

Chronic high-fat diet affects intestinal fat absorption and postprandial triglyceride levels in the mouse

Valerie Petit,* Laurent Arnould,[†] Pascal Martin,[§] Marie-Claude Monnot,* Thierry Pineau,[§] Philippe Besnard,* and Isabelle Niot^{1,*}

Physiologie de la Nutrition,* Ecole Nationale Supérieure de Biologie Appliquée à la Nutrition et à l'Alimentation, Unité Mixte de Recherche Centre Européen des Sciences du Gout 5170-Centre National de la Recherche Scientifique/1214 Institut National de la Recherche Agronomique/Université de Bourgogne, Dijon; France; Department of Pathology,[†] Centre G-F Leclerc, Dijon, France; and Laboratoire de Pharmacologie et Toxicologie,[§] Institut National de la Recherche Agronomique, Toulouse, France

Abstract The effects of chronic fat overconsumption on intestinal physiology and lipid metabolism remain elusive. It is unknown whether a fat-mediated adaptation to lipid absorption takes place. To address this issue, mice fed a high-fat diet (40%, w/w) were refed or not a control diet (3%, w/w) for 3 additive weeks. Despite daily lipid intake 7.7-fold higher than in controls, fecal lipid output remained unchanged in mice fed the triglyceride (TG)-rich diet. In situ isolated jejunal loops revealed greater [¹⁴C]linoleic acid uptake without TG accumulation in mucosa, suggesting an increase in lipid absorption capacity. Induction both in intestinal mitotic index and in the expression of genes involved in fatty acid uptake, trafficking, and lipoprotein synthesis was found in high-fat diet mice. These changes were lipid-mediated, in that they were fully abolished in mice refed the control diet. A lipid load test performed in the presence or absence of the LPL inhibitor tyloxapol showed a sustained blood TG clearance in fat-fed mice likely attributable to intestinal modulation of LPL regulators (apolipoproteins C-II and C-III). These data demonstrate that a chronic high-fat diet greatly affects intestinal physiology and body lipid use in the mouse.—Petit, V., L. Arnould, P. Martin, M-C. Monnot, T. Pineau, P. Besnard, and I. Niot. Chronic high-fat diet affects intestinal fat absorption and postprandial triglyceride levels in the mouse. *J. Lipid Res.* 2007. 48: 278–287.

Supplementary key words small intestine • intestinal proliferation • lipid binding proteins • triglyceridemia

It is well established that fat overconsumption leads to obesity in a number of animal models, including mice (1). Although the small intestine is responsible for body fat disposal, its role in this phenomenon has been neglected. The fact that the small intestine has long been considered a simple selective barrier able to efficiently absorb dietary

fat explains this paradox. However, recent insights into intestinal physiology demonstrate that triglyceride (TG) absorption is more complex than initially believed. It is now well established that several membrane and soluble lipid binding proteins (LBPs) are involved in this process (2). By reason of their location throughout the enterocyte and their high binding affinity for long-chain fatty acids (LCFAs), LBPs are thought to play a direct or indirect role in each step of lipid absorption: uptake, trafficking, lipoprotein synthesis, and secretion. This physiological involvement has especially been highlighted by the generation of knockout mice. For instance, the invalidation of genes encoding the intestinal fatty acid binding protein (I-FABP) (3), the fatty acid transporter (FAT/CD36) (4), and the microsomal triglyceride transfer protein (MTP) (5) is associated with deep alterations of postprandial triglyceridemia. It is noteworthy that the gene expression of several intestinal LBPs is upregulated by LCFA through the activation of nuclear receptors, the peroxisome proliferator-activated receptors (PPARs) (2).

Therefore, although the intestinal functions of most LBPs remain elusive, it can be hypothesized that a high fat supply triggers a coordinated change in LBP expression, increasing intestinal absorption capacity. Moreover, it is well known that the intestinal epithelium is characterized by a dramatic cellular turnover, the whole mucosa being renewed every 3 days in the mouse (6). This organ exhibits a remarkable capacity to adapt its morphology to nutritional status. Fasting decreases cell proliferation, leading to a progressive atrophy of rat intestinal epithelium (7). Conversely, refeeding restores the proliferative activity,

Abbreviations: apoA-IV, apolipoprotein A-IV; FAT/CD36, fatty acid transporter; FATP-4, fatty acid transport protein 4; I-FABP, intestinal fatty acid binding protein; LBP, lipid binding protein; LCFA, long-chain fatty acid; L-FABP, liver fatty acid binding protein; LPL, lipoprotein lipase; MTP, microsomal triglyceride transfer protein; PPAR, peroxisome proliferator-activated receptor; TG, triglyceride.

¹To whom correspondence should be addressed:
e-mail: niot@u-bourgogne.fr

Manuscript received 30 June 2006 and in revised form 19 October 2006 and in re-revised form 10 November 2006.

Published, JLR Papers in Press, November 18, 2006.
DOI 10.1194/jlr.M600283-JLR200

dietary lipids being the strongest stimulators of mucosal regeneration (8). Together, these observations strongly suggest that the high TG bioavailability of gut might not be attributable to inborn properties but to acquired properties. To determine whether such a lipid-mediated adaptation exists, a chronic high-fat diet effect on absorption capacity of the small intestine was studied in mice. Because the absorption efficiency could affect intestinal TG secretion and/or clearance (4), lipid load tests were also performed to explore the high-fat intestinal effect on postprandial triglyceridemia. The data reported here show that the small intestine can adapt its absorption capacity to the fat content of the diet in the mouse. They provide evidence that the small intestine plays an active role in the regulation of triglyceridemia, especially during the postprandial state.

MATERIALS AND METHODS

Animals and experimental treatment

French guidelines for the use and care of laboratory animals were followed. Protocols were approved by the ethics committee of the University of Burgundy. Five week old male B6D2F1 mice weighing 20–25 g were purchased from Janvier. Mice were housed in a controlled environment that provided constant temperature and humidity and a period of darkness from 6 PM to 6 AM. Mice were acclimated individually in metabolic cages and fed a semipurified control diet for 1 week (Table 1). Then, mice were fed for 3 weeks a semipurified control diet containing 3% lipids (w/w), a high-fat diet containing 40% lipids (w/w) (Table 1), or refed the control diet for 3 weeks after the high-fat diet. The diets were nutritionally adequate, providing for all known essential nutrient requirements.

[1-¹⁴C]linoleic acid uptake in the jejunum

To study the fatty acid uptake capacity of small intestine, an in situ isolated jejunal loop was realized in mice subjected to a control diet, a high-fat diet, or the high-fat diet followed by the control diet. After 16 h of starvation, mice were anesthetized with isoflurane and laparotomized. A 10 cm segment of jejunum was isolated between two ligatures. The in situ isolated segment was infused with 0.5 ml of linoleic acid in 2.19 mM solution containing 10% [1-¹⁴C]linoleic acid (51 mCi/mmol; Perkin-Elmer Life Sciences, Inc.) emulsified with 10 mM taurocholic acid. After an incubation period of 5 min, the intestinal loop was removed and the intestinal lumen was collected. The mucosa from the in situ isolated jejunal segment was then scraped off. Lipids contained in lumen and mucosa were extracted by Delsal's method (9), and radioactivity was deter-

mined by liquid scintillation. The [1-¹⁴C]linoleic acid uptake by the jejunal segment corresponds to the difference between the quantity of [1-¹⁴C]linoleic acid infused and that remaining in the intestinal lumen at the end of the experiment.

