Fatty acid transport protein 4 is dispensable for intestinal lipid absorption in mice[S]

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Abstract **FA transport protein 4 (FATP4), one member of a multigene family of FA transporters, was proposed as a major FA transporter in intestinal lipid absorption. Due to the fact that *Fatp4*$^{-/-}$ mice die because of a perinatal skin defect, we rescued the skin phenotype using an FATP4 transgene driven by a keratinocyte-specific promoter (*Fatp4*$^{-/-}$*;Ivl-Fatp4*$^{tg/+}$ mice) to elucidate the role of intestinal FATP4 in dietary lipid absorption. *Fatp4*$^{-/-}$*;Ivl-Fatp4*$^{tg/+}$ mice and wild-type littermates displayed indistinguishable food consumption, growth, and weight gain on either low or high fat (Western) diets, with no differences in intestinal triglyceride (TG) absorption or fecal fat losses. Cholesterol absorption and intestinal TG absorption kinetics were indistinguishable between the genotypes, although Western diet fed *Fatp4*$^{-/-}$*;Ivl-Fatp4*$^{tg/+}$ mice showed a significant increase in enterocyte TG and FA content. There was no compensatory upregulation of other FATP family members or any other FA or cholesterol transporters in *Fatp4*$^{-/-}$*;Ivl-Fatp4*$^{tg/+}$ mice. Furthermore, although serum cholesterol levels were lower in *Fatp4*$^{-/-}$*;Ivl-Fatp4*$^{tg/+}$ mice, there was no difference in hepatic VLDL secretion in-vivo or in hepatic lipid content on either a chow or Western diet. Taken together, our studies find no evidence for a physiological role of intestinal FATP4 in dietary lipid absorption in mice.—**Shim, J., C. L. Moulson, E. P. Newberry, M-H. Lin, Y. Xie, S. M. Kennedy, J. H. Miner, and N. O. Davidson. **Fatty acid transport protein 4 is dispensable for intestinal lipid absorption in mice.** ***J. Lipid Res.*** **2009.** 50: **491–500.**

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A major fraction of the daily caloric intake in Western societies comes from dietary lipid, whose absorption by the small intestine is normally extremely efficient (>95%) and virtually independent of the amount of fat consumed (1). In view of the rampant epidemic of obesity and the abundance of dietary fat in the average diet, considerable attention has focused on targets that might limit intestinal lipid absorption as a means of reducing caloric intake. However, our understanding of the molecular mechanisms of intestinal fat absorption is remarkably incomplete, despite many years of investigation. It remains unclear, for example, the extent to which intestinal uptake of dietary long-chain FA occurs via diffusional or protein-mediated mechanisms, since both have been proposed (as reviewed in Ref. 2). As regards protein-mediated FA transport by the mammalian intestine, the identity of the protein(s) responsible has been intensively investigated over the years. Among these candidates, CD36, FABP-pm, and Caveolin1 (3–6) have been proposed as potential intestinal FA transporters. Recent evidence suggests a role for CD36 in very long chain FA (VLCFA) uptake (7) and in regulating regional transport kinetics (i.e., proximal jejunum) of FA (8) and cholesterol (9). However, to date, none of these candidates has been proven indispensable for physiological intestinal FA transport in vivo.

Several years ago, Hirsch, Stahl, and Lodish (10) identified and characterized a family of FA transport proteins (FATPs), each with a tissue specific pattern of expression and a range of possible functions in regard to long-chain FA transport (as reviewed in Ref. 6). One such gene, FATP4 (FA transport protein 4, Slc27a4) is ~60% homologous to the founding member of the family, FATP1, and is abundantly expressed in the small intestine (11), and at lower levels in brain, kidney, liver, and skin (6, 12). Earlier studies suggested that FATP4 is the major FATP expressed in the intestine, with protein expression localized to the apical and microvillus border of enterocytes (11). In addition, FATP4 knockdown in isolated enterocytes pro-

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Abbreviations: FATP, fatty acid transport protein; Ivl, involucrin; TG, triglyceride.

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[S] The online version of this article (available at http://www.jlr.org) contains supplementary data in the form of one table and one figure.

duced a corresponding, dose-dependent decrease in the uptake of radiolabeled long-chain FA (11). Based on these and other findings, it was proposed that FATP4 was the long sought intestinal FA transport protein.

FATP4$^{-/-}$ mice have been generated by several groups and die either embryonically (13) or perinatally with thickened, malformed skin that restricts the ability of the animals to breathe and suckle and is ineffective as a barrier to prevent dehydration (12, 14). The perinatal lethality appears to be due exclusively to absence of FATP4 in skin, as knockout mice can be rescued by transgenic expression of FATP4 from a keratinocyte specific promoter, yielding mice (*Fatp4*$^{-/-}$*;Ivl-Fatp4*$^{tg/+}$ mice) that are viable and have only a mild skin/hair phenotype (15). Because *Fatp4*$^{-/-}$*;Ivl-Fatp4*$^{tg/+}$ mice lacking FATP4 in the intestine suckle normally and develop to adulthood, it was unclear to what extent the presence of FATP4 in the intestine is required for normal fat absorption. Further complicating our understanding of the role of FATP4 was the observation that its localization was confined to the endoplasmic reticulum in fibroblasts and COS cells (16, 17) and not to the plasma membrane as originally proposed (11). In addition, Lobo et al. (18) demonstrated no change in basal or insulin-stimulated FA influx in 3T3-L1 adipocytes following knockdown of FATP4. Collectively, these studies suggest that the physiological role of FATP4 remains open to question. The transgenic rescue of the perinatal lethality in *Fatp4*$^{-/-}$*;Ivl-Fatp4*$^{tg/+}$ mice afforded us the opportunity to clarify the role of FATP4 in FA uptake and metabolism in the intestine and liver using this line. Our findings cast doubt on the suggestion that FATP4 is required for intestinal lipid absorption.

MATERIALS AND METHODS

Materials

Anti-FATP4 antiserum was generated as described (19). Anti-heat shock protein 40 antiserum was obtained from Stressgen Biotechnology (Ann Arbor, MI) and anti-α-tubulin antiserum was from Santa Cruz Biotechnology (sc 5286; Santa Cruz, CA). Anti-Collagen IV monoclonal antibody, the generous gift of Yoshifumi Ninomiya and Yoshikazu Sado, was used as described (20). Biochemical assays for measurement of triglyceride (TG), total cholesterol, free cholesterol, and free FA levels in serum and tissue were performed using kits obtained from Wako Chemical USA (Richmond, VA).

