the quality of a high-fat diet considering the small sample size analyzed in this study. However, microbiota of the P group mice shows an *E. coli* population superior with regard to those of the MF, R, and S groups (Fig. 4). Furthermore, dietary fat composition seems to modify significantly the quantity of the *Lactobacillus/Leuconostoc* group (dominant group in rodent microbiota) vs. the chow diet. In our study, *Bifidobacterium* genus and *F. prausnitzii* species were not detected in gut microbiota. Principal component analysis, based on the qPCR bacterial quantifications, was carried out, and two first components (PC1 and PC2) plotted represented 87.4% of whole inertia (insert Fig. 4). It shows that the *E. coli* and *Lactobacillus/Leuconostoc* populations allow distinguishing the Ch and P diets of the others.

To test whether gut LPS could be partly absorbed by enterocytes, imaging was performed in the jejunum in high-

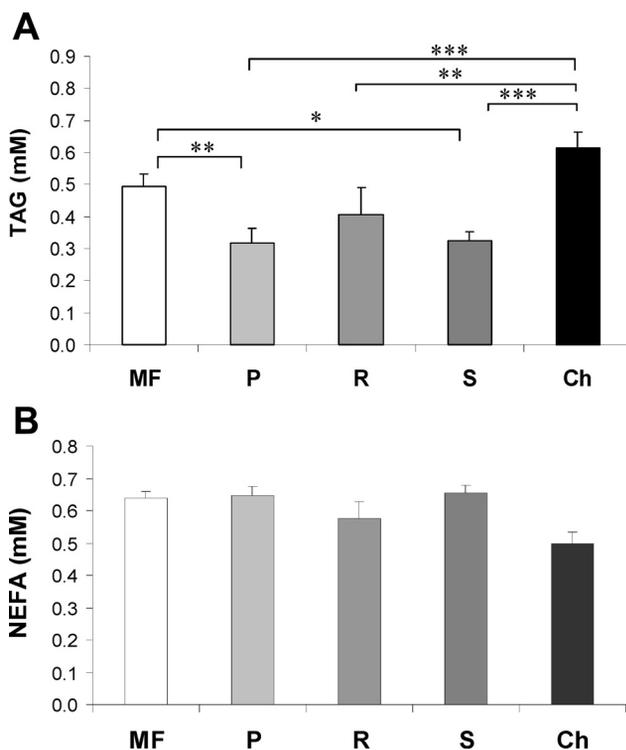


Fig. 1. Plasma lipids in mice fed diets with milk fat (MF), palm oil (P), rapeseed oil (R), sunflower oil (S), or normal low-fat chow (Ch). A: plasma triacylglycerols (TAG; mM). B: plasma nonesterified fatty acids (NEFA; mM). Data are means  $\pm$  SE. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ;  $n = 7$ . ANOVA followed by Fisher test.

