Short-term adaptation of postprandial lipoprotein secretion and intestinal gene expression to a high-fat diet

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1Université Pierre et Marie Curie-Paris 6, UMR S 872, Les Cordeliers, 75006, Paris; 2INSERM, U 872, Paris; 3Université Paris Descartes, UMR S 872, Paris; 4Cardiovascular and Urogenital Center of Excellence for Drug Discovery, GlaxoSmithKline, Les Ulis; 5Université Paris-VII, CNRS UMR 7059, Paris; and 6Clermont Université; UMR CNRS6247; CRNH-Auvergne, Aubière, France

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Hernández-Vallejo SJ, Alqub M, Luquet S, Cruciani-Guglielmacci C, Delerive P, Lobaccaro J, Kalopissis A, Chambaz J, Rousset M, Lacorte J. Short-term adaptation of postprandial lipoprotein secretion and intestinal gene expression to a high-fat diet. Am J Physiol Gastrointest Liver Physiol 296: G782–G792, 2009. First published February 5, 2009; doi:10.1152/ajpgi.90324.2008.—Western diet is characterized by a hypercaloric and hyperlipidic intake, enriched in saturated fats, that is associated with the increased occurrence of metabolic diseases. To cope with this overload of dietary lipids, the intestine, which delivers dietary lipids to the body, has to adapt its capacity in lipid absorption and lipoprotein synthesis. We have studied the early effects of a high-fat diet (HFD) on intestinal lipid metabolism in mice. After 7 days of HFD, mice displayed normal fasting triglyceridemia but postprandial hypertriglyceridemia. HFD induced a decreased number of secreted chylomicrons with increased associated triglycerides. Secretion of larger chylomicrons was correlated with increased intestinal microsomal triglyceride transfer protein (MTP) content and activity. Seven days of HFD induced a repression of genes involved in fatty acid synthesis (FAS, ACC) and an increased expression of genes involved in lipoprotein assembly (apoB, MTP, and apoA-IV), suggesting a coordinated control of intestinal lipid metabolism to manage a high-fat loading. Of note, the mature form of the transcription factor SREBP-1c was increased and translocated to the nucleus, suggesting that it could be involved in the coordinated control of gene transcription. Activation of SREBP-1c was partly independent of LXR. Moreover, HFD induced hepatic insulin resistance whereas intestine remained insulin sensitive. Altogether, these results demonstrate that a short-term HFD is sufficient to impact intestinal lipid metabolism, which might participate in the development of dyslipidemia and metabolic diseases.

enterocyte; chylomicron; lipid metabolism; SREBP-1C

IMPORTANT CHANGES IN HUMAN nutrition have occurred these last decades in most parts of the world, among which a significant increment in caloric intake and an increase in saturated fatty acid intake (8). These changes occurred concomitantly with a rise in metabolic diseases, such as obesity, metabolic syndrome, and diabetes, that are risk factors of atherosclerosis and cardiovascular diseases. Several studies have established that the development of these pathologies is associated with prolonged postprandial hypertriglyceridemia (12, 19, 26), even in patients without fasting hypertriglyceridemia (11). Dramatic postprandial hypertriglyceridemia is frequently associated with impaired catabolism by lipoprotein lipase (LPL) of triglyceride-rich lipoproteins (TRL) and/or uptake of the ensuing remnants by tissues mainly by the LDL receptor (31). Little attention was paid in these studies to the role that intestine, the first organ to face nutrients, could play in these metabolic disorders. Yet dyslipidemia may result from overproduction or modifications of the postprandial TRL secreted by the intestine. TRL synthesis and secretion are complex processes. Indeed, after hydrolysis of dietary fat and emulsification, resulting fatty acids and monoglycerides are taken up by enterocytes and used for triglyceride (TG) synthesis by the successive actions of monoacylglycerol acyltransferase and diacylglycerol acyltransferase at the membrane of the smooth endoplasmic reticulum (ER). After transfer in the ER lumen, TG droplets associate with primordial lipoproteins comprising apoB48 and phospholipids, through the action of microsomal triglyceride transfer protein (MTP), to form TRL. After further processing in the secretory pathway, mature chylomicrons are secreted into the lymph (17).