Animals

Fatp4$^{-/-}$*;Ivl-Fatp4*$^{tg/+}$ mice were generated as described and maintained on a mixed 129/B6/CBA strain background (15). Both *Fatp4*$^{+/-}$*;Ivl-Fatp4*$^{tg/+}$ and *Fatp4*$^{+/-}$ or Fatp4$^{+/+}$ mice (without the *Ivl-Fatp4* transgene) were used as control mice, with no difference in phenotype observed between these genotypes. Unless otherwise indicated, studies were performed on male and female mice, and the data were pooled. For dietary studies, mice were started on a Western diet (Adjusted Calories diet, TD 88137, Harlan Tecklad) at 8–10 weeks of age and weighed weekly. In addition, female C57BL/6J mice (Jackson Laboratories) were fed high-saturated fat or high-cholesterol diet to investigate diet-induced changes in FATP4 gene expression (21, 22). Mice were housed in a full barrier facility with a 14/10-h light/dark cycle. All animal protocols were approved by the Washington University Animal Studies Committee and conformed to criteria outlined in the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*.

Dietary lipid absorption studies

For fat balance assays, mice were placed in metabolic cages 2 days prior to the start of the experiment. Food intake was measured, and feces were collected every 2 days for 8 days. No difference in food consumption was observed between the genotypes. Fecal lipid content was determined gravimetrically following Folch extraction as described (21, 22). Lipid mass was normalized to food consumption and dietary fat content for determination of percent fat absorption. For dietary fat absorption studies on mice fed a Western diet, mice were fed the diet for 11 weeks prior to analysis. For sucrose polybehenate (SPB) studies, mice were fed the Western diet for 11 weeks and then switched to a low (5%) fat diet containing olestra/SPB and essential nutrients. The ratio of fecal FAs vs. SPB (a nonabsorbable fat marker) was determined using gas chromatography as described (23).

Determination of intestinal FA absorption in vivo

For long-chain FA gavage experiments, chow-fed female mice were fasted overnight and intravenously injected with 500 mg/kg Tyloxapol (T0307, Sigma-Aldrich, St. Louis, MO) to block serum lipase activity and allow serum lipid accumulation to be monitored. After 30 min, mice were gavaged with 500 μl of a mixture containing 4 parts Intralipid® 20% IV Fat Emulsion (~50% linoleic acid (C18:2); Baxter, Deerfield, IL), 1 part corn oil, and 10 μCi [^{3}H]triolein (American Radiolabeled Chemicals, St Louis, MO). Blood samples were collected at time zero (prior to Tyloxapol injection) and every h after gavage for 4 h. Mice were sacrificed after the final bleed, and serum radioactivity and TG content were determined. The intestine was divided into three equal segments and the intestinal mucosa scraped and frozen in liquid nitrogen for later analysis. For VLCFA absorption experiments, 10–12-month-old male mice were fasted overnight and gavaged with 200 μl of Menhaden fish oil (Sigma-Aldrich) containing 1.5μCi [^{14}C]lignoceric acid (American Radiolabeled Chemicals) in the absence of Tyloxapol. Menhaden oil contains C22:6 (~18%), C22:5 (~2%), and C20:5 (~14%). Blood samples were collected at time zero (prior to gavage) and at 1, 3, and 5 h after gavage. At sacrifice, the stomach and intestine were flushed, and the intestine was divided into three equal segments and scraped. Hepatic and intestinal lipid content was measured as described (22). Radioactivity remaining in the intestinal lumen or mucosa was determined by scintillation counting.

Analysis of serum and tissue

Serum, intestine, and hepatic lipid levels were examined in mice fed a chow diet or fed a Western diet for 11 weeks. Lipids were extracted from liver and intestinal tissue segments as described (22). Protein concentration was determined by D_C Protein Assay (Bio-Rad, Hercules, CA). A portion of the organic phase was dried and resuspended in 1% Triton X-100 for enzymatic determination of tissue lipid content. Western blotting and immunohistochemical staining of intestinal extracts or tissues for FATP4 was conducted as detailed (15).

Cholesterol absorption

Cholesterol absorption was measured using the fecal dual-isotope method (24, 25). Mice were gavaged with 150 μl of corn oil containing 1 μCi [^{14}C]cholesterol (PerkinElmer, Waltham, MA) and 2 μCi of [^{3}H]sitostanol (American Radiolabeled Chemicals) and individually housed in metabolic cages. Feces were

collected for 2 days following gavage and extracted using chloroform:methanol as described (24). The ^{14}C to ^{3}H ratio for each sample was determined and corrected to the dosing mixture ratio, with percent cholesterol absorption calculated as described (25).

Ezetimibe treatment

Ezetimibe, which inhibits intestinal cholesterol uptake through inhibition of Npc1L1 (26), was the kind gift of Schering-Plough Research Institute. Baseline fat balance and cholesterol absorption studies were performed 1 week prior to the start of ezetimibe feeding. Mice were fed a chow diet containing ezetimibe (0.05 mg/g) ad libitum for 3 weeks prior to sacrifice. Fat and cholesterol absorption studies on ezetimibe-fed mice were started ~1 week prior to sacrifice.

RNA Isolation and real-time quantitative PCR

Total RNA was extracted and treated with DNase I (DNA-free, Ambion). Two micrograms of total RNA was reverse transcribed with random hexamers using Superscript II Reverse Transcriptase (Invitrogen, Carlsbad, CA). Real-time quantitative PCR was performed using SYBR green PCR Master Mix (Applied Biosystems, Foster City, CA) on an ABI Prism 7000 Sequence Detection System (Applied Biosystems). Individual mRNAs were quantified and normalized to 18S mRNA; relative mRNA expression levels were calculated as fold change compared with control mice. Primer sequences are listed in supplementary Table I.

Determination of VLDL secretion in vivo

Mice fed either chow or Western diet (9 weeks) were fasted 4 h, then intravenously injected with 20 mg Tyloxapol in 100 μl phosphate buffered saline. Blood was collected from mice prior to Tyloxapol injection and every 30 min following injection up to 120 min. Serum TG content at each time point was determined enzymatically.