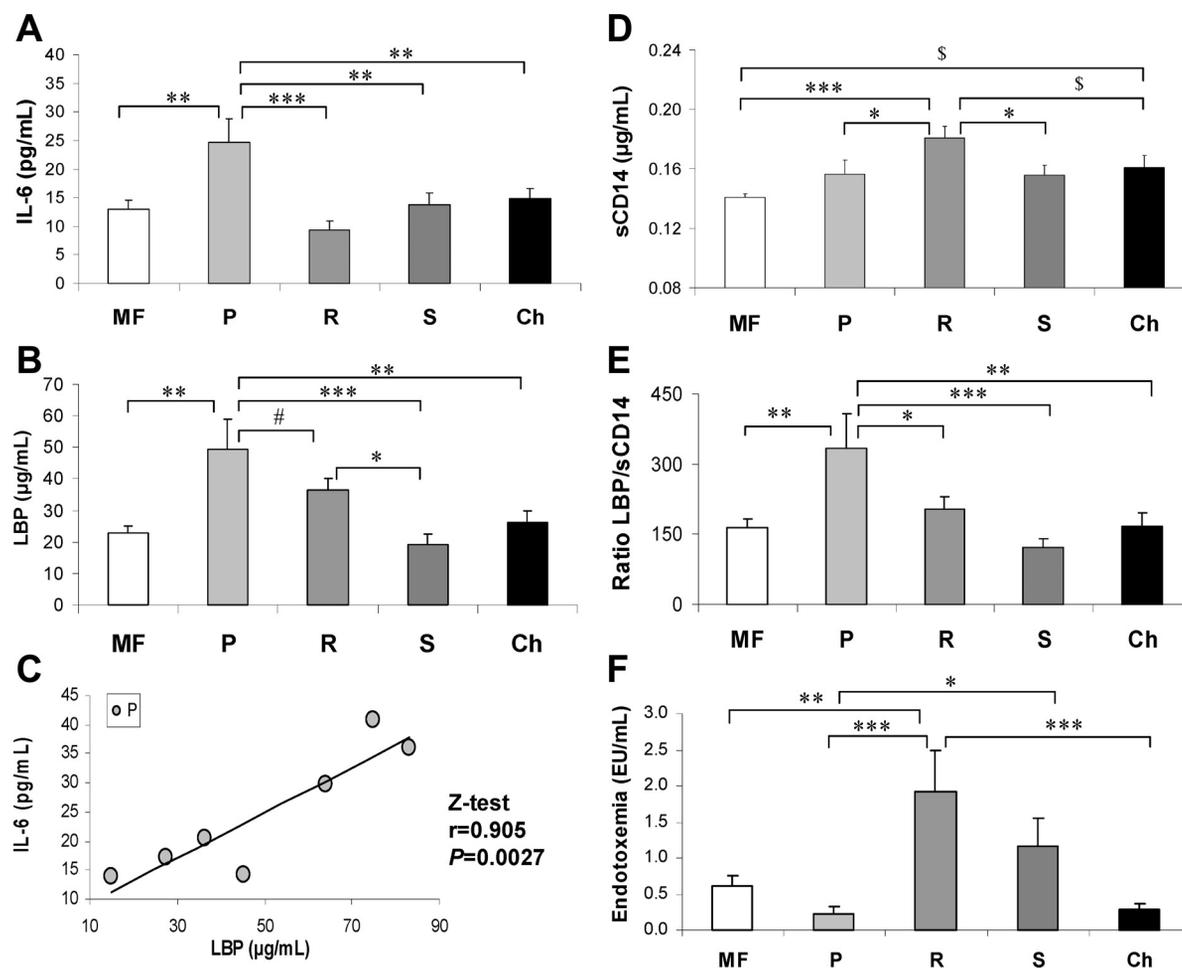


Fig. 2. Inflammation and endotoxin metabolism parameters in plasma of mice fed diet with MF, P, R, S, or Ch. A: IL-6 (pg/ml). B: lipopolysaccharide (LPS)-binding protein (LBP; µg/ml). C: correlation of IL-6 vs. LBP for individual mice in P group. D: sCD14 (soluble cluster of differentiation 14; µg/ml). E: ratio of LBP/sCD14. F: endotoxemia (EU/ml). Data are means  $\pm$  SE. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , # $P = 0.08$ , \$ $P < 0.06$ ;  $n = 7$ . ANOVA followed by Fisher test.

fat-fed mice. Electronic microscopy using an anti-LPS antibody revealed the presence of such LPS in enterocytes of the jejunal section, the major area of lipid absorption, regardless of the high-fat group (Fig. 5).

*FA structure modulates inflammatory markers in vitro on 3T3-L1 adipocytes.* We studied the impact of myristic (14:0; present in milk fat), palmitic (16:0; present mostly in milk fat and palm oil), linoleic (18:2 n-6; present mostly in sunflower oil), and  $\alpha$ -linolenic (18:3 n-3; present mostly in rapeseed oil) acids on MCP-1 and IL-6 secretion by 3T3-L1 cells. Stimulation with LPS increased MCP-1 secretion, but no differences were observed between the different FA treatments (results not shown). Figure 6 shows that myristic and palmitic acids increased IL-6 accumulation 22.5- and 54.3-fold, respectively, in the culture media compared with the control ( $P < 0.05$ ), whereas linoleic and linolenic acids had no effects. As expected, the addition of LPS was correlated with a significant accumulation of IL-6 in the culture medium for each FA (Fig. 6). Some FA appeared to interact with LPS to induce a greater increase in IL-6 than LPS stimulation alone, such as palmitic and  $\alpha$ -linolenic acids. These data indicate that FAs were able to participate in induced inflammation in adipocytes in vitro either alone or synergistically with endotoxins.

## DISCUSSION

The novel feature of the present study is that it focuses on the effects of dietary oil composition on LPS-related inflammation, an aspect that has thus far been widely ignored. This is an important issue for human nutrition, because in Western diets vegetable oils, including palm oil, are increasingly widely used conversely to animal fats such as milk fat (2, 19). FAs can be divided into four groups: saturated FA, among which short-, medium-, and long-chain saturated FAs (e.g., myristic, palmitic acids) can be distinguished; monounsaturated FAs; n-6 polyunsaturated FAs (e.g., linoleic acid); and n-3 polyunsaturated FAs (e.g.,  $\alpha$ -linolenic acid). FAs and their metabolites are known to act directly and indirectly to regulate metabolism. Among diet-induced metabolic diseases, obesity is characterized by low-grade inflammation and, as revealed recently, by metabolic endotoxemia. Different works show a link between low-grade inflammation or related metabolic disorders and plasma endotoxemia or plasma LBP (9, 18, 25, 43, 64, 69). However, to our knowledge, nothing to date is known about the importance of the composition of dietary fats and oils on such endotoxin-linked low-grade inflammation. The present data lead us to propose a relationship between the lipid composition