Metabolic fate of [1-¹⁴C]linoleic acid in jejunal mucosa

A 100 μ l aliquot of lipids contained in mucosa was evaporated to dryness under a nitrogen stream. Pellet was dissolved in 40 μ l of chloroform, then separated by thin-monolayer chromatography in hexane-ethyl ether-glacial acetic acid (140:60:2, v/v/v). After migration, the different lipid species were identified according to standards, [³H]triolein, and [1-¹⁴C]linoleic acid. Then, the percentage of radioactivity found in TGs, fatty acids, diglycerides/monoglycerides, and phospholipids was quantified using a Berthold scanner.

Gene expression analysis using INRAArray 01.4

To identify gene expression changes in jejunal mucosa, removed according to previous procedures (10) in mice subjected to a high-fat diet compared with a control diet, the low-density microarray INRAArray 01.4 dedicated to lipid metabolism was used (11). The full list of 320 selected cDNA probes spotted onto the INRAArray 01.4 is available at www.inra.fr/Internet/Centres/toulouse/pharmacologie/lpt.htm. The two independent diet studies were analyzed (total of $n = 11$ /group). Total RNA from jejunal mucosa extracted with Trizol reagent (Invitrogen Life Technologies) was controlled with a Bioanalyzer 2100 (Agilent Technologies, Massy, France). For each sample, 3 μ g of total RNA along with a fixed amount of 12 spiked-in yeast RNAs (used for normalization) were labeled by reverse transcription with Superscript II reverse transcriptase (Invitrogen Life Technologies) in the presence of 40 μ Ci of [α -³³P]dCTP (ICN, Orsay, France). Radiolabeled cDNA purification as well as hybridization, washing, scanning, and image analysis with the INRAArray were performed according to previously described methods (11). Statistical analysis of microarray data was performed under R (www.r-project.org) using Bioconductor packages (www.bioconductor.org). Data were log-transformed and normalized by the mean log(signals) for the 12 spiked-in yeast RNAs. A total of 137 genes exhibiting log(signals) significantly above background intensities were further analyzed. An ANOVA with the experiment and diet factors was performed for each gene. Eighteen genes whose transcripts displayed a significant diet effect ($P < 0.01$) and exhibited at least a 1.5-fold change between the high-fat and control diets were declared differentially expressed. The expression data for these 18 genes were then transformed to Z-scores and clustered as a heat map using the Euclidean distance and the Ward criterion.

Northern blot analysis

Total RNA from jejunal mucosa of mice subjected to one of the three diets was extracted with Trizol Reagent (Invitrogen Life Technologies). Total RNA was denatured, subjected to electrophoresis on a 1% agarose gel, and transferred to a GeneScreen membrane (New England Nuclear) using 20-fold concentrated 3 mM NaCl and 0.3 mM trisodium citrate, pH 7 (NaCl-citrate). cDNAs of mouse fatty acid transport protein 4 (FATP-4) (12), mouse FAT/CD36 (a gift from Dr. P. Grimaldi, Nice, France), rat intestinal and liver fatty acid binding proteins (I-FABP and L-FABP; provided by Dr. J. I. Gordon, St. Louis, MO), and mouse apolipoprotein A-IV (apoA-IV; a gift from Dr. T. Pineau, Toulouse, France) were used as probes. MTP cDNA was obtained by Superscript II reverse transcriptase (Invitrogen Life Technologies). MTP primer sequences used were described by Sellers and Shelness (13). The reverse transcription reaction was carried

TABLE 1. Composition of the experimental diets

Ingredients	Control Diet	High-Fat Diet
Casein	21.8	13.5
Glucose + starch	61.6	38.1
Cellulose	5.8	3.6
Minerals	6.8	4.2
Vitamins	1	0.6
Lipids	3	40
Saturated fatty acids	0.3	4
Monounsaturated fatty acids	1.8	24
Polyunsaturated fatty acids	0.9	12

Values shown are in g/100 g of dry powder.

out at 48°C for 45 min followed by 95°C for 2 min. The conditions of PCR were 95°C for 30 s, 55°C for 1 min, 72°C for 1 min (35 cycles) and 72°C for 10 min. The expected size of PCR products was 699 bp for MTP. Probes were labeled with [α -³²P]dCTP (3,000 Ci/mmol; Amersham) by a Megaprime kit (Amersham). A 24 residue oligonucleotide specific for 18S rRNA was used as a probe to ensure that equivalent quantities of RNA were loaded and transferred. This oligonucleotide was 5' end-labeled with T₄ polynucleotide kinase and [γ -³²P]ATP (3,000 Ci/mmol; Amersham). Filters were prehybridized for 4 h and hybridized for 16 h at 42°C according to previously published procedures (14). Filters were washed successively twice in 2× NaCl-citrate at room temperature, twice in 2× NaCl-citrate with 1% sodium dodecyl sulfate at 60°C for 30 min, and finally once in 0.1× NaCl-citrate at room temperature. Autoradiograms were quantified with a calibrated densitometric scanner (Bio-Rad GS-800).

Real-time PCR analysis of jejunal and hepatic apoC-II and apoC-III mRNA levels

cDNA was reverse transcribed from 1 µg of total RNA pretreated with DNase [DNase I Amplification grade (Invitrogen Life Technologies) and Omniscript reverse transcriptase (Qiagen)]. cDNA was diluted to 25 ng/µl using sterilized water, and real-time PCR was done in duplicate with 1 µl of cDNA, 12.5 µl of SYBR Green PCR Master Mix (qPCR™ Mastermix Plus for SYBR® Green I Fluorescein; Eurogentec), 10.5 µl of distilled water, and 1 µl of forward and reverse primers (200 nM) for a final reaction volume of 25 µl. The primer sequences were as follows: apoC-II, 5'-ACTGGAGTGAGCCAGGATAG-3' and 5'-ACATCAGGATGAC-CAGGAAT-3'; apoC-III, 5'-TCAGATCCCTGAAAGGCTAC-3' and 5'-ATAGCTGGAGTTGGTTGGTC-3'. PCR was run on the iCycler iQ system (Bio-Rad Laboratories, Inc.) using the following conditions: 50°C for 2 min, 95°C for 10 min, and 45 cycles of 95°C for 15 s and 60°C for 30 s. The fluorescence measurement used to calculate threshold cycle (Ct) was made at 60°C. Quantification of data was done by the comparative $\Delta\Delta$ Ct method (15).

Determination of jejunal proliferative activity

After animals were euthanized, a 1 cm length of jejunum was taken from mice in each dietary group. Tissues were placed in 4% formalin overnight and incubated in 70% ethanol, in a graded series of ethanol from 80% to 100%, and finally in xylene before it was embedded in paraffin. Paraffin sections (4 µm thick) were cut perpendicularly to the mucosal surface and fixed to polylysine-coated slides. Proliferative activity was then measured by labeling of Ki-67 antigen, which is expressed in nuclei of all cells in G1, S, and G2 phases and mitosis. After removal of paraffin with xylene, sections were rehydrated in a graded series of ethanol from 100% to 95%, and endogenous peroxidase was blocked with 3% H₂O₂ in methanol. All sections were pretreated in a microwave oven in 10 mM citrate buffer at pH 6 for 10 min at 100°C. Unspecific binding sites were blocked using 10% sheep serum before an incubation of 2 h at room temperature with the primary antibody, the monoclonal mouse antibody TEC3 (M7248; DAKO). The antibody was then linked with biotinylated rat anti-mouse IgG secondary antibody (E0464; DAKO), which was then labeled with streptavidin conjugated to horseradish peroxidase (P0397; DAKO). Ki-67-positive cells were visualized by exposing the peroxidase to 3,3'-diaminobenzidine hydrochloride chromogen substrate (K3467; DAKO) followed by counterstaining in hematoxylin. Slides were washed with deionized water, dehydrated, cleared, and mounted with permanent medium (S3026; DAKO).