Statistical analysis

Statistical significance was determined with an unpaired, two-tailed Students *t*-test. Data are expressed as the mean ± SEM unless otherwise noted.

RESULTS

Expression of FATP4 in the small intestine of control and *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$ mice

Fatp4$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$ mice are viable and fertile, with FATP4 expression restricted to keratinocytes within the granular layer of the epidermis (15). FATP4 was detected in enterocytes from control (**Fig. 1A, E**), but not *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$ mice (Fig. 1B, E). No change was observed in intestinal morphology or villus architecture in *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$ mice, as suggested by immunostaining with anti-collagen IV antiserum (Fig. 1C, D) (as well as measurement of villus height/crypt depth and intestine length; data not shown). FATP4 protein abundance was comparable in control Fatp4$^{+/+}$ mice proximally to distally across the small intestine, although the duodenum (section 1) displayed lower FATP4 expression (Fig. 1F). These data confirm that *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$ mice express neither endogenous nor transgenic FATP4 mRNA (15) or protein in the small intestine and suggest that there is no gradient of FATP4

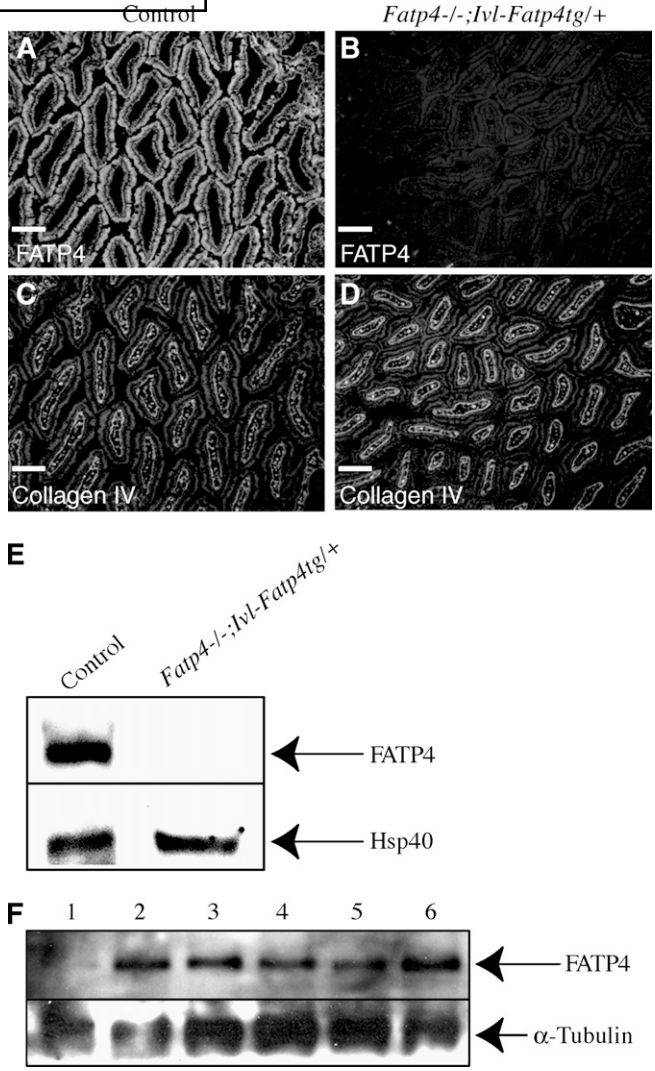

Fig. 1. Expression of FA transport protein 4 (FATP4) in control and *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$ intestine. FATP4 immunofluorescence in the small intestine of adult control mice (A) and *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$ mice (B). Immunofluorescent localization of collagen IV shows comparable appearance of villi in both control (C) and *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$ sections (D). E: One hundred micrograms of scraped mucosal proteins from control and *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$ mice were separated by SDS-PAGE and Western-blotted for FATP4 (top) and Hsp40 as a loading control (bottom). F: Control small intestine was divided into six sections. Scraped mucosal proteins from four mice were pooled, separated by SDS-PAGE, and Western-blotted for FATP4. The Western blot was stripped and probed again for α-tubulin.

expression in the intestine of wild-type mice. Furthermore, there was no change in FATP4 mRNA or protein expression in the intestine of wild-type mice fed a range of fat and/or cholesterol supplemented diets (including Western, high cholesterol, or high saturated fat), compared with mice fed a low-fat, low-cholesterol, chow diet (data not shown).

Intestinal fat absorption and weight gain are not altered in *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$ mice

Because of the predicted role of FATP4 in intestinal FA trafficking we examined whether the absorption of dietary

fat was altered in *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$ mice. Surprisingly, there was no difference in fecal fat content (expressed as percent of fecal weight) in chow fed *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$ mice compared with control mice (6.12% ± 0.30, control; 5.64% ± 0.16, *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$, n = 6–7). To examine fat absorption more closely, fat balance studies were conducted using the nonabsorbable lipid marker SPB. As shown in **Fig. 2A**, ~98% of dietary fat was absorbed in *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$ mice fed a low (5%) fat, SPB-containing diet. Dietary fat absorption was also examined gravimetrically in mice fed a high-fat Western diet for 11 weeks. Again we observed ~98% absorption of dietary fat (Fig. 2A). Moreover, fecal TG, cholesterol, and FFA concentration per mg stool were no different between the genotypes (data not shown). There was no significant difference in body weight or weight gain between control and *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$ mice fed either a chow (data not shown) or a high-fat Western diet (Fig. 2B, C), although there was a trend toward decreased weight gain in Western diet-fed *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$ mice. This trend was observed primarily in female *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$ mice, although the data reached statistical significance only at one time point (see supplementary Fig. I for gender-specific weight data). By contrast, body weight and weight gain were virtually superimposable in male *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$ and control mice fed a Western diet (see supplementary Fig. I). Overall, these data suggest that there is no disruption of dietary FA absorption in *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$ mice.