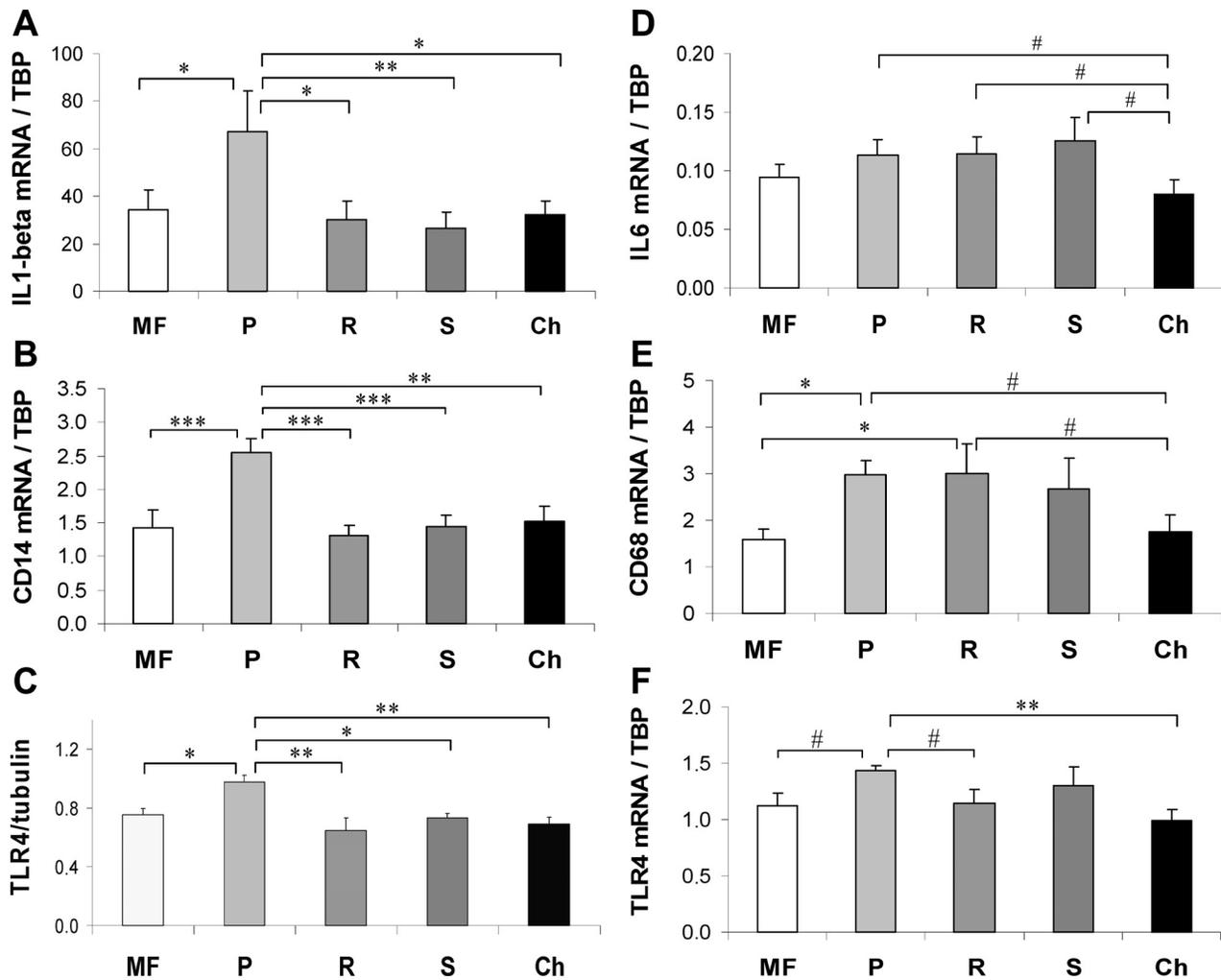


Fig. 3. Inflammation and macrophage markers and endotoxin membrane receptors in white adipose tissue (WAT) in mice fed diet with MF, P, R, S, or Ch; RT-quantitative PCR results of mRNA expression ( $n = 7$ ). A: IL-1 $\beta$ . B: cluster of differentiation 14 (CD14). C: representative Western Blot analysis of Toll-like receptor 4 (TLR4)/tubulin in WAT ( $n = 4$  for densitometry analysis). D: IL-6. E: cluster of differentiation 68 (CD68). F: TLR4. Data are means  $\pm$  SE. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , # $P < 0.09$ . ANOVA followed by Fisher test. TBP, TATA box-binding protein.

of a high-fat diet and inflammation in connection with endotoxin receptors.

We first submitted mice to four different high-fat diets enriched with milk fat, palm oil, rapeseed oil, and sunflower oil compared with regular chow. Then, we examined the role of major dietary FAs on adipose tissue inflammation by using a model of adipose cells. The studied FAs differed mainly by the number of carbons and unsaturations, and the studied oils differed by their FA profile and by the position of FAs on the triacylglycerol backbones (17, 51). Two hypotheses can be formulated. 1) The different FAs can be transported and metabolized by different mechanisms, leading to different inflammatory processes, depending on their nature (long-chain saturated FAs; n-6 vs. n-3 FAs); and/or 2) the different dietary FAs can induce some metabolic changes that will secondarily lead to changes in lipid and LPS absorption and transport and ultimately contribute to inflammation.