All cells in the active phases of the cell cycle stained brown except for G0-phase cells, which remained blue. To determine

mitotic activity, an average of 30 crypts were analyzed per animal. The labeling index (%) was defined as Ki-67-positive cells/total cryptic cells.

Biochemical analysis

Plasma samples were collected from the axillar vein on heparinized propylene tubes. Serum TG and free fatty acid concentrations were determined using a commercial kit (Biomerieux; Wako).

Stool lipids were extracted from an aliquot of feces (0.75 g) collected during the last 3 days of the control diet or the high-fat diet using the methanol/chloroform method (16). Resulting solvent was transferred to a balloon, evaporated under a nitrogen stream, placed in a vacuum desiccator overnight, and weighed. The weight difference between the starting empty balloon and the balloon containing the dried lipid was the fecal lipid amount. [¹⁴C]oleic acid (51 mCi/mmol) was added during lipid extraction to evaluate the efficiency of the process.

Lipids were extracted from mucosa and dried. Extracts were dissolved into 1% Triton X-100/chloroform, dried under vacuum, and redissolved in water for the determination of TG using a commercial kit (Biomerieux). The cellular protein content was determined by bicinchoninic acid assay (Pierce). The mucosal content of TG is expressed as mg TG/g protein.

Lipid load test

Mice were maintained on a control diet or a high-fat diet for 21 days. After a 16 h fast, mice were weighed and received an intragastric bolus of lipids (0.5 ml of oil). Blood samples were collected from the tail vein before gavage and at 0.5, 1, 2, 3, and 4 h after gavage. Plasma TG concentration was assayed using a commercial kit (Biomerieux). The same experiment was performed in mice previously subjected to an intraperitoneal injection of tyloxapol (Sigma) in saline (500 mg/kg body weight) to block LPL activity.

Statistical methods

The results are expressed as means \pm SEM. The significance of differences between groups was determined by Student's *t*-test.

RESULTS

High-fat feeding does not lead to steatorrhea

To determine whether the fat content of the diet can affect the TG absorption capacity of the small intestine, B6D2F1 mice, individually housed in metabolic cages, were

TABLE 2. Impact of a chronic high-fat diet on body mass, caloric and lipid intake, and fecal lipid excretion

Parameters	Control Diet (3% Fat, w/w)	High-Fat Diet (40% Fat, w/w)
Body mass (g)		
Before	19.97 \pm 0.6	19.97 \pm 0.70
After	22.96 \pm 0.78	22.60 \pm 0.92
Caloric intake (kcal/day)	12.39 \pm 0.91	10.92 \pm 0.91
Lipid intake (mg/day)	129.40 \pm 8.59	996.14 \pm 73.83 ^a
Total lipid content in feces (mg/day)	31.95 \pm 1.14	30.88 \pm 0.49

Mice, individually housed in metabolic cages, were fed for 3 weeks either a control diet or a high-fat diet. Lipids in feces collected during the 3 last days of treatment were extracted by the method Folch, Lees, and Sloane Stanley (16). Values shown are means \pm SEM (n = 5).

^a *P* < 0.001.

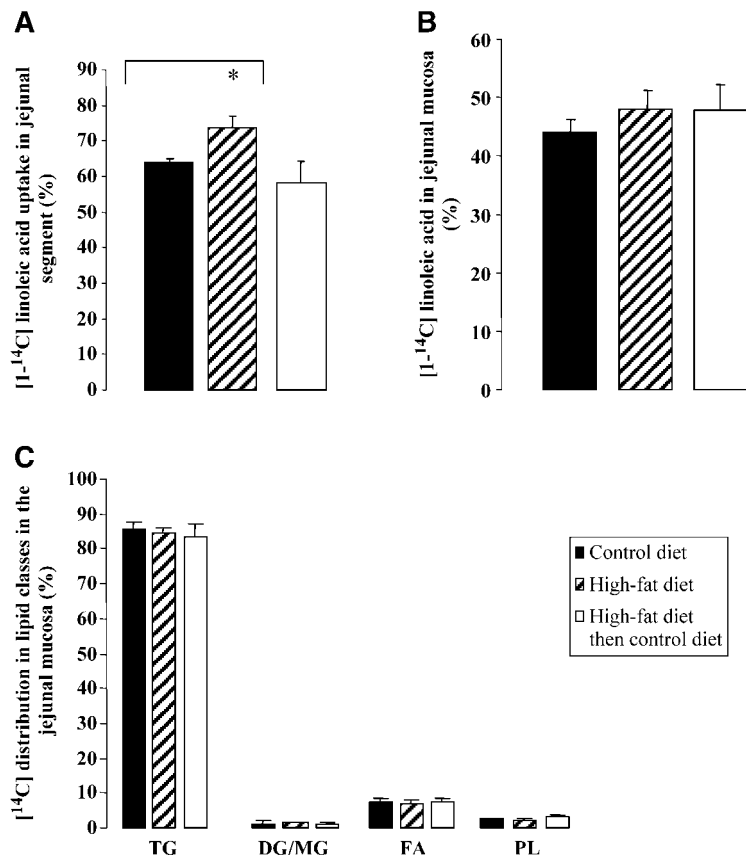


Fig. 1. Effects of a chronic high-fat diet on fatty acid uptake and processing in an in situ-isolated jejunal loop. Mice were fed a control diet (black bars), a high-fat diet (hatched bars), or refed the control diet after the high-fat diet (white bars). A total of 0.5 ml of linoleic acid (2.18 mM) in solution containing 10% [^{1-¹⁴C}]linoleic acid (51 mCi/mmol) emulsified with 10 mM taurocholic acid was infused into isolated jejunal loops of fasted mice. Radioactivity in lumen and mucosa was determined at 5 min after the infusion. Lipids were extracted by Delsal's method (9), and the amount of [^{1-¹⁴C}]linoleic acid was assayed by liquid scintillation. A: Jejunal [^{1-¹⁴C}]linoleic acid uptake was determined by subtracting the quantity of [^{1-¹⁴C}]linoleic acid remaining in the intestinal lumen at the end of the experiment. B: Amount of [^{1-¹⁴C}]linoleic acid found in mucosa. C: ¹⁴C distribution in the main lipids of the mucosa. DG/MG, diglycerides/monoglycerides; FA, fatty acid; PL, phospholipid; TG, triglyceride. Values shown are means \pm SEM (n = 4). * $P < 0.05$.

subjected to the control or high-fat diet (Table 1) for 3 weeks followed or not by an additive period of 3 weeks on the control diet. In this strain, the high-fat diet did not affect the body mass, the daily caloric intake being similar regardless of the diet (Table 2). Despite a daily lipid intake 7.7-fold higher than in controls, the lipid elimination in feces remained unchanged in mice subjected to a chronic high fat supply. These data suggest either

the existence in the mouse of an innate high absorption capacity for lipids or a fat-dependent adaptation of small intestine to the lipid content of the diet in the mouse.