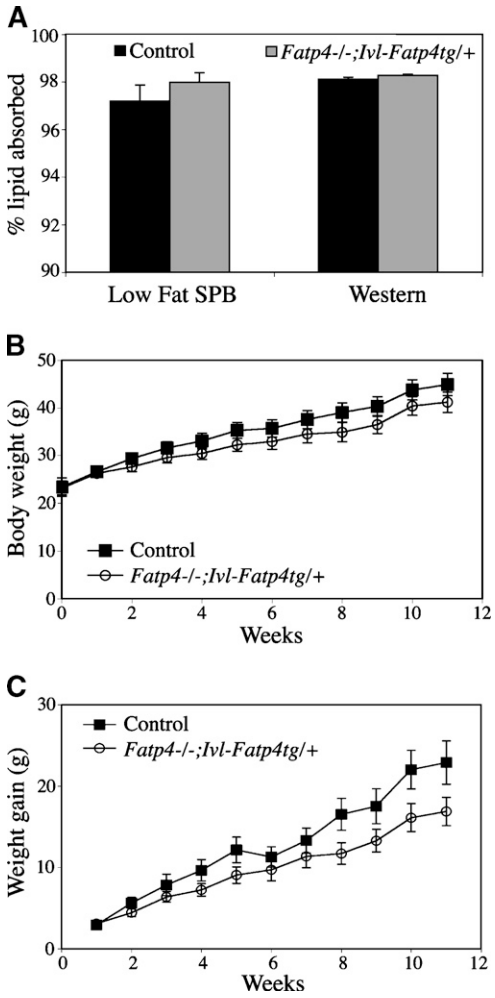

Fig. 2. Intestinal lipid absorption in control and *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$ mice fed a Western diet. A: Dietary fat absorption in mice fed a low-fat, sucrose polybehenate (SPB)-containing diet (left panel) or a high-fat, Western diet (right panel). n = 6–7 mice/genotype for SPB diet and n = 10–11 mice/genotype for Western diet studies. Body weight (B) and weight gain (C) were measured in control (n = 14, black squares) and *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$ (n = 16, open circles) mice fed a Western diet for 10–12 weeks. Data were recorded weekly. The trend toward decreased body weight and weight gain in Western diet-fed *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$ mice was primarily due to female mice (n = 8 females in control group, 9 in *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$ group), though the differences still did not reach statistical significance. Separate graphs for male and female mice are shown in supplementary Figure I. Data are shown as mean ± SE.

Subtle alterations in intestinal lipid content in *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$ mice

Intestinal lipid content was examined in *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$ mice fed a chow or Western diet to investigate possible alterations in intracellular lipid trafficking. We observed no difference between the genotypes in TG, cholesterol, or FFA content of scraped mucosa obtained from mice fed a chow diet (**Fig. 3A**), but *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$ mice fed a Western diet demonstrated increased intestinal TG and FFA content compared with controls (Fig. 3B). These data suggest that there may be a subtle alteration in FA trafficking in *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$ mice, resulting in accumulation of excess lipid in the proximal intestine of these mice.

No alteration in kinetics of intestinal FA uptake and secretion in *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$ mice

Previous studies in CD36$^{-/-}$ mice demonstrated no gross abnormalities in overall intestinal fat absorption, but rather showed a defect in the *rate* at which dietary FAs are transported into the lymph and plasma compartments (3). To examine whether the kinetics of intestinal FA absorption were altered in *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$ mice, animals were first challenged with an acute lipid bolus containing [^{3}H]triolein in 0.5ml intralipid/ corn oil, which contains primarily long-chain unsaturated FA (i.e., C18:2). Serum radioactivity (**Fig. 4A**) and TG mass (Fig. 4B) increased with a similar time course in both genotypes, indicating that there is no alteration in the rate of intestinal FA uptake and secretion of long-chain FAs in *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$ mice. We also found that serum radioactivity was predominantly (~85%) distributed into TG in both genotypes, implying that uptake of dietary FA and resynthesis into TG following ingestion of [^{3}H] triolein was not impaired in *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$ mice. Moreover, no differences in mucosal TG content in proximal, middle, or distal intestinal segments were observed between the genotypes 4 h after the lipid bolus (Fig. 4C), suggesting that there is no residual accumulation of lipid in *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$ mice, analogous to that previously observed in similar studies with CD36$^{-/-}$ mice (9).

Because previous studies have implicated a role for FATP4 in uptake and acylation of VLCFAs (17, 27), specifically

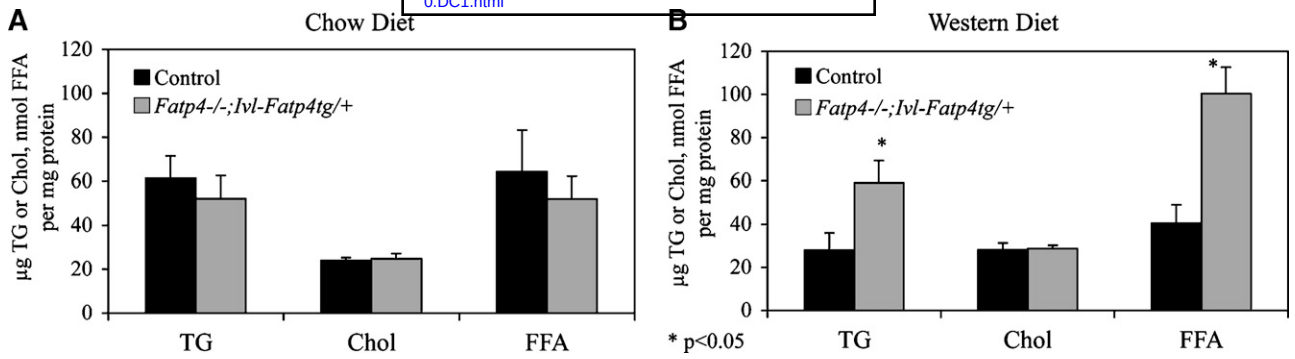

Fig. 3. Intestinal lipid content in chow and Western diet-fed control and *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$ mice. Intracellular triglyceride (TG), cholesterol, and free FA content of scraped mucosa from mice fed a chow (A) (control, n = 6 and *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$, n = 5–6) or Western diet (B) (control, n = 5–6 and *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$, n = 5–7) were measured. Mice were fasted for 4 h prior to sacrifice. * $P < 0.05$. Data are shown as mean ± SE.