Our results show that, depending on oil composition in the diet, inflammation and endotoxin transporters were modulated differently. We showed in vitro that palmitic acid elicited the most important effect on IL-6 secretion by adipocytes, even

if acute in vitro effects cannot reflect in vivo mechanisms. Moreover, we showed in vivo that the palm oil-enriched diet induced higher IL-6 than other diets, including chow. This is consistent with epidemiological observations suggesting a positive association between diet and plasma IL-6 concentrations (5). Inflammation onset was not correlated with body weight gain. The n-6 FAs are reported to be proinflammatory, whereas n-3 FAs are anti-inflammatory (28, 61). However, in this study, the FA status in WAT-PL did not appear to contribute to plasma and WAT inflammation either; indeed, no differences were observed for the percentage of arachidonic acid between the four groups, and the ratio of n-6/n-3 FA was not correlated with the measured markers of circulating and WAT inflammation. Also, palmitate concentration in plasma was not correlated with inflammation because circulating palmitate was similar in the P, MF, and Ch groups. Moreover, when LPS was added to FAs in vitro, we observed the highest IL-6 production for palmitic and  $\alpha$ -linolenic acids. These results support the concept of a synergistic action of LPS and FAs on the secretion of proinflammatory cytokines.

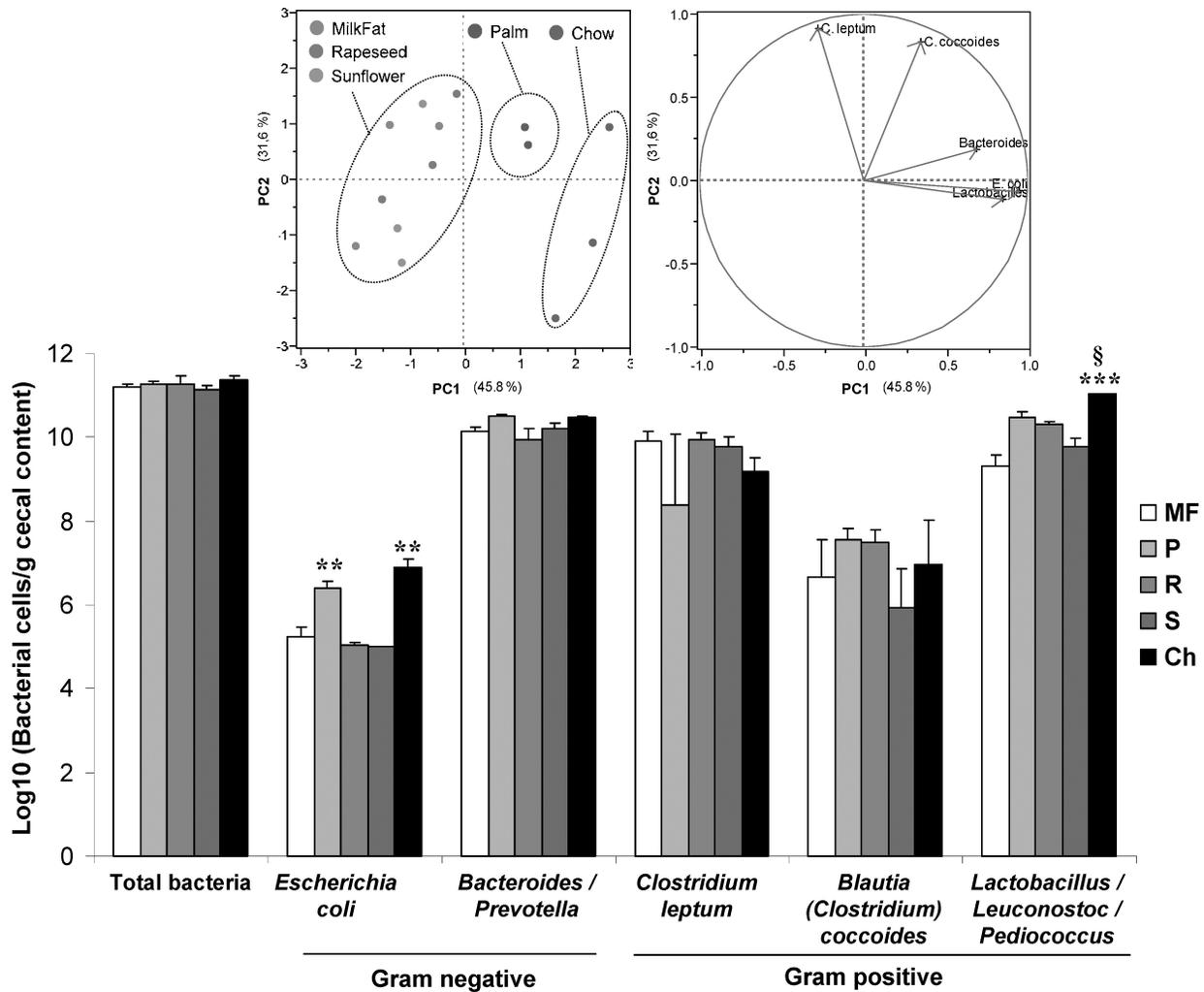


Fig. 4. Groups of bacteria in cecal content of mice fed diet with MF, P, R, S, or Ch. Bacterial numbers are expressed as log<sub>10</sub> (bacterial cells/g cecal content wet wt)  $\pm$  SE;  $n = 3$ . *Bottom*: the LPS-containing bacteria (gram negative) are highlighted. Bifidobacteria and *F. prausnitzii* were measured but not detectable. *Top*: principal component analysis highlighting global differences among groups. \*\* $P < 0.01$  vs. MF, S, and R. \*\*\* $P < 0.01$  vs. MF, S, and R. § $P < 0.05$  vs. P. Data were compared using Student's *t*-test.