Changes in intestinal lipoprotein secretion have already been reported in the context of insulin resistance or diabetes, in animal models as in humans, involving enhanced activity of intestinal MTP in association with an oversecretion of apoB48 lipoproteins (23, 27). Such studies were performed when pathologies were already established. By contrast, little is known about early changes of intestinal lipid metabolism that could occur in response to high-fat diet. These potential changes deserve to be characterized, especially when considering the short lifetime of enterocytes, with a complete renewal of the intestinal epithelium within 3–4 days in mouse or 5–6 days in humans.

For this study, which intended to analyze whether such early changes in the intestinal function of dietary lipid transfer occurred, we used a diet enriched in cholesterol and in medium-chain fatty acid-containing coconut oil (Table 1), which is largely used as frying oil and in food manufacturing to provide firmness and texture. This diet was reported to induce fasting hypertriglyceridemia, hypercholesterolemia, and lipid accumulation in macrophages in rodent models (25, 33). It is known that short-chain fatty acids (C:10 and less) are directly transported through the enterocyte to circulation and are not inte-

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Table 1. Composition of experimental diets

<table>
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<tr>
<th></th>
<th>CD, g/100 g</th>
<th>HFD, g/100 g</th>
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<tr>
<td>Proteins</td>
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<td>Fibers</td>
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</tr>
<tr>
<td>Ash</td>
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<tr>
<td>Carbohydrates</td>
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<td>39.8</td>
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<td>Moisture</td>
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<td>Cholesterol</td>
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<tr>
<td>Fat</td>
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<tr>
<td>Caloric density, kcal/g</td>
<td>3.38</td>
<td>4.52</td>
</tr>
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</table>

Fatty acid composition, % of total fatty acids:

- 8:0
- 10:0
- 12:0
- 14:0
- 16:0
- 16:1
- 18:0
- 18:1
- 18:2

150 mM NaCl, 1 mM EDTA, and 2% protease inhibitor cocktail (Sigma-Aldrich). MTP activity was measured with an MTP assay kit (Roar Biomedical, New York, NY) by incubating, for different times at 37°C, 100 μg of proteins of intestinal epithelium homogenates with 4 μl of donor solution and 4 μl of acceptor solution (according to manufacturer’s instructions) in homogenization buffer (total volume 200 μl). Fluorescence was measured (485-nm excitation wavelength and 538-nm emission wavelength) at 37°C every 15 min for 115 min using the Fluostar Ascent FL (Labsystems, Paris, France).

Preparation of microsomes. The first third of the small intestine from five mice was excised (7–10 cm length), flushed with 10 ml of washing buffer (117 mM NaCl, 5.4 mM KCl, 2.6 mM NaHCO₃, 5 mM HEPES, 5.5 mM glucose, 0.96 mM Na₂HPO₄) via a syringe-attached catheter. Intestine was then everted and cut into 3-mm pieces that were then incubated with Matrisperse (BD Biosciences) at 4°C overnight. The intestinal epithelium was filtered and washed with PBS. The filtrate was homogenized in 0.25 M sucrose, 10 mM Tris-Cl, pH 7.5, 3 mM MgCl₂ and 2% protease inhibitor cocktail by 10 strokes up and down, 800 rpm at 4°C in a glass Potter homogenizer. The homogenate was centrifuged (300 g for 5 min at 2°C). The supernatant was then centrifuged (100,000 g for 1 h at 4°C) to obtain microsomes.

Extraction of nuclear proteins. The filtered epithelium (as described above) was homogenized with buffer A (0.02 M HEPES pH 7.9, 1.5 mM MgCl₂, 0.01 KCl, 0.05 M NaF, 2 mM orthovanadate, 0.5 mM DTT, 0.5 mM spermidine, 0.15 mM spermine, 5 μg/ml leupeptin, and 2% protease inhibitors), vortexed, and incubated at 4°C for 10 min and then centrifuged (3,000 rpm for 10 min at 4°C). The supernatant was removed and the pellet was homogenized with buffer B (0.02 M HEPES pH 7.9, 1.5 mM MgCl₂, 0.05 M NaF, 0.5 M NaCl, 2 mM orthovanadate, 0.5 mM spermidine, 0.15 mM spermine, 0.5 mM EDTA, 25% glycerol, 5 μg/ml leupeptin, and 2% protease inhibitor cocktail) and centrifuged at 13,000 rpm for 30 min at 4°C; the supernatant containing nuclear proteins was recovered and stored at −80°C.