C24:0, *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$ mice were also challenged with a lipid bolus containing [^{14}C]lignoceric acid (C24:0) in Menhaden fish oil, which is enriched in eicosapentaenoic acid (EPA, C20:5) and docosahexaenoic acid (DHA, C22:6). Mice were sacrificed after 5 h, and intestinal lipid mass and radioactivity were measured. We observed no difference in the mass of TG contained within enterocytes from each section of the small intestine (Fig. 4D), with a slight decrease in FFA content in the proximal intestine of *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$ mice (Fig. 4E). However, there was no difference between the genotypes in the amount of [^{14}C]lignoceric acid remaining in the intestinal lumen 5 h after gavage (30.2 ± 2.5%, control; 29.0 ± 1.6%, *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$, expressed as percent of total radioactivity administered) and no difference in the amount of radioactivity present in each of the intestinal segments (Fig. 4F), indicating that there is no defect in [^{14}C]lignoceric acid absorption in *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$ mice. Finally, we observed similar increases in serum TG and FFA levels in both genotypes (data not shown). Taken together, these data strongly suggest that there is no kinetic defect in intestinal absorption or secretion of VLCFA, including C24:0, in *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$mice.

No defect in intestinal cholesterol absorption in *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$ mice

We observed that total serum cholesterol levels were reduced in *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$ mice fed either a chow or Western diet (**Table 1**), with no difference in serum TG levels between the genotypes. Decreased serum cholesterol appears to correlate with a change in cholesterol ester levels, due to the fact that no difference in free cholesterol was observed between the genotypes (Table 1). In order to investigate the reason for the reduced serum cholesterol, we examined whether intestinal cholesterol absorption was altered in *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$ mice. As shown in **Fig. 5A**, cholesterol absorption was similar in *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$ and control mice, and was almost completely eliminated in both genotypes when mice were fed an ezetimibe-containing diet for 3 weeks. In addition, we observed no differences in the expression of a range of cholesterol or FA transporter mRNAs in proximal small intestinal mucosa from either genotype (Fig. 5B), suggesting in particular that there is no compensatory induction of FATP1, FATP2, FATP3, FATP5, CD36, or Caveolin1 expression in the proximal intestine of *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$ mice.

Hepatic lipid content and VLDL secretion are unchanged in *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$ mice

As an alternative explanation for reduced serum cholesterol levels in *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$ mice, we examined whether hepatic lipid content or VLDL production were altered in chow and Western diet fed *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$ mice. Hepatic TG secretion rates were similar in both chow and Western diet fed mice of both genotypes (**Fig. 6A, C**), although there was a trend to lower TG secretion rates in *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$ mice fed Western diet. In addition, we observed no difference in hepatic TG, cholesterol, or FFA content between control and *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$ mice fed either a chow diet or Western diet (Fig. 6B, D) and no compensatory changes in mRNA expression of other FATPs or in a panel of lipid metabolism-related genes (detailed in Fig. 5), including apoB and microsomal TG transfer protein (data not shown). These data demonstrate that hepatic lipid content and VLDL production are not significantly different in *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$ mice fed either a low-fat chow diet or high-fat Western diet. Thus, we conclude that the absence of FATP4 in liver does not dramatically alter hepatic lipid metabolism.

DISCUSSION

The mechanisms and pathways by which long-chain FA are absorbed across the brush border of the mammalian small intestine have yet to be completely resolved. Experimental evidence using a variety of models (including isolated loops, pooled enterocytes, and even purified apical membrane vesicles) has supported the concepts of both diffusional and saturable components of intestinal FA uptake (4, 28–31), although there are inherent reservations with some of the underlying assumptions for these kinetic models, as recently summarized by Nassir et al. (8). That said, in view of the importance and efficiency of intestinal FA uptake, it seems reasonable to predict that a high affinity