We further investigated *in vivo* endotoxin metabolism, depending on dietary oil composition. Indeed, proinflammatory LPS have been reported to be able to diffuse from the gut to the bloodstream during high-fat diets either by direct diffusion through intestinal paracellular permeability or through absorption by enterocytes during chylomicron secretion (10, 27, 39). In this study, the presence of LPS inside jejunal enterocytes was indeed detected by electronic microscopy in all groups.

In this context, LBP and sCD14 appear to be key players in determining the inflammatory response to plasma LPS exposure. LBP and sCD14 are reported to function as molecules that shuttle LPS, having both activatory and suppressive effects via mCD14 activation and HDL-mediated clearance, respectively (35, 70). Very recently, a higher LBP/sCD14 ratio has been observed in patients suffering from gut-derived inflammation compared with controls (37).

LBP is actually increasingly used as a marker of metabolic endotoxemia in clinical and rodent studies (43, 53, 56, 64, 69) because it is a major proinflammatory LPS transporter in plasma. In our study, the P group was the only group to present significantly higher LBP compared with Ch and higher than

other high-fat groups. Moreover, in P group, a strong correlation between plasma LBP and plasma IL-6 was observed. The P group was also the only one to present higher inflammation in plasma and IL-1 $\beta$ , TLR4, and CD14 expression in WAT compared with Ch. Consistently, we highlight that mice of the P group also had a greater LBP/sCD14 ratio than mice fed other diets. LBP is an acute-phase protein (72) whose plasma concentration increases during inflammation (23) and that delivers circulating LPS to CD14 or HDL (67). During an acute inflammatory response, the LBP/sCD14 ratio is known to increase (67). Moreover, the binding of LPS to LBP-sCD14 conducts to the activation of proinflammatory cells via the TLR4 pathway, which results in production of proinflammatory markers, including IL-6 and IL-1 $\beta$ . Thus the LPS induce a higher inflammatory reaction when bound to LBP than in the free unbound form (48, 71). Moreover, we cannot rule out the possibility that LBP increase in P group was secondarily enhanced due to sensitivity to overall inflammatory status; IL-6 and IL-1 $\beta$  are indeed reported to induce the expression of LBP by airway epithelial cells (63), and IL-22 induces LBP in hepatocytes (78).