Western blot analysis. The first third of the small intestine was excised (7–10 cm length), flushed with 10 ml of washing buffer. Intestine was then everted, rinsed with cold PBS, and scraped with a glass slide. The scraped tissue was homogenized by sonication in 1 ml lysis buffer containing 5% protease inhibitor cocktail (Sigma-Aldrich), 1% Triton X-100, 5 mM EDTA, and PBS.

Forty micrograms of total proteins or 2 μl of plasma (containing protease inhibitor cocktail) were loaded on 5% SDS-PAGE gels for apoB and E-cadherin detection or 12% SDS-PAGE gels for MTP and α-actin detection. For apoB, blots were probed by using goat polyclonal anti-human apoB antibody (1/10,000) (Chemicon) and then peroxidase-conjugated horse anti-goat IgG (1/10,000) (Vector Laboratories, AbCys). For MTP, blots were probed with a mouse monoclonal anti-mouse MTP antibody (BD Biosciences, 1/2,500) and then peroxidase-conjugated sheep anti-mouse IgG antibody (1/10,000) (Amersham). ApoB and MTP levels were normalized by α-actin or E-cadherin expression by use of mouse anti-actin monoclonal antibody (Chemicon, 1/2,000) or mouse anti-E-cadherin monoclonal antibody (Takara Bio, 1/2,500), respectively. For sterol regulatory element binding protein (SREBP)-1c, 80 μg of microsomal fractions and 60 μg of nuclear extracts were subjected to 12% SDS-PAGE and probed by use of mouse monoclonal anti-mouse SREBP-1c antibody. The mature form of SREBP-1c in nuclear extracts was normalized by C/EBPα expression (antibody anti-C/EBPα from Santa Cruz). The blots were developed with ECL Western blotting reagents according to the manufacturer’s instructions (Amersham). Films were scanned and quantified by using Image-Quant software (Molecular Dynamics).

Analysis of postprandial lipoprotein secretion. After 4 h of fasting, between 8:00 and 12:00 AM mice fed CD or HFD received 150 μl of coconut oil bolus by gavage. For each feeding condition, five mice were killed before the lipid bolus, to determine baseline parameters...
time 0), and at the indicated times after bolus (30, 60, 90, 180, and 270 min).

To determine lipoprotein secretion, mice received an intraperito-
neal injection of 1 μg Tyloxapol WR-1339 (Sigma-Aldrich) per gram of
body weight and 30 min later mice were force fed with 150 μl of
coconut oil containing [1-C14]oleic acid (2 μCi, specific activity 50
μCi/μmol, 8.17% of total fat of the bolus) (PerkinElmer). Two hours
after Triton injection, animals were euthanized. EDTA plasma from
10 mice per group were pooled. Chylomicrons were prepared by
ultracentrifugation at 10,000 g for 30 min at 20°C. A second ultra-
centrifugation was performed at 100,000 g for 18 h at 10°C to isolate
VLDL. Lipids were extracted from each fraction and separated by
TLC as previously described (39). After autoradiography, radioactive
spots corresponding to TG were excised and counted in a scintillation
counter (Beckman).

RNA extraction and semiquantification by real-time RT-PCR. Total
RNA was extracted from liver and scraped mucosa of small intestine
by using Tri Reagent (Euromedex) according to the manufacturer’s
instructions. cDNA was synthesized from 1 μg of total RNA in 20 μl
by using random hexamers and murine Moloney leukemia virus
reverse transcriptase (Invitrogen, Cergy Pontoise, France) as recom-