A chronic high-fat diet affects jejunal fatty acid uptake and processing

To address this question, the impact of a chronic high-fat supply on intestinal LCFA uptake was studied using an

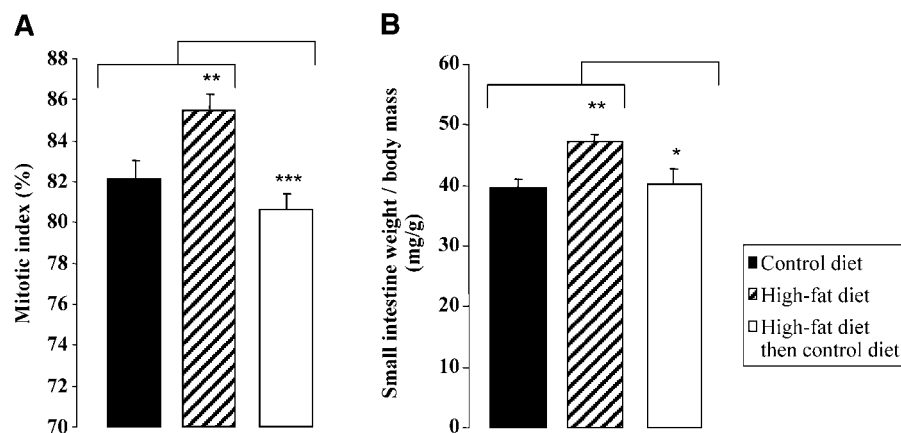


Fig. 2. Effects of a chronic high-fat diet on jejunal absorptive area. Mice were fed a control diet (black bars), a high-fat diet (hatched bars), or refed the control diet after the high fat diet (white bars). A: The mitotic index was defined as the Ki-67-positive cell/total cryptic cell ratio. Values shown are means \pm SEM (n = 3 with 30 crypts/animals). B: Intestinal relative mass. After weighing, animals were euthanized, and the small intestine was removed, rinsed with 0.9% NaCl, and then weighed. Values shown are means \pm SEM (n = 5). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

in situ isolated intestinal loop. By keeping intact both the luminal microenvironment (i.e., the low pH microclimate of the unstirred water layer lining the enterocytes) and the enteric lymph and blood circulation, this method allows the determination of intestinal LCFA uptake in a physiological context. As shown in **Fig. 1A**, mice chronically subjected to a high-fat diet exhibited a higher [^{14}C]linoleic acid uptake capacity than controls. The fact that this increase was fully blunted in animals refed the control diet demonstrated the link between the intestinal LCFA uptake capacity and the TG content of the diet. To further assess the metabolic fate of LCFA in enterocytes, [^{14}C]linoleic acid was assayed in the intestinal mucosa at 5 min after infusion of the lipid emulsion. No difference was found regardless of the experimental conditions used (**Fig. 1B**). This result suggests that the greater lipid influx into enterocytes found in the high-fat-fed mice was followed by a rapid cellular output. Moreover, the chronic high-fat supply did not affect the ^{14}C distribution into the main classes of lipids found in intestinal mucosa (**Fig. 1C**). The fact that radioactivity was retrieved mainly in the TG fraction confirms the physiological relevance of this model.

Intestinal cell proliferation is enhanced by a chronic high-fat diet

Intestinal epithelium undergoes rapid renewal. To explore whether a change in the cell proliferation rate of intestinal cells might contribute to the fat-mediated increase in TG absorption, the jejunal mitotic index was determined using the Ki-67 antigen method. Ki-67 protein is specifically expressed in the nucleus of cells in division. As shown in **Fig. 2A**, the mitotic index increased significantly in the jejunum of mice chronically subjected to the high-fat diet compared with the control diet. This change was reversible, because a return to the control value was found when mice were refed the low-fat diet. Lipid-induced mitotic activity might lead to an increase in villi size and, thus, in absorptive area. The fact that the relative intestinal mass was increased by the fat content of the diet correlates quite well with this assumption (**Fig. 2B**).

A chronic high-fat diet affects the expression of key genes for fat absorption

To examine whether a chronic high-fat supply affects the intestinal gene profile and thereby influences intestinal TG absorption, the low-density microarray INRArray 01.4, dedicated to genes mainly involved in lipid metabolism, was used (11). As shown in **Fig. 3**, two groups of genes were easily identified. Two genes implicated in the metabolic fate of intestinal TG-rich lipoproteins, apoA-I and apoC-III, were repressed by the high-fat diet. Conversely, this diet upregulated several genes known to play a crucial role in LCFA uptake/trafficking (FATP-4, I-FABP, L-FABP) and lipoprotein synthesis (apoA-IV). This global gene analysis highlights the strong effect of dietary fat on intestinal TG metabolism. To further analyze this regulation, a kinetic study was undertaken in a larger set of

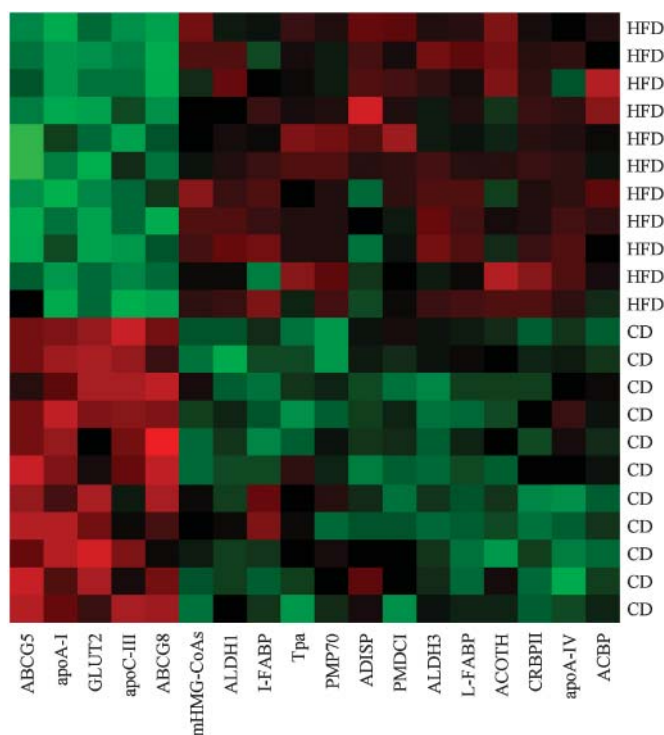


Fig. 3. Hierarchical clustering of gene expression in jejunum from mice subjected to standard chow or high-fat diet using the INRArray 01.4 dedicated to lipid metabolism. Z-scores for 18 differentially expressed genes, 22 samples ($n = 11$ per group) clustered using the Euclidean distance, and the Ward criterion were represented as a heat map. The green areas indicate a low Z-score, and the red areas indicate a high Z-score. Samples are well clustered based on the diet factor, and two groups of genes are easily identified. The first group is composed of the five following genes: ABCG5 and ABCG8, glucose transporter 2 (GLUT2), and apolipoproteins A-I and C-III (apoA-I and apoC-III), which are all repressed under the high-fat diet (HFD). The 13 remaining genes form the second cluster and are all induced under the high-fat diet. ACBP, acyl-CoA binding protein; ACOTH, cytosolic acyl-CoA thioesterase; ADISP, adipocyte-specific mRNA; ALDH1, aldehyde dehydrogenase 1; CD, control diet; CRBP II, cellular retinal binding protein II; mHMG-CoAs, mitochondrial 3-hydroxy 3-methylglutaryl-CoA synthase; PMDC1, peroxisomal/mitochondrial dienoyl-CoA isomerase; PMP70, peroxisomal membrane protein; Tpa, mitochondrial trifunctional protein α -subunit.

prototypical genes. As shown in **Fig. 4**, chronic high-fat diet led to a dramatic upregulation of FATP-4, FAT/CD36, I-FABP, L-FABP, MTP, and apoA-IV genes. This induction was rapid and maintained throughout the exposure to the high-fat diet. Except for FATP-4, a rapid return to basal values was observed as soon as mice were refed the control diet.