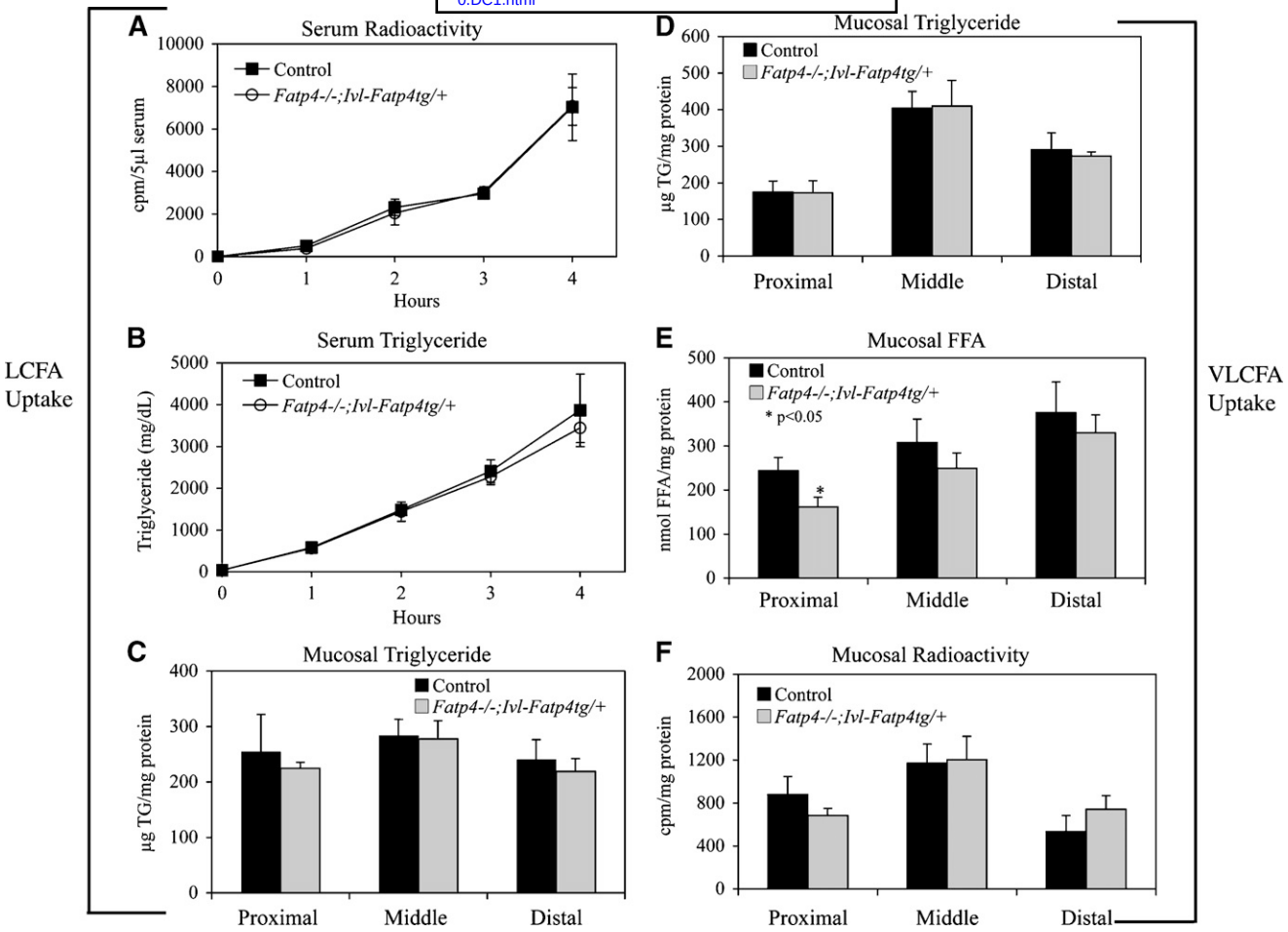

Fig. 4. Intestinal lipid trafficking in control and *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$ mice. A–C: Control (n = 3, black squares) and *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$ (n = 4, open circles) mice were fasted overnight, injected intravenously with Tyloxapol to block serum lipase activity, then gavaged with a long-chain FA lipid bolus containing [^{3}H]triolein. Serum radioactivity (A) and TG (B) were measured every h for 4 h following the gavage. Following the last time point, animals were sacrificed, and intestinal TG content of each third of the intestine was determined (C). D–F: Control (n = 5, black bars) and *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$ (n = 6, gray bars) mice were fasted overnight, then gavaged with a VLCFA bolus containing [^{14}C]lignoceric acid in Menhaden oil. Intracellular TG (D) and FFA (E) content of each third of the intestine 5 h after bolus was determined and normalized to protein concentration. Cellular radioactivity (F) was determined by scintillation counting and normalized to protein content. * $P < 0.05$. Data are shown as mean ± SE.

apical FA transport protein(s) would be expressed in mammalian enterocytes and by corollary, that its genetic deletion might interfere with FA uptake. The current studies detail our findings in regard to a physiological role for one such candidate intestinal FA transport protein, FATP4.

FATP4 was identified several years ago as the predominant FATP in the small intestine, an abundant, apically localized FA transport protein whose knockdown in isolated enterocytes resulted in decreased uptake of long-chain FA (11). These findings, coupled with studies in heterozygous *Fatp4*$^{+/-}$ mice (13) in which there was reduced long-chain

TABLE 1. Serum lipid levels in wild-type and *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$ mice

Chow Diet	Control	*Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$	*P* value
Triglyceride (mg/dl)	131 ± 8 (24)	119 ± 11 (16)	NS
Total Cholesterol (mg/dl)	121 ± 5 (29)	95 ± 6 (22)	<0.008
Free Cholesterol (mg/dl)	18 ± 0.6 (5)	16 ± 1.3 (6)	NS
Western Diet	**Control**	***Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$**	***P* value**
Triglyceride (mg/dl)	118 ± 4 (11)	125 ± 7 (11)	NS
Cholesterol (mg/dl)	165 ± 39(10)	86 ± 1 (10)	<0.0002

NS = not significant. Data is expressed as mean ± SE, with the number of animals indicated in the parentheses. Serum was collected following at least a 4 h fast.

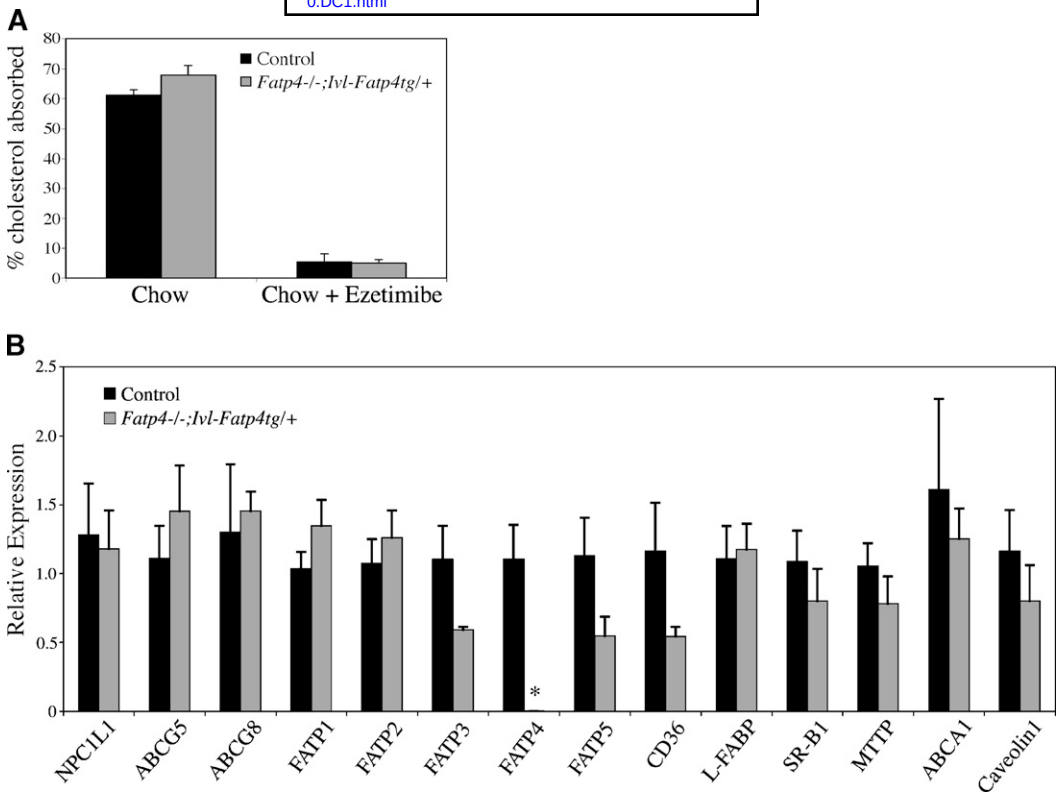

Fig. 5. Cholesterol absorption and relative mRNA expression of genes involved in lipid metabolism in control and *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$ mice. A: Cholesterol absorption in control and *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$ was measured using a fecal dual isotope assay on mice fed a chow diet (control n = 13, *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$ n = 10) and on mice fed a chow diet containing ezetimibe to block cholesterol absorption (control n = 7, *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$ n = 6). B: Relative mRNA levels of genes involved in lipid metabolism in scraped mucosa from proximal small intestine (control n = 5–6, *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$ n = 5–6) were measured using real-time quantitative PCR. Relative expression is expressed as fold change normalized to enterocytes from control mice. * $P < 0.007$. Data are shown as mean ± SE.