Table 2. Sequence of primers for semiquantitative RT-PCR

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<th>Gene</th>
<th>mRNA Transcript Size</th>
<th>Primer Sense 5'→3'</th>
<th>Primer Antisense 5'→3'</th>
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Animals were individually housed after surgery and their body
weight was monitored daily. Animals were excluded from the study at
day 6 postsurgery if weight loss was greater than 10% of presurgery
weight. After being fasted for 6 h, awake animals were placed
unrestrained in their home cage for the duration of the clamp ex-
periment. After a bolus infusion of 5 μCi of [3-3H]glucose (Amersham
Biosciences) tracer solution and 80 μM/kg insulin (1), the tracer was
infused continuously (0.025 μCi/min, at a constant rate of 5 μl/min)
for the duration of the experiment and insulin infusion was kept
constant at 0.2 IU·kg⁻¹·h⁻¹ (3.33 mU·kg⁻¹·min⁻¹). Blood glucose
levels were determined from tail blood samples (5 μl) at t = 0 and
then every 15 min (glucose analyzer Accu-chek, Roche). Steady state
was ascertained when glucose measurements were constant for at
least 20 min at a fixed glucose infusion rate, and this was achieved within
50 to 80 min. At steady state, two blood samples (25 μl) were
collected for determination of basal parameters, followed by a bolus
injection of 2-deoxy-D-[1-14C]glucose (2DG) (5 μCi, Amersham). Blood
samples (20 μl) were collected from the tail at 0, 5, 10, 20, 30,
40, 50, and 60 min until the end of the experiment, when mice were
killed by pentobarbital injection and tissues were collected. Basal and
steady-state plasma [3-3H]glucose radioactivity was measured as
described (21). Tissue glucose turnover rate (mg·kg⁻¹·min⁻¹) was
calculated as described (21). In vivo glucose uptake (ng·mg of
muscle⁻¹·min⁻¹) for muscle (tibialis anterior, soleus, extensor
digitorum longus), white adipose tissue (subcutaneous, periepi-
didymal, visceral), intestinal muscle and intestinal epithelium was
calculated on the basis of the accumulation of 2DG-6-phosphate in
the respective tissue and the disappearance rate of 2DG from
plasma as described (21).

For [3-3H]glucose determination, plasma was deproteinized
with Ba(OH)₂ and ZnSO₄. For each sample, an aliquot of the supernatant
was counted directly and another was dried to remove 3H₂O. Plasma
3H₂O was determined as a difference between dried and undried
samples. Immuno reactive insulin was determined as described (21).

Glucose rates of appearance (Rₐ) and disappearance (Rₜ) were
determined by using Steel’s non-steady-state equation. Endogenous
glucose production (EndoRₐ, given as mg·kg⁻¹·min⁻¹) was deter-
mined by subtracting the glucose infusion rate from total Rₐ. Glyco-
lytic rates were estimated from the increment per unit of time of
3H₂O multiplied by the estimated body water divided by [3-3H]glucose
specific activity. 3H₂O appearance was determined by linear regres-
sion of the measurement at t = 80 to t = 120 min. Body water was
assumed as 60% of body weight.
Statistical analysis. Results are given as means ± SE. Statistical analysis was performed with Excel software (Microsoft), and differences were determined with the t-test for nonpaired samples.

RESULTS

Effects of a short-term HFD on body weight and plasma parameters. C57Bl/6 mice were fed, for 7 days, either a CD with lipids accounting for 13% of total energy or HFD with lipids accounting for 48% of total energy and 0.15% (wt/wt) cholesterol. As shown in Fig. 1A, weight gain over 7 days was similar with both diets. In the HFD group, fasting plasma total cholesterol and nonesterified fatty acids were increased (Fig. 1, C and F), without significant change in fasting triglyceridemia (Fig. 1B). In addition, glycemia and insulinemia were also increased, indicating that a short-term HFD might have a possible effect on insulin sensitivity (Fig. 1, D and E). Moreover, analysis of lipoprotein profile of fasting mice plasma showed that the increase in plasma cholesterol was correlated with increased in HDL-cholesterol (data not shown). Since mice do not naturally express cholesteryl ester transfer protein, the normal fasting TG-cholesterol and the increase of HDL are consistent with an adequate LPL activity, as was previously described (37).

Short-term HFD induces changes in postprandial TRL. To characterize the postprandial kinetics of TRL after a short-term HFD, mice were fasted for 4 h and then received an oral lipid bolus of coconut oil. In mice fed CD, triglyceridemia increased moderately 30 min after the oral lipid load and decreased rapidly thereafter (Fig. 2A). This modest peak of TG, which differs from the elevated peak occurring 3 h after the oral load

![Graphs showing weight gain, triglycerides, cholesterol, glucose, insulin, and NEFA levels for CD and HF diets.](http://ajpgi.physiology.org/)