Plasma TG clearance is improved in mice chronically fed a high-fat diet

ApoC-II and apoC-III are known to be a strong activator and a strong inhibitor, respectively, of LPL, an enzyme responsible for blood chylomicron clearance (17, 18). Because microarray analysis showed that the high-fat diet decreased intestinal apoC-III gene expression (**Fig. 3**),

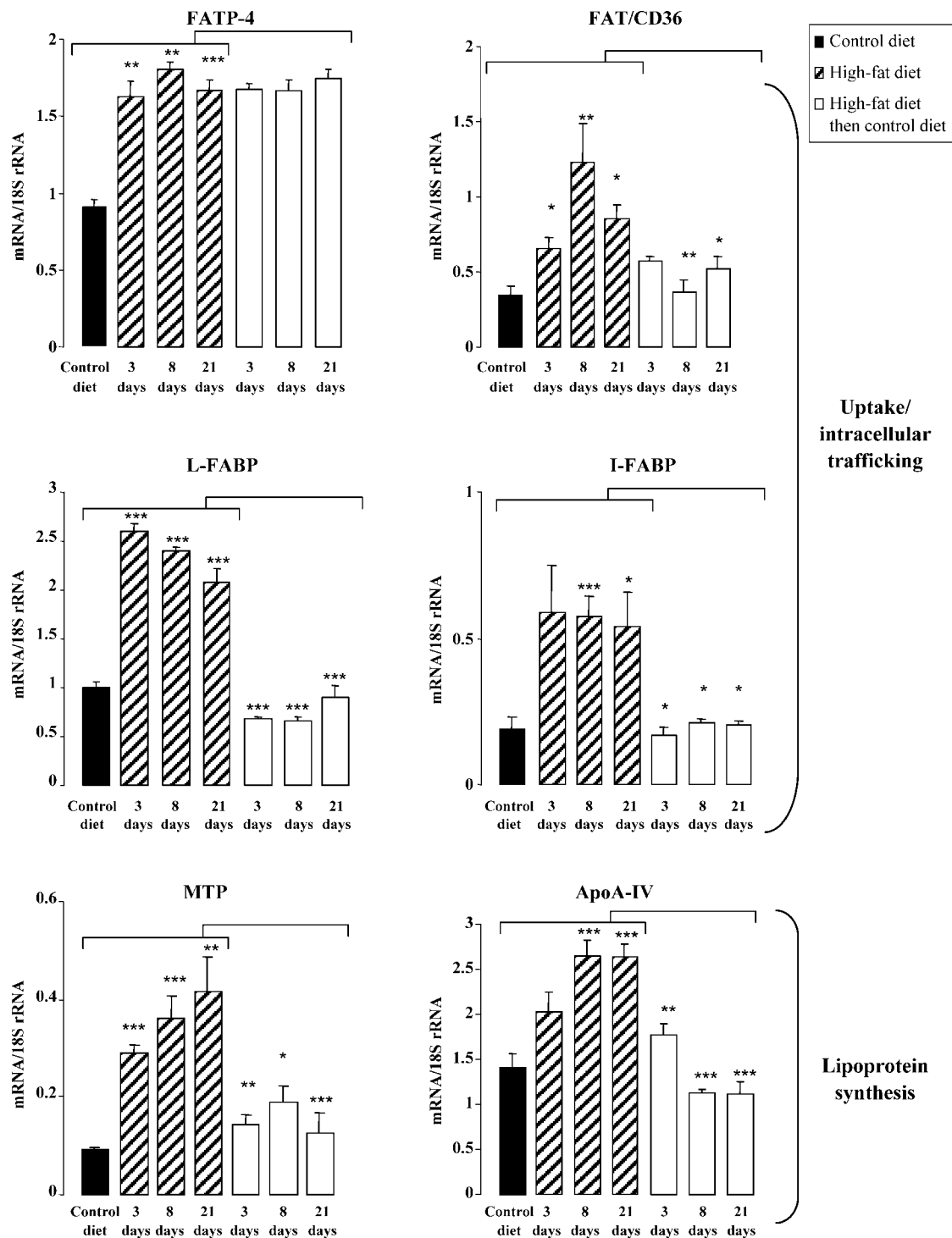


Fig. 4. Effects of a chronic high-fat diet on intestinal mRNA levels of genes involved in lipid absorption. Mice were fed a control diet (black bars), a high-fat diet (hatched bars), or refed the control diet after the high-fat diet (white bars). Total RNA (50 μ g) isolated from jejunum of mice after different times of treatment (3, 8, 21 days) was analyzed by Northern blotting using murine cDNAs for fatty acid transport protein 4 (FATP-4), fatty acid transporter (FAT/CD36), intestinal and liver fatty acid binding proteins (I-FABP and L-FABP), microsomal triglyceride transfer protein (MTP), and apoA-IV. Values were normalized to the 18S rRNA. Values shown are means \pm SEM (n = 5). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

mRNA levels of apoC-II and apoC-III were assayed in jejunum by quantitative PCR. As shown in **Fig. 5A**, a huge increase in apoC-II mRNA levels was found in mice fed the TG-rich diet, whereas apoC-III mRNA levels decreased

according to the microarray analysis. A return to control values was observed in mice refed standard laboratory chow, demonstrating that this gene regulation is adaptive. Because chylomicrons can also be enriched in apoC