FA uptake, led to the prediction that FATP4 was directly involved in dietary FA uptake and utilization (6). However, the studies presented in this manuscript are at odds with those previous findings and thus do not support this prediction. *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$ mice express FATP4 only in the skin, with no detectable expression of FATP4 mRNA or protein in the liver or intestine. Despite this, *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$ mice absorb ~98% of dietary fat, both on a low-fat chow diet and on a high-fat, high-cholesterol Western diet, and manifest similar weight gain and growth on both diets. Following gavage of a long-chain FA lipid bolus, *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$ mice display no differences in the rate of appearance of either TG mass or radioactivity (predominantly as [^{3}H]TG) in serum compared with controls (Fig. 4A–C). Similarly, *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$ mice displayed no defect in intestinal uptake and trafficking of a lipid bolus containing VLCFA (Fig. 4D-F). The most straightforward interpretation of these data is that there is no obvious defect in the ability of FATP4-deficient enterocytes, at least in the whole animal, to absorb dietary long-chain FA, direct these FA for resynthesis into complex lipid including TG, and to assemble and secrete TG-rich lipoprotein particles into the serum. Some elements of these conclusions, however, merit further discussion.

As noted above, we and others reported the phenotype of FATP4 null mice, which included perinatal lethality resulting from a severe skin defect, characterized by altered VLCFA composition (primarily ≥C26) in epidermal ceramides, which in turn led to defective formation of the cornified envelope that forms the skin barrier (12, 14, 15, 32). This phenotype was rescued by FATP4 expression in the suprabasal keratinocytes within stratified squamous epithelium and hair follicles and required an intact acyl-CoA synthetase domain within the FATP4 transgene (15). Other studies using fibroblasts isolated from *Fatp4*$^{-/-}$ mice demonstrated a significant decrease in the levels of C20 and C22 FA compared with FA from *Fatp4*$^{+/+}$ fibroblasts (17). An important conclusion stemming from these as well as other studies is that FATP4 may play a specific role in the metabolism of VLCFA, specifically ≥C22. However, we did not observe a marked difference in intestinal lipid content or radioactivity in *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$ mice following administration of a lipid bolus containing [^{14}C]lignoceric acid (C24:0), as well as C20:5 and C22:6 FA, suggesting no defect in intestinal absorption, processing, or secretion of these FA. Nevertheless, it remains formally possible that loss of FATP4 function in the small intestine may target the absorption of select, VLCFAs (specifically ≥C26), rather

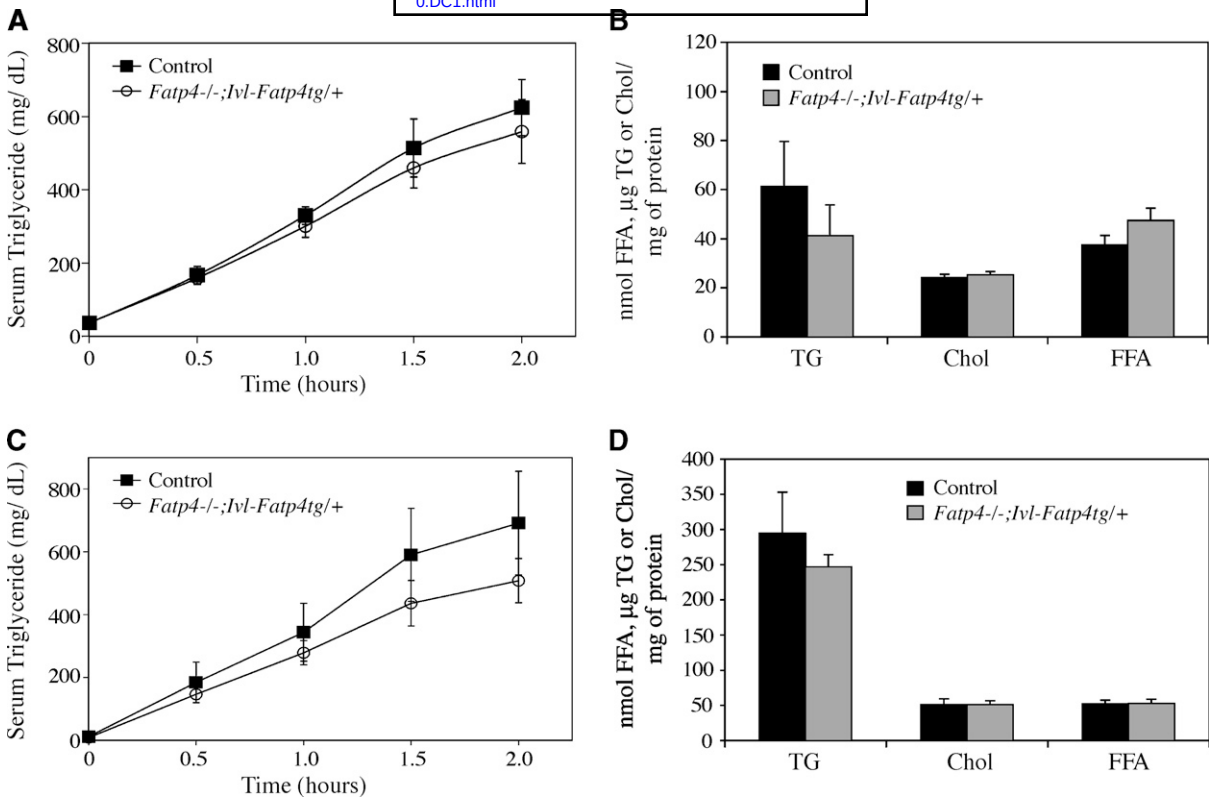

Fig. 6. Hepatic lipid secretion in control and *Fatp4*$^{-/-}$*;Ivl-Fatp4*$^{tg/+}$ mice. A, C: Control (n = 3, black squares) and *Fatp4*$^{-/-}$*;Ivl-Fatp4*$^{tg/+}$ (n = 4, open circles) female mice fed a chow diet (A) and a Western diet (C) were fasted for 4 h and intravenously injected with Tyloxapol. Blood was taken from mice every 30 min for 120 min. Serum TG was measured at each time point. Time zero blood samples were taken prior to Tyloxapol injections. B, D: Hepatic TG, cholesterol, and free FA content in livers of mice fed a chow (B, n = 5) or Western diet (D, n = 9-10) were measured. Data are shown as mean ± SE.

than absorption of 16–18 carbon FA more typically found in dietary fat.