Fig. 1. Body weight and plasma parameters during short-term, high-fat (HF) diet (HFD). Individually housed mice, were fed either a chow diet (CD) or a HFD during 7 days. Body weight was measured every day. Weight gain (A) represents the difference between the initial weight and the weight at the end of the regimens. Plasma triglycerides (B), cholesterol (C), glucose (D), insulin (E), and nonesterified fatty acids (NEFA; F) were measured at 12:00, after 4 h of fasting. Data are means ± SE obtained from 15–20 mice. *P < 0.05, **P < 0.01, ***P < 0.001 HFD vs. CD.
of long-chain fatty acids (6), may be due to the use of coconut oil, composed of ~60% of medium-chain fatty acids that are in part directly delivered to the portal vein and in part incorporated into chylomicron-associated TG (24). CD animals also exhibit an early increase in apoB-100 protein, which has an exclusive hepatic origin, coincident with an increased apoB48 (Fig. 2, B and C). These results suggest that, in mice fed CD, liver has the capability to secrete apoB-100-containing particles relatively early by using the fatty acids delivered in portal vein. Seven days of coconut oil HFD resulted in dramatic changes of postprandial triglyceridemia, without affecting fasting TG levels (Fig. 1). Plasma TG peaked between 60 to 90 min and the peak was 1.6-fold higher than in CD-fed mice (Fig. 2A), suggesting that, after a short-term HFD, enterocytes and/or hepatocytes may become able to efficiently use medium-chain fatty acid for TG synthesis. Nevertheless, after 7 days of HFD, the increase of triglycerides occurred within 60 min after lipid bolus, a too short time lapse for the digestion of TG from bolus, the intestinal absorption of resulting fatty acids and monoglycerides, the liver uptake of fatty acids, the synthesis of triglycerides, and the secretion of corresponding VLDL. Moreover, apoB-100 levels in HFD mice at 60 min and 90 min after the bolus were not significantly different from basal apoB-100 at 0 min (Fig. 2C), arguing in favor of an intestinal origin of the observed TG peak.

At the same time, fasting and postprandial apoB48 and apoB100 were assessed in CD or HFD fed mice. In CD mice, apoB48 peaked between 30 and 60 min after the lipid bolus, then decreasing below the fasting levels between 90 and 270 min (Fig. 2B). Interestingly, HFD mice displayed lower fasting apoB48 levels compared with CD (Fig. 2B, time 0) and dramatically different kinetics after the bolus: a small increase at 30 and 60 min, and sustained levels of corresponding apoB48 between 90 and 270 min, which are higher than CD mice. At the same time, fasting apoB100 was considerably lower in HFD mice than in CD mice, with the virtual absence of an early peak at 30 and 60 min (Fig. 2C). These results suggest that elevated postprandial triglyceridemia after 7 days of HFD is likely associated with apoB48-containing TRL rather than apoB100-containing TRL.

Short-term HFD induces increased intestinal synthesis of triglycerides and decreased number of secreted chylomicrons. To measure TRL secretion, mice were injected with Triton WR-1339, which inhibits lipoprotein lipase activity and thus TRL catabolism. In addition, mice were force fed with a bolus of coconut oil. To avoid bias due to the direct transfer of medium-chain fatty acid to portal vein and to strictly quantify intestinal TG synthesis, long-chain [1-14C]oleic acid was used as a radiolabeled tracer. This does not preclude that differential incorporation of the two types of fatty acid into the particle during assembly and differential removal during catabolism in the two dietary groups may occur. Ninety minutes after bolus, postprandial TRL were isolated by sequential ultracentrifugation to separate chylomicrons of intestinal origin and VLDL of both hepatic and intestinal origins.

The apoB48 content of the chylomicron fraction was lower in HFD compared with CD mice whereas newly synthesized [1-14C]TG and total TG were greatly increased (Fig. 3, A–C). By contrast, there was no significant change in the amounts of apoB48 and newly synthesized [1-14C]TG in the VLDL frac-
Levels were measured in chylomicrons and VLDL. This decrease in total VLDL-TG after 7 days of HFD (Fig. 3) was set at 100%, whereas the total VLDL-TG content was even decreased in mice fed HFD compared with CD.