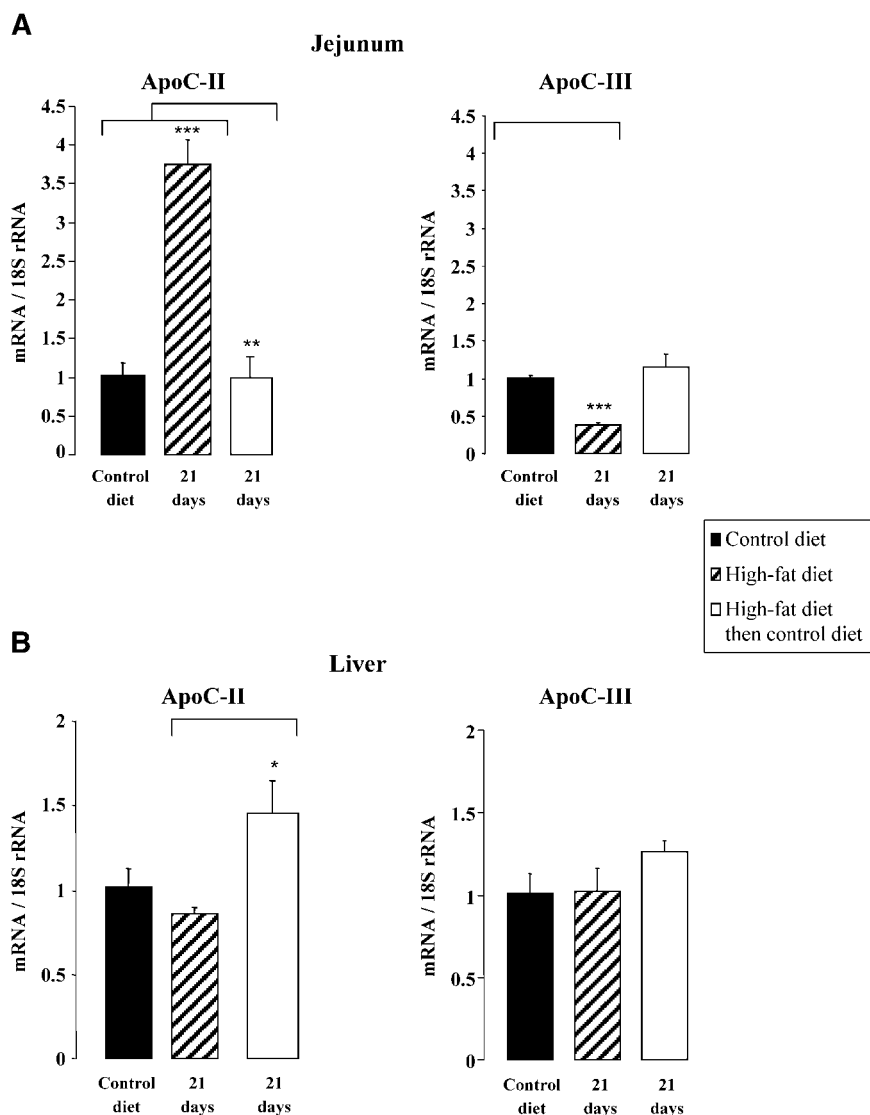


Fig. 5. Effects of a chronic high-fat diet on apoC-II and apoC-III mRNA levels in jejunum (A) and liver (B). Jejunal and hepatic apoC-II and apoC-III mRNA levels were assayed by quantitative PCR after 3 weeks of treatment. Mice were fed a control diet (black bars), a high-fat diet (hatched bars), or the high-fat diet and then the control diet (white bars). Values shown are means \pm SEM ($n = 4$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

by exchanges with hepatic lipoproteins, apoC-II and apoC-III gene expression was next determined in liver, but no change was found (Fig. 5B).

A high apoC-II/apoC-III ratio in chylomicrons is expected to increase their clearance from blood and, thereby, decrease postprandial triglyceridemia. To explore this assumption, plasma TG levels were assayed in overnight-fasted mice force-fed 0.5 ml of oil. Mice subjected to the high-fat diet displayed plasma TG levels lower than mice fed the control diet (Fig. 6A). This finding might be attributable to a decrease in intestinal TG release and/or an increase in blood TG hydrolysis by peripheral tissues. To address this question, the LPL inhibitor tyloxapol was injected intraperitoneally at 30 min before the intragastric lipid load was administered (0.5 ml). In contrast to the previous experiment, plasma TG levels from chronically fat-fed mice were similar to those of mice fed the control

diet (Fig. 6B). Because no TG retention was observed in intestinal mucosa at 4 h after the fat load (Fig. 6C), the low plasma TG levels found in mice fed the high-fat diet were attributable to the more efficient TG clearance from blood. These intestinally mediated changes in the chylomicron composition might account for the lower postprandial TG levels found in mice chronically subjected to the high-fat diet. This phenomenon was rapid, because a significant decrease in blood TG levels was found as early as 3 days after high-fat feeding and was reversible (Fig. 6D).

DISCUSSION

This report demonstrates that the intestinal absorption capacity can be adapted to the fat content of the diet in the mouse. This fat-mediated adaptation takes place through

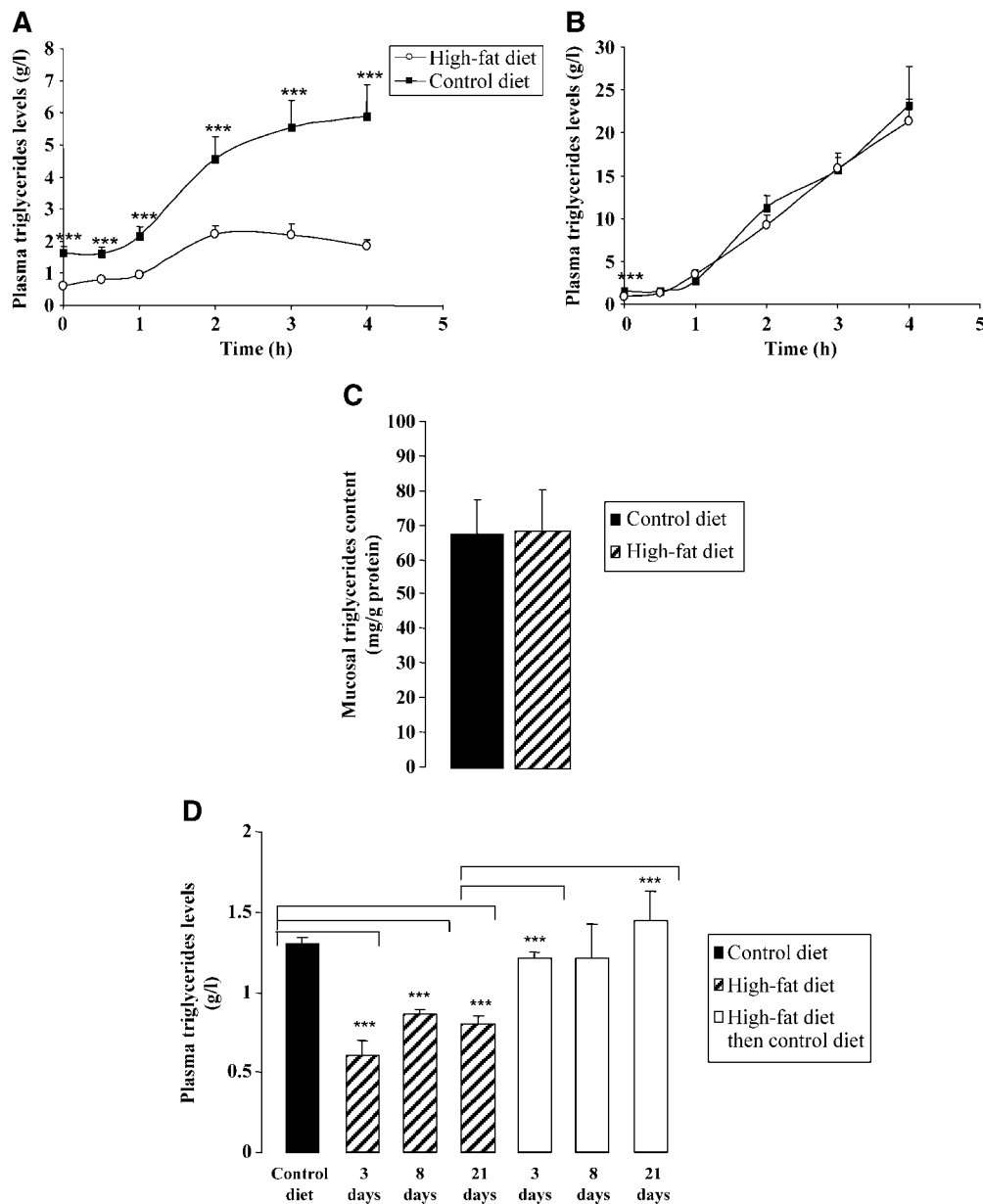


Fig. 6. Effects of a chronic high-fat diet on triglyceridemia. **A:** Postprandial plasma TG levels were determined after an oral lipid bolus (0.5 ml) in mice fed a control diet (black squares) or a high-fat diet (white circles). Blood obtained from the tail vein was collected at 0, 0.5, 1, 2, 3, and 4 h after gavage, and plasma TG concentrations were measured ($n = 11$). **B:** The same protocol was performed in mice previously subjected to an intraperitoneal injection of the LPL inhibitor tyloxapol ($n = 11$). **C:** Mucosal TG levels were determined in tyloxapol-treated mice 4 h after the lipid bolus ($n = 11$). **D:** Triglyceridemia of fed mice subjected to a control diet (black bars), a high-fat diet (hatched bars), or the high-fat diet and then the control diet (white bars) for 3, 8, and 21 days. Values shown are means \pm SEM ($n = 5$). *** $P < 0.001$.