Similar approaches to those used in the current study have been employed to elicit a functional role for the FA transport protein CD36, and the conclusions from these studies bear comparison to our findings. Studies by Drover et al. (3) demonstrated subtle defects in intestinal chylomicron secretion from CD36$^{-/-}$ mice as manifested by intestinal lipid accumulation following an oral lipid bolus. Further study however revealed no defect in intestinal FA absorption in CD36$^{-/-}$ mice using SPB determination on fecal samples (analogous to that used in the current study), but rather demonstrated that the rate of transport into lymph and serum of TG and cholesterol was reduced in CD36$^{-/-}$ mice (9). More recent studies refined these conclusions by demonstrating a role for CD36 in FA and cholesterol uptake in the proximal but not distal intestine, suggesting that there is a region-specific function for CD36 in the transport and secretion of intestinal chylomicrons (8), while yet other studies demonstrated that CD36$^{-/-}$ mice are protected against diet induced obesity (33). These studies collectively imply a robust role for CD36 in the regulation of intestinal lipid transport. In regard to the role of FATP4, our findings reveal several striking departures from the phenotypes described above in CD36$^{-/-}$ mice. Specifically, we find no evidence for intestinal lipid accumulation following a fat bolus, no evidence for delayed appearance of radiolabel or TG mass in the serum, and no evidence for protection against Western diet-induced obesity. The slight, albeit statistically significant increase in mucosal TG and free FA content in Western diet-fed *Fatp4*$^{-/-}$*;Ivl-Fatp4*$^{tg/+}$ mice (Fig. 3B) suggests that there may be a subtle effect on intestinal lipid accumulation and transport, but it is difficult to assign a physiological interpretation to these findings given the magnitude of the differences. Further study is required to determine whether there are differences (i.e., in chain length or degree of saturation) in the intestinal lipid species that accumulate in Western diet-fed *Fatp4*$^{-/-}$*;Ivl-Fatp4*$^{tg/+}$ mice compared with controls.

The finding that *Fatp4*$^{-/-}$*;Ivl-Fatp4*$^{tg/+}$ mice manifest lower plasma total cholesterol levels on a chow and a high-fat Western diet is unexplained, though the differences appear to be due to altered levels of esterified cholesterol, because free cholesterol levels were unchanged (Table 1). We examined the possibility that *Fatp4*$^{-/-}$*;Ivl-Fatp4*$^{tg/+}$ mice might exhibit reduced cholesterol absorption (analogous to some of the findings alluded to above in CD36$^{-/-}$ mice), but this was not the case. Our findings demonstrate that *Fatp4*$^{-/-}$*;Ivl-Fatp4*$^{tg/+}$ mice exhibit no defect in intestinal cholesterol absorption as compared with littermate controls, with no compensatory change in the expression of other cholesterol transporters. We also examined the possibility that hepatic TG secretion was

reduced in *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$ mice, but yet again we found no differences from control mice. It is worth noting that targeted deletion of *Fatp5* revealed a dramatic liver phenotype with reduced steatosis and decreased TG secretion following high-fat feeding (34), while knockdown of FATP5 successfully reversed diet-induced steatosis in mice (35). These findings, taken in conjunction with the skin phenotype in *Fatp4* $^{-/-}$ mice, suggest that there are nonredundant functions for FATP family members despite overlapping expression patterns.

In conclusion, our findings demonstrate that intestinal lipid transport is preserved in *Fatp4*$^{-/-}$;*Ivl-Fatp4*$^{tg/+}$ mice. These mice exhibit no protection against high-fat diet induced weight gain and display grossly similar transport characteristics for intestinal TG and cholesterol. Although the evidence does not permit us to conclude that there is no role for FATP4 in intestinal or hepatic lipid metabolism, the findings suggest that FATP4 is dispensable for intestinal lipid transport.

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REFERENCES

1. Chen, Z., and N. O. Davidson. 2006. Genetic regulation of intestinal lipid transport and metabolism. *In* Physiology of the Gastrointestinal Tract. L. R. Johnson, editor. Elsevier Academic Press, Boston, MA 1711–1734.
2. Mansbach 2nd, C. M., and F. Gorelick. 2007. Development and physiological regulation of intestinal lipid absorption. II. Dietary lipid absorption, complex lipid synthesis, and the intracellular packaging and secretion of chylomicrons. *Am. J. Physiol.* **293:** G645–G650.
3. 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.
4. Stremmel, W. 1988. Uptake of fatty acids by jejunal mucosal cells is mediated by a fatty acid binding membrane protein. *J. Clin. Invest.* **82:** 2001–2010.
5. Pohl, J., A. Ring, R. Ehehalt, T. Herrmann, and W. Stremmel. 2004. New concepts of cellular fatty acid uptake: role of fatty acid transport proteins and of caveolae. *Proc. Nutr. Soc.* **63:** 259–262.
6. Doege, H., and A. Stahl. 2006. Protein-mediated fatty acid uptake: novel insights from in vivo models. *Physiology (Bethesda, MD).* **21:** 259–268.
7. Drover, V. A., D. V. Nguyen, C. C. Bastie, Y. F. Darlington, N. A. Abumrad, J. E. Pessin, E. London, D. Sahoo, and M. C. Phillips. 2008. CD36 mediates both cellular uptake of very long chain fatty acids and their intestinal absorption in mice. *J. Biol. Chem.* **283:** 13108–13115.
8. Nassir, F., B. Wilson, X. Han, R. W. Gross, and N. A. Abumrad. 2007. CD36 is important for fatty acid and cholesterol uptake by the proximal but not distal intestine. *J. Biol. Chem.* **282:** 19493–19501.
9. 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.
10. Hirsch, D., A. Stahl, and H. F. Lodish. 1998. A family of fatty acid transporters conserved from mycobacterium to man. *Proc. Natl. Acad. Sci. USA.