**Short-term HFD increases apoB isoforms, total triglyceride (TG), and [1-14C]triglyceride of chylomicrons and VLDL.**

Mice were fed CD or HFD for 7 days. At 3 h 30 min after food withdrawal, mice received an intraperitoneal injection of Triton WR-1339 (1 µg/g body wt) and 30 min later received a bolus of coconut oil (150 µl) containing [1-14C]oleic acid (OA). Ninety minutes later, blood was collected from the abdominal vein. Plasma from 10 mice was pooled and lipoproteins were separated by sequential ultracentrifugation. The level of apoB48 in chylomicrons and VLDL was analyzed by Western blot and quantified by densitometric scanning. The level of apoB48 in CD fed mice was set at 100%. Lipids were extracted from chylomicrons and VLDL and, after separation by TLC, [1-14C]triglyceride was counted. Therefore, these results suggest that the early intestinal response to a lipid overload consisted of an increased TG synthesis, a decreased secretion of chylomicrons overloaded with TG, and a maintained or increased secretion of apoB48-containing TRL in the density range of VLDL.

**Short-term HFD increases MTP amount and activity.** We then analyzed whether the 7 days of HFD induced an augmentation of MTP activity that would be consistent with increased chylomicron lipidation. Results obtained with microsomal fractions of the intestinal mucosa showed that both MTP amount (Fig. 4A) and activity (Fig. 4B) were increased in mice fed HFD compared with CD.

**Short-term HFD induces hepatic but not intestinal resistance to insulin.** As shown in Fig. 1, HFD induced fasting hyperinsulinemia that might reveal insulin resistance. However, this result could not explain easily the effects on intestinal lipid metabolism. Indeed, it has been recently reported that a bolus of insulin decreased plasma apoB48 (7), whereas increased intestinal MTP mass and activity have been correlated with insulin deficiency or insulin resistance (10, 34). To evaluate the consequences of the short-term HFD on insulin sensitivity, we performed hyperinsulinemic-euglycemic clamp analysis. In this experiment, the determination of glucose infusion rate (GIR) required to maintain euglycemia, when insulin is maintained high (0.2 IU·kg⁻¹·min⁻¹), allows the determination of EndoR₈ by subtracting the GIR from the R₈. Under hyperinsulinemia, endogenous glucose production, measured at steady state, reflects insulin’s ability to suppress hepatic glucose production. As shown in Fig. 5, a short-term HFD is sufficient to induce hepatic insulin resistance, since EndoR₈ was higher in mice fed HFD compared with mice fed CD (18.84 ± 1.15 vs. 14.45 ± 0.85 mg·kg⁻¹·min⁻¹, P = 0.02). However, whole body glucose uptake was similar in both groups (29.36 ± 0.99 vs. 26.79 ± 0.49 mg·kg⁻¹·min⁻¹, not significant), and the insulin-stimulated glucose uptake rates in skeletal muscle, adipose tissue, intestinal epithelium, and liver or intestine, it is not possible to distinguish in plasma the proportions of apoB48-containing VLDL originating from liver or intestine.

Whatever the case, our results showed changes in the distribution of postprandial TRL between chylomicrons and VLDL under a short-term HFD. By contrast, Western blot analysis revealed no difference in the amounts of apoB48 in the intestinal mucosa after 7 days of HFD compared with CD (0.98 ± 0.08 vs. 0.87 ± 0.05 au, respectively; P = 0.28).

Under hyperinsulinemia, endogenous glucose production, measured at steady state, reflects insulin’s ability to suppress hepatic glucose production. As shown in Fig. 5, a short-term HFD is sufficient to induce hepatic insulin resistance, since EndoR₈ was higher in mice fed HFD compared with mice fed CD (18.84 ± 1.15 vs. 14.45 ± 0.85 mg·kg⁻¹·min⁻¹, P = 0.02). However, whole body glucose uptake was similar in both groups (29.36 ± 0.99 vs. 26.79 ± 0.49 mg·kg⁻¹·min⁻¹, not significant), and the insulin-stimulated glucose uptake rates in skeletal muscle, adipose tissue, intestinal epithelium, and liver or intestine.