two complementary events. First, there is an enhancement of intestinal cell proliferation, which might lead to an increased absorptive area. Consistent with this assumption, rat studies show that fatty acid-enriched diets increase the height of villi and induce the rate of enterocyte migration along the crypt-to-villus axis (19, 20). Interestingly, the effect of dietary lipid on intestinal trophism appears to be more efficient than that of other nutrients. It is especially evident in situations in which the absorptive area has been

deeply altered, as after long-term starvation or a drastic intestinal resection (8, 21). Second, our data show the existence of a fat-induced coordination of genes known to play a significant role in intestinal fatty acid uptake or intracellular processing (i.e., FATP4, FAT/CD36, I-FABP, L-FABP) and lipoprotein secretion (i.e., MTP, apoA-IV). FATP-4 upregulation might facilitate LCFA uptake by enterocytes through its acyl-CoA synthetase activity (12). Plasma membrane being impermeable to long-chain acyl-

CoA, a FATP-4-mediated esterification of LCFA must not only prevent their enterocyte efflux but also create a concentration gradient favoring LCFA influx (2). The fact that a positive relationship exists between FATP-4 protein and LCFA uptake by enterocytes in FATP4^{+/-} mice is consistent with this assumption (22).

The efficiency of LCFA uptake is also modulated by I-FABP and L-FABP expression levels, which are known to facilitate cellular LCFA trapping and trafficking (23). Transfection studies suggest that I-FABP and L-FABP preferentially target LCFA to the reesterification pathway, facilitating lipoprotein synthesis (23, 24). A tight correlation between I-FABP expression and TG synthesis was reported in human enterocyte-like Caco-2 cells (25). Similarly, the use of FAT/CD36-null mice revealed an involvement in intestinal lipoprotein metabolism (4). Moreover, the resident endoplasmic reticulum protein MTP, which controls the neutral lipid transfer to apoB-48 (26), was dramatically upregulated in high-fat-fed mice (Fig. 4). This protein plays a crucial role in chylomicron synthesis. Indeed, an intestine-specific MTP gene invalidation produces a dramatic decrease in chylomicron synthesis and secretion (5). Finally, the induction of apoA-IV expression found in lipid-fed mice might also facilitate intestinal lipoprotein production (27). Altogether, these lipid-mediated changes in gene expression must improve the intestinal lipid absorption and, thereby, deeply affect the postprandial triglyceridemia. Interestingly, an increase in blood TG levels is systematically found after a targeted deletion of genes encoding FAT/CD36 (4), I-FABP (3), and MTP (5). In contrast, these last findings might explain why a low triglyceridemia level was found in mice receiving the TG-enriched diet (Fig. 5D). Such a result has already been observed in rats, especially when, as in our conditions, a high monounsaturated fat diet was used (28). A higher efficiency of TG clearance by LPL is suggested by the concomitant increase in apoC-II and decrease in apoC-III gene expression. Indeed, these apolipoproteins are known to activate and inhibit LPL activity, respectively (17, 18).

Chylomicron size is also known to affect blood TG clearance, larger chylomicrons exhibiting a greater affinity for LPL than smaller lipoprotein particles (29). Chylomicron size is highly dependent on expression levels of a few LBPs and apolipoproteins. For instance, FAT/CD36 knockout mice secrete smaller chylomicrons than wild-type mice (30), and the induction of intestinal apoA-IV has been reported to lead to larger particles (27, 31). According to these data, the fat-mediated increase of genes encoding FAT/CD36 and apoA-IV might produce large chylomicrons rapidly hydrolyzed by LPL. This report provides evidence implicating the small intestine in postprandial triglyceridemia.

The fact that in the rodent insulin resistance model of hypertriglyceridemia has been demonstrated to result from the hypersecretion of intestinal lipoproteins indicates that the small intestine could participate in metabolic lipid disorders (32). Consistent with this assumption, Kondo and coworkers (33) reported, during the review of this work, that the fat-mediated adaptation of intestinal lipid metabolism is associated with susceptibility to obesity in the mouse. The molecular basis for this adaptation remains to be de-

termined. The coordinated regulation of gene expression reported here suggests the involvement of a common regulatory factor. PPAR β/δ is a plausible candidate for such a function. Indeed, this nuclear receptor, which is especially highly expressed in the small intestine, is known to be involved in both the regulation of cell proliferation (34–36) and the gene expression of several intestinal LBPs (10, 37). The generation of mice in which the PPAR β/δ gene is invalidated or, conversely, overexpressed specifically in the small intestine might provide a useful tool to explore this hypothesis. ■

V.P. is supported by a fellowship from the Council of Burgundy and GlaxoSmithKline. The authors thank Alfrediam, which also supported her work. The authors are grateful to Frederic Lassère and Sylvie Baumann for their technical assistance.

REFERENCES

1. West, D. B., and B. York. 1998. Dietary fat, genetic predisposition, and obesity: lessons from animal models. *Am. J. Clin. Nutr.* **67** (Suppl. 3): 505–512.
2. Niot, I., and P. Besnard. 2003. Intestinal fat absorption: roles of intracellular lipid-binding proteins and peroxisome proliferator-activated receptors. In *Cellular Proteins and Their Fatty Acids in Health and Disease*. A. K. Duttaroy, and F. Spener, editors. Wiley-VCH Verlag, Germany. 359–381.
3. Vassileva, G., L. Huwyler, K. Poirier, L. B. Agellon, and M. J. Toth. 2000. The intestinal fatty acid binding protein is not essential for dietary fat absorption in mice. *FASEB J.* **14**: 2040–2046.
4. Drover, V. A., M. Ajmal, F. Nassir, N. O. Davidson, A. M. Nauli, D. Sahoo, P. Tso, and N. A. Abumrad. 2005. CD36 deficiency impairs intestinal lipid secretion and clearance of chylomicrons from the blood. *J. Clin. Invest.* **115**: 1290–1297.
5. Xie, Y., E. P. Newberry, S. G. Young, S. Robine, R. L. Hamilton, J. S. Wong, J. Luo, S. Kennedy, and N. O. Davidson. 2006. Compensatory increase in hepatic lipogenesis in mice with conditional intestine-specific Mtp deficiency. *J. Biol. Chem.* **281**: 4075–4086.
6. Marshman, E., C. Booth, and C. S. Potten. 2002. The intestinal epithelial stem cell. *Bioessays*. **24**: 91–98.
7. Dunel-Erb, S., C. Chevalier, P. Laurent, A. Bach, F. Decrock, and Y. Le Maho. 2001. Restoration of the jejunal mucosa in rats refed after prolonged fasting. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **129**: 933–947.
8. Buts, J. P., V. Vijverman, C. Barudi, N. De Keyser, P. Maldague, and C. Dive. 1990. Refeeding after starvation in the rat: comparative effects of lipids, proteins and carbohydrates on jejunal and ileal mucosal adaptation. *Eur. J. Clin. Invest.* **20**: 441–452.
9. Delsal, J. L. 1944. Nouveau procédé d'extraction des lipides du sérum par le méthylal. Application aux microdosages du cholestérol total, des phosphoaminolipides et des protéides. *Bull. Soc. Chim.* **26**: 99–105.
10. Poirier, H., I. Niot, M. C. Monnot, O. Braissant, C. Meunier-Durmort, P. Costet, T. Pineau, W. Wahli, T. M. Willson, and P. Besnard. 2001. Differential involvement of peroxisome-proliferator-activated receptors alpha and delta in fibrate and fatty-acid-mediated inductions of the gene encoding liver fatty-acid-binding protein in the liver and the small intestine. *Biochem. J.* **355**: 481–488.
11. Martin, P. G., F. Lasserre, C. Calleja, A. Van Es, A. Roulet, D. Concordet, M. Cantiello, R. Barnouin, B. Gauthier, and T. Pineau. 2005. Transcriptional modulations by RXR agonists are only partially subordinated to PPARalpha signaling and attest additional, organ-specific, molecular cross-talks. *Gene Expr.* **12**: 177–192.
12. Herrmann, T., F. Buchkremer, I. Gosch, A. M. Hall, D. A. Bernlohr, and W. Stremmel. 2001. Mouse fatty acid transport protein 4 (FATP4): characterization of the gene and functional assessment as a very long chain acyl-CoA synthetase. *Gene*. **270**: 31–40.
13. Sellers, J. A., and G. S. Shelness. 2001. Lipoprotein assembly capacity of the mammary tumor-derived cell line C127 is due to