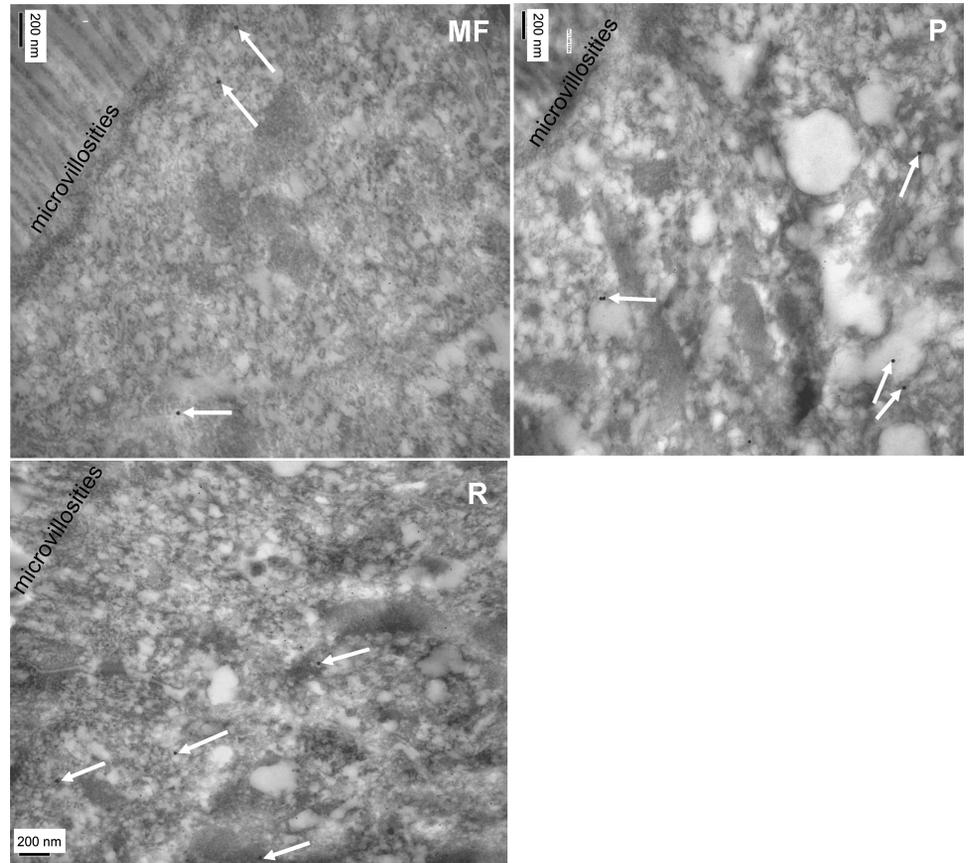


Fig. 5. Electronic micrograph of a jejunal section of mice fed diet with MF, P, or R (point examples representative of images obtained on 40 enterocytes/group). White arrows show gold beads linked to *E. coli*-LPS.

In turn, sCD14 recently appeared to provide protective effects against LPS response (20, 79). An excess of sCD14 in plasma is actually reported to buffer the inflammatory signals, avoiding LPS exposure with cell-anchored mCD14 (20). Fernández-Real et al. (20) revealed recently that, in mice, treatment with recombinant human sCD14 improves glucose tolerance following a high-fat diet and decreases IL-1 $\beta$  expres-

sion in epididymal WAT. Similarly, in the present study, high sCD14 in the R group was associated with low IL-1 $\beta$  expression in WAT. In humans, the administration of sCD14 is now suggested as a therapeutic strategy to ameliorate phenotypes associated with the metabolic syndrome (20). Also, the increased ability of plasma to eliminate endotoxins by sCD14 after gastrointestinal surgery was reported to be more important than endotoxemia itself in patient clinical outcome; besides, sCD14 levels were beneficially increased by using a nutritional strategy in these patients to improve their clinical outcome (79). Altogether, in our study, using dietary lipid modifications we observe that the lower inflammation of the R group compared with the P group is associated with significantly higher sCD14 in the R group despite apparently elevated metabolic endotoxemia.

In our diets, plasma endotoxemia was not associated with fat content in the diet (22 vs. 3%) but rather with lipid quality, with plasma endotoxemia being on the whole higher in the unsaturated R and S groups. In the literature, Amar et al. (4) have shown that plasma endotoxemia was lower after the control diet (~2 EU/ml) compared with the 35% fat/37% carbohydrate/28% protein diet (~3 EU/ml) or high-fat (72%)/high-protein (28%) diet (~6 EU/ml). Cani and colleagues (9, 11, 14) reported higher plasma endotoxemia in the high-fat (in the range of 5–10 EU/ml) vs. the chow diet (<5 EU/ml). Importantly, these authors used high-fat diets that were very different from the present study, i.e. up to 72% energy as fat and almost devoid of carbohydrates (9, 10, 12, 13). In contrast, our diets contained 38% energy as fat, and the nonfat components were balanced using chow ingredients. The latter include

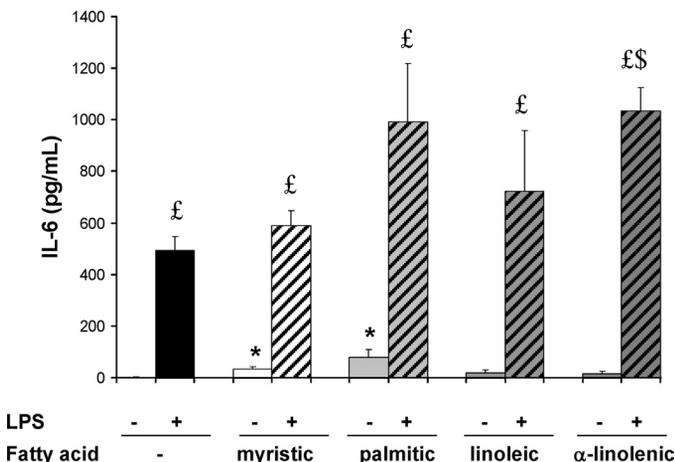


Fig. 6. IL-6 (pg/ml) in 3T3-L1 adipocytes treated for 24 h with myristic acid, palmitic acid, linoleic acid,  $\alpha$ -linolenic acid, or control medium. \* $P < 0.05$ , myristic acid vs. control medium, linoleic acid, and  $\alpha$ -linolenic acid and palmitic acid vs. control, linoleic acid, and  $\alpha$ -linolenic acid;  $\$P < 0.01$   $\alpha$ -linolenic acid + LPS vs. control + LPS. £ $P < 0.001$ , fatty acids vs. fatty acids + LPS. Data were compared using Student's *t*-test. Data are means  $\pm$  SE;  $n = 3$ .

wheat and barley that can contain nondigestible fermentable carbohydrates reported to be beneficial for the gut barrier and to lower endotoxemia (11). Because all of our diets contained the same amount of such carbohydrates, our results suggest that oil composition in the diet may modulate their endotoxemia-lowering effects.