**Short-term HFD increases microsomal triglyceride transfer protein (MTP) amount and activity.** A: 40 µg of intestinal microsomal proteins, prepared from mice fed HFD or CD for 7 days, were separated by SDS-PAGE and immunoblotted with antibody against MTP and α-actin. Representative Western blot (from 3 independent experiments) shows increased MTP mass in microsomal fraction. B: MTP activity in intestinal epithelium homogenates of mice fed HFD or CD for 7 days. Results are expressed as fluorescence (F) units·min⁻¹·µg of protein⁻¹; n = 5 mice for each condition. **P < 0.01, MW, molecular weight expressed as kDa.
Adaptation of intestinal gene expression under HFD. Changes in intestinal lipid metabolism might be associated with a coordinated control of gene expression. We therefore quantified the expression of genes involved in lipid and lipoprotein synthesis and secretion. As shown in Fig. 6A, HFD induced an increase in the expression of apoA-IV, apoB, and MTP genes and a decrease in the expression of apoC-III, FAS, and ACC genes in intestine. This pattern suggested that the short-term intestinal adaptation to HFD led to the inhibition of de novo fatty acid synthesis, as expected, and to the stimulation of lipoprotein assembly and secretion. In parallel, we quantified the expression of lipid-sensor or lipid-dependent transcription factor genes. We noted a dramatic increase in the expression of SREBP-1c in the intestine of HFD compared with CD mice. In addition, short-term HFD moderately raised LXRα expression in the intestine and had no effect on SREBP-2, HNF4α, PPARγ2, and PPARα. Besides, with the exception of FAS mRNA, no significant change was observed in the expression of these genes in liver (Fig. 6B), stressing that liver and intestine respond differently to a short-term HFD.

HFD induces an increased amount of SREBP-1c and its translocation to the nucleus. To address the question of whether the increased level of intestinal SREBP-1c mRNA was physiologically relevant, the quantification of SREBP-1c protein was performed in microsomal preparations and in nuclear
cholesterol addition, increased fasting triglyceridemia and cholesterolemia (15, 20, 36). Our study focused on the first steps of intestinal adaptation and postprandial response to HFD before establishment of pathologies.

We report for the first time that, during a short-term HFD, intestine adapts its postprandial secretion of TRL by decreasing the number of apoB48-containing particles in the chylomicron fraction and by increasing triglyceride synthesis and secretion. Since it is known that there is one molecule of apoB48 per chylomicron (29), this adaptation led to the secretion of a smaller number of larger-sized chylomicrons. Moreover, an increased intestinal MTP content and activity correlated with the increased lipolysis of chylomicrons. These results are interesting in the light of studies in rodents and humans reporting an increased postprandial intestinal lipoprotein secretion through the increased synthesis and secretion of both triglycerides and apoB48 after a long-term HFD containing long-chain fatty acids (3, 18, 40). To our knowledge, only one report described the effects of coconut oil on postprandial lipid metabolism and showed a greater increase in triglyceride response in rabbits fed a long-term coconut oil-containing regimen than in rabbits fed olive oil (38). However, in this report, regimens were administered for 4 wk. Therefore our results obtained after a short-term HFD suggest that intestinal adaptation to HFD has at least two phases: the first phase, which may be considered as an emergency step, is to increase the size of postprandial TRL to manage lipid overloading. This early adaptive response of intestine may also facilitate TRL catabolism since it is known that the activity of LPL, the enzyme responsible for the first and rate-limiting step of TRL-TG hydrolysis, depends on physicochemical characteristics of TRL, such as particle size and composition (2, 30, 41), large chylomicrons being more rapidly catabolized than smaller ones. A second phase of intestinal adaptation appears upon longer term maintenance of dietary lipid loading when intestine probably amplify lipid delivery efficiency by increasing numbers of postprandial apoB48-TRL as usually reported. However, we cannot exclude that the early changes we report in postprandial lipoproteins might be a specific effect of coconut oil on intestinal lipoprotein secretion or clearance. Further studies using different dietary fatty acids should be performed to address the question of whether these changes in postprandial lipoproteins are a more general phenomenon.