- the expression of functional microsomal triglyceride transfer protein. *J. Lipid Res.* **42**: 1897–1904.
14. Besnard, P., A. Mallordy, and H. Carlier. 1993. Transcriptional induction of the fatty acid binding protein gene in mouse liver by bezafibrate. *FEBS Lett.* **327**: 219–223.
 15. Livak, K. J., and T. D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods.* **25**: 402–408.
 16. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226**: 497–509.
 17. Jong, M. C., M. H. Hofker, and L. M. Havekes. 1999. Role of ApoCs in lipoprotein metabolism: functional differences between ApoC1, ApoC2, and ApoC3. *Arterioscler. Thromb. Vasc. Biol.* **19**: 472–484.
 18. Shachter, N. S. 2001. Apolipoproteins C-I and C-III as important modulators of lipoprotein metabolism. *Curr. Opin. Lipidol.* **12**: 297–304.
 19. Thomson, A. B., C. I. Cheeseman, M. Keelan, R. Fedorak, and M. T. Clandinin. 1994. Crypt cell production rate, enterocyte turnover time and appearance of transport along the jejunal villus of the rat. *Biochim. Biophys. Acta.* **1191**: 197–204.
 20. Thomson, A. B., M. Keelan, M. T. Clandinin, and K. Walker. 1986. Dietary fat selectively alters transport properties of rat jejunum. *J. Clin. Invest.* **77**: 279–288.
 21. Buts, J. P., C. L. Morin, and V. Ling. 1979. Influence of dietary components on intestinal adaptation after small bowel resection in rats. *Clin. Invest. Med.* **2**: 59–66.
 22. Gimeno, R. E., D. J. Hirsch, S. Punreddy, Y. Sun, A. M. Ortegon, H. Wu, T. Daniels, A. Stricker-Krongrad, H. F. Lodish, and A. Stahl. 2003. Targeted deletion of fatty acid transport protein-4 results in early embryonic lethality. *J. Biol. Chem.* **278**: 49512–49516.
 23. Prows, D. R., E. J. Murphy, and F. Schroeder. 1995. Intestinal and liver fatty acid binding proteins differentially affect fatty acid uptake and esterification in L-cells. *Lipids.* **30**: 907–910.
 24. Alpers, D. H., N. M. Bass, M. J. Engle, and K. DeSchryver-Kecsckemeti. 2000. Intestinal fatty acid binding protein may favor differential apical fatty acid binding in the intestine. *Biochim. Biophys. Acta.* **1483**: 352–362.
 25. Darimont, C., N. Gradoux, and A. de Pover. 1999. Epidermal growth factor regulates fatty acid uptake and metabolism in Caco-2 cells. *Am. J. Physiol.* **276**: G606–G612.
 26. Hussain, M. M., J. Shi, and P. Dreizen. 2003. Microsomal triglyceride transfer protein and its role in apoB-lipoprotein assembly. *J. Lipid Res.* **44**: 22–32.
 27. Lu, S., Y. Yao, X. Cheng, S. Mitchell, S. Leng, S. Meng, J. W. Gallagher, G. S. Shelness, G. S. Morris, J. Mahan, et al. 2006. Overexpression of apolipoprotein A-IV enhances lipid secretion in IPEC-1 cells by increasing chylomicron size. *J. Biol. Chem.* **281**: 3473–3483.
 28. Hsu, S. C., and C. J. Huang. 2006. Reduced fat mass in rats fed a high oleic acid-rich safflower oil diet is associated with changes in expression of hepatic PPARalpha and adipose SREBP-1c-regulated genes. *J. Nutr.* **136**: 1779–1785.
 29. Xiang, S. Q., K. Cianflone, D. Kalant, and A. D. Sniderman. 1999. Differential binding of triglyceride-rich lipoproteins to lipoprotein lipase. *J. Lipid Res.* **40**: 1655–1663.
 30. Nauli, A. M., F. Nassir, S. Zheng, Q. Yang, C. M. Lo, S. B. Vonlehmden, D. Lee, R. J. Jandacek, N. A. Abumrad, and P. Tso. 2006. CD36 is important for chylomicron formation and secretion and may mediate cholesterol uptake in the proximal intestine. *Gastroenterology.* **131**: 1197–1207.
 31. Lu, S., Y. Yao, S. Meng, X. Cheng, and D. D. Black. 2002. Overexpression of apolipoprotein A-IV enhances lipid transport in newborn swine intestinal epithelial cells. *J. Biol. Chem.* **277**: 31929–31937.
 32. Haidari, M., N. Leung, F. Mahbub, K. D. Uffelman, R. Kohen-Avramoglu, G. F. Lewis, and K. Adeli. 2002. Fasting and postprandial overproduction of intestinally derived lipoproteins in an animal model of insulin resistance. Evidence that chronic fructose feeding in the hamster is accompanied by enhanced intestinal de novo lipogenesis and ApoB48-containing lipoprotein overproduction. *J. Biol. Chem.* **277**: 31646–31655.
 33. Kondo, H., Y. Minegishi, Y. Komine, T. Mori, I. Matsumoto, K. Abe, I. Tokimitsu, T. Hase, and T. Murase. 2006. Differential regulation of intestinal lipid metabolism-related genes in obesity-resistant A/J vs. obesity-prone C57BL/6J mice. *Am. J. Physiol. Endocrinol. Metab.* **291**: E1092–E1099.
 34. Jehl-Pietri, C., C. Bastie, I. Gillot, S. Luquet, and P. A. Grimaldi. 2000. Peroxisome-proliferator-activated receptor delta mediates the effects of long-chain fatty acids on post-confluent cell proliferation. *Biochem. J.* **350**: 93–98.
 35. Hellemans, K., L. Michalik, A. Dittie, A. Knorr, K. Rombouts, J. De Jong, C. Heirman, E. Quartier, F. Schuit, W. Wahli, et al. 2003. Peroxisome proliferator-activated receptor-beta signaling contributes to enhanced proliferation of hepatic stellate cells. *Gastroenterology.* **124**: 184–201.
 36. Park, B. H., B. Vogelstein, and K. W. Kinzler. 2001. Genetic disruption of PPARdelta decreases the tumorigenicity of human colon cancer cells. *Proc. Natl. Acad. Sci. USA.* **98**: 2598–2603.
 37. Bastie, C., D. Holst, D. Gaillard, C. Jehl-Pietri, and P. A. Grimaldi. 1999. Expression of peroxisome proliferator-activated receptor PPARdelta promotes induction of PPARgamma and adipocyte differentiation in 3T3C2 fibroblasts. *J. Biol. Chem.* **274**: 21920–21925.