However, importantly, plasma endotoxemia is transient in nature, with a reported half-life of LPS in plasma of only <8 min (49) because of LPS uptake by various receptors and transporters in plasma (18, 39). In sepsis, the severity of inflammatory reaction cannot be correlated to the blood levels of endotoxins (33). Indeed, endotoxins do not necessarily have to be detected in blood to confirm that endotoxins have been absorbed into blood, and this is mainly because plasma endotoxemia fluctuates during the day and during digestion (11, 18, 26, 39). Moreover, even using great caution such as sample heating, there are known difficulties in removing interference in blood regarding endotoxemia analysis (59). According to Sun et al. (69), the short half-life of LPS and the disadvantages associated with the LAL assay have limited its potential applications in routine clinical settings and large-scale studies.

Therefore, the observed differences in plasma endotoxemia between the R group and other groups in this study may have been enhanced by a combination of 1) diurnal variations in detectable LPS concentrations and 2) different digestion times at the moment of euthanization and blood sampling. Indeed, mice were euthanized in the fed state to be in the phase of intestinal lipid absorption, where acute LPS absorption had been described (18, 27, 39). Conversely, having a relatively long half-life and reliable measurements, LBP might serve as a marker reflecting an "effective" LPS level and innate immune response triggered by LPS (69). As such, LBP is reported to be a marker better reflecting long-term exposure to endotoxins rather than plasma endotoxemia itself (18, 33, 60, 69).

Altogether, our observation of different LBP/sCD14 ratios after different high-fat diets is an indicator of specific metabolic adaptations to circulating endotoxin transport that can either efficiently trigger inflammation (high LBP in the P group) or prevent inflammation (high sCD14 in the R group). The different inflammation profiles between the R and P groups may be due to the different impacts of these diets on the ability of plasma to bind endotoxins with a more important contribution of sCD14 compared with LBP for the R group. Importantly, elevated plasma levels of LBP have been reported recently to be associated with obesity, metabolic syndrome, type 2 diabetes, and cardiovascular mortality in humans (43, 69). Therefore, our results support such a role of LBP in metabolic disorders, which could be modulated by the quality of dietary fats and oils.

Consistent with LBP plasma levels, we show that TLR4 and CD14 expression in epididymal adipose tissue were the highest following the palm-rich diet. This result suggests that the WAT of the P group was more able to respond to circulating LPS by favoring the transduction of the inflammatory signal. It is important to note that 1) TLR4 and CD14 expression in WAT are modulated, depending on oil composition of the diet, and 2) this profile of TLR4 and CD14 expression in WAT among the different groups is similar to the plasma IL-6 profile.

The observed difference in plasma IL-6, depending on the dietary oil composition, can be explained by different mechanisms. First, the ability of long-chain saturated FAs to trigger

inflammation via TLR4 was already shown in vitro (16, 66, 68, 74). Actually, TLR4 is known to be a potential candidate to initiate inflammation and insulin resistance via its activation by FAs (3, 40, 66). However, in this study, palmitic acid alone cannot explain differences in inflammation via TLR4 activation because plasma palmitate levels were not higher in the P group compared with the Ch and MF groups. Thus, evidence converges on the possibility that both FAs and LPS can activate TLR4 and trigger inflammation in different tissues. Second, in vitro results show that differences in IL-6 release by adipose cells may be provoked by a synergistic action of LPS and FAs. However, in vivo, differences of WAT inflammatory status regarding IL-1 $\beta$  were observed, whereas IL-6 expression was modest, certainly because IL-6 is expressed mostly in macrophages that are diluted in WAT. Therefore, elevated plasma IL-6 in the P group cannot be explained directly by IL-6 production by WAT. However, it may be due to an indirect effect of IL-1 $\beta$  that is secreted by WAT on the secretion of IL-6 by the liver (47). Finally, we cannot rule out a possible contribution of gut microbiota on plasma inflammatory markers. Indeed, the literature shows that proinflammatory high-fat diets induce intestinal dysbiosis in C57Bl6/J mice, albeit with discrepancies such as decreased *Bacteroides* after 4 wk (58) or increased *Bacteroides* after 12 wk (62) using the same high-fat diet containing >60% of energy as lipids (mainly lard), caseins, and starch. In other studies with 72% of energy as lipids (mainly lard), *Bacteroides* and *Enterobacteriaceae* did not vary after 4 wk (9), whereas they decreased after 14 wk (11). In this study, with intermediate duration and lower fat intake using different fat sources, *Bacteroides* did not seem to vary. However, *E. coli* tended to be higher in the dominant populations of gut microbiota in the few palm oil-fed mice analyzed. Because *E. coli* is reported to increase in the case of intestinal inflammation (45), it would be interesting to investigate further whether high *E. coli* after high-fat diet can contribute to metabolic inflammation. In this respect, we must note that the Ch diet, which is richer in carbohydrates and lower in fat, shows a significantly different gut microbiota profile compared with the high-fat diets. Ch mice had higher numbers of *Lactobacilli* and LPS-containing *E. coli*; moreover, the *Bacteroidetes*, *E. coli*, and *Lactobacilli* on the PCA scores plot track with the Ch diet. Because Ch contains more carbohydrates, including nondigestible fermentable carbohydrates, than high-fat diets, this result highlights the importance of carbohydrate fermentation in regulating gut health and its subsequent implications for metabolic risk, in agreement with Cani et al. (11). *Bifidobacteria* have been reported to lower plasma endotoxemia and inflammation (11); however, this genus was undetectable in the presently studied mice. Altogether, considering the different reported effects of high-fat diets on gut microbiota, we raise the question of whether not only the amount but also the biochemical composition and biological activity of gut-derived LPS would be modified by the diet; this should be the subject of further studies.