We observed that the early adaptation of intestine to HFD was associated with coordinated changes in the expression of genes involved in lipid metabolism, e.g., a markedly decreased expression of genes involved in fatty acid synthesis (FAS and ACC), and an increased expression of genes involved in TG transport and delivery (apoB and MTP) and/or signaling (apoA-IV). Several transcription factors have been described as modulators of lipid metabolism, among which the members of nuclear receptor family HNF-4, PPARα/β/γ, LXRα/β, farnesyl X receptor (FXR), and SREBP-1/2. We show that, after 7 days of HFD, the mature form of SREBP-1c is increased and translocated to the nucleus where it might be transcriptionally active, as suggested by the increase of SCD1 expression, one of its target genes. However, activation of SREBP-1c is not sufficient to explain all of the observed transcriptional changes in intestinal cells during HFD, such as the decreased FAS mRNA level or the increased MTP mRNA level (22, 32). Other transcription factors may also play a role in these effects, such as PPARα, which has been shown to decrease FAS expression and increase MTP expression.

**DISCUSSION**

It is commonly admitted that high-fat diets administered over several weeks induce features of metabolic syndrome, e.g., hypertriglyceridemia, hypercholesterolemia, and hyperglycemia, and increase the risk of developing diabetes and obesity. Several studies in human and animal models demonstrated that a long-term administration of coconut diet, which is enriched in medium-chain saturated fatty acids, with or without

Fig. 7. Short-term HFD increases precursor and mature forms of sterol regulatory element binding protein (SREBP)-1c in intestine. A: after preparation of microsomal fractions from intestinal mucosa of mice fed HFD or CD for 7 days, 80 μg of microsomal proteins were separated by SDS-PAGE and immunoblotted with antibody against SREBP-1c. Representative Western blot (from 3 independent experiments) shows precursor form of SREBP-1c (pSREBP-1c) in microsomal fraction. B: representative Western blot (from 2 independent experiments) shows levels of mature form of SREBP-1c (mSREBP-1c) in the nuclear extracts prepared from intestinal mucosa of mice fed HFD or CD for 7 days.
Fig. 8. Liver X receptor (LXR) and SREBP-1c activation after 7 days of HFD. After oral administration of LXR agonist T0901317 for 2 days, relative gene expressions were quantified by real-time PCR in liver (A) and intestine (B). C: relative intestinal gene expressions in LXRα/β knockout mice and their wild-type littermates fed CD (open bars) or HFD (solid bars) for 7 days. Values were normalized to the 18S rRNA and expressed in arbitrary units; n = 5 for each condition. *P < 0.05; **P < 0.01; ***P < 0.001 vs. CD.
as Sp1, NFY, or USF, which have been shown to bind to the proximal promoter of FAS and to control its transcription in response to dietary fatty acids in hepatocytes (9, 35), or HNF4, which is known to enhance the promoter activity of MTP gene (16). Further studies will clarify the mechanisms underlying the coordinated control of intestinal gene transcription during short-term HFD, a process that obviously involves complex interactions between transcription factors and coactivators or corepressors.

We report here that 7 days of HFD are sufficient to increase glycemia and insulinemia and to induce hepatic but not intestinal insulin resistance. Because it is known that insulin activates the transcription and the proteolytic maturation of SREBP-1c in several cell types (14), we suggest that activation of SREBP-1c in intestine may have resulted from the increased insulinemia, associated with a maintained sensitivity of intestine to insulin. Moreover, the decreased intestinal apoB48-TRL secretion could be related to the hyperinsulinemia and the intestinal insulin sensitivity. It was recently reported that a bolus of insulin induced a downregulation of apoB48-lipoprotein production in chow-fed hamsters. By contrast, during insulin-resistant state, intestine was not responsive to the inhibitory effect of insulin (7). However, in animal models and in humans, several studies have shown that diabetes or insulin resistance is associated with postprandial hypertriglyceridemia and with an increased secretion of apoB48-containing intestinal lipoproteins (5, 13, 28). Again, short-term HFD seems to be a particular period that could represent a transient state during which intestine is still sensitive to insulin and produces large chylomicrons whereas liver is already resistant to insulin but secretes less apoB100-containing lipoproteins. The physiological consequences of this short-term adaptation of intestinal lipid metabolism to HFD have to be evaluated. Indeed, it must be determined whether these changes represent the contribution of intestine to detrimental mechanisms that promote metabolic diseases and atherosclerosis or a beneficial adaptation that counteracts the lipid toxicity of high-fat diets.

In conclusion, the present study highlights the role that intestine plays, very early, in the adaptation to the fat content of the diet as well as in the control of postprandial triglyceridemia and questions the potential long-term consequences on metabolic diseases.

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REFERENCES


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