Importantly, lipid metabolism is now recognized to interact with endotoxemia. Apart from endotoxin receptors characterized in the present study, HDLs are also reported to contribute to LPS clearance from plasma (67, 70). Thus it would be interesting to conduct studies on interactions between plasma lipoprotein metabolism and LPS receptors after different high-fat diets. Moreover, as discussed above, plasma endotoxemia

varies according to the feeding status of mice, and chylomicrons contribute to LPS transport in plasma. In the present study, plasma has been characterized in the fed state. To gain deeper knowledge of fasting and postprandial endotoxemia according to the diet, further studies should aim at measuring fasting and postprandial endotoxin metabolism after oil gavage following different high-fat diets. Dose response designs for the different high-fat diets would also allow us to conclude on the relative impact of fat composition and fat content on metabolic inflammation related to endotoxin receptors.

In humans, epidemiological and intervention studies often discuss that inflammation-related cardiovascular diseases would be positively related to consumption of saturated FA and negatively to consumption of polyunsaturated FA (1, 7, 15, 65, 77). However, recent data show that 1) substituting saturated FA with carbohydrates is inefficient or even deleterious regarding cardiovascular disease risk (50, 54) so that the quality of fat (saturated FA vs. polyunsaturated FA) should be discussed on an isolipidic basis and 2) saturated FAs correspond to different individual molecular species, including short- and medium-chain FAs whose health effects should be considered independently (42, 50). Both the isolipidic design and existing differences in saturated FA chain length in saturated fats (MF, P) were accounted for in the present study. Noticeably, medium-chain triglycerides were shown to protect rats from LPS-induced injury of gut and liver, contrary to corn oil (36). Moreover, saturated fat structure can be very different among fats and oils, including FA position on the triglycerides (51, 55). In this respect, we must note that a greater inflammation was observed with the palm oil diet compared with the milk fat diet. This indicates that low-grade inflammation induced by FAs and endotoxin transport by LBP and sCD14 is not a common characteristic of all saturated FA but specific of each fat composition. Moreover, rapeseed oil, sunflower oil, and milk fat were found to result in similar plasma levels of proinflammatory cytokine despite their different FA and TAG structures, which was associated with similar LBP/sCD14 ratio. Importantly, rapeseed oil is rich in  $\alpha$ -linolenic acid. This n-3 PUFA is described to present beneficial metabolic effects to prevent low-grade inflammation (61). The present results suggest a new beneficial effect of  $\alpha$ -linolenic acid-rich oils that can enhance plasma sCD14 and thus may buffer LPS and lower inflammatory response.

In conclusion, we demonstrate that compared with milk fat, rapeseed oil, or sunflower oil, a high-fat diet formulated with palm oil results in higher inflammation in plasma and adipose tissue and a higher circulating LBP/sCD14 ratio, reflecting proinflammatory endotoxin transport. In contrast, rapeseed oil resulted in lower inflammation through higher levels of sCD14 despite elevated endotoxemia. Therefore, the onset of high-fat diet-induced inflammation in mice appears to depend on dietary fat composition partly through plasma endotoxin receptors, and transporters sCD14 and LBP oriented toward TLR4 and CD14 activation rather than being directly correlated with gut-derived plasma endotoxemia. Thus nutritional strategies can be envisaged by optimizing dietary lipid sources to blunt the metabolic path involving endotoxin transport that contributes to low-grade inflammation in metabolic diseases.

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## DISCLOSURES

The authors have no conflict of interest to declare.

## AUTHOR CONTRIBUTIONS

F.L. and M.-C.M. did the conception and design of the research; F.L., J.-P.F., P.D., E.L., A.G., C.O.S., C.S., J.L.-L., and M.-C.M. performed the experiments; F.L., J.-P.F., C.D., and M.-C.M. analyzed the data; F.L., J.-P.F., C.D., N.B.-H., J.B., N.P., H.V., and M.-C.M. interpreted the results of the experiments; F.L. and M.-C.M. prepared the figures; F.L. and M.-C.M. drafted the manuscript; F.L., J.-P.F., and M.-C.M. edited and revised the manuscript; F.L., J.-P.F., C.D., P.D., E.L., A.G., C.O.S., C.S., J.L.-L., N.B.-H., J.B., N.P., H.V., and M.-C.M. approved the final version of the manuscript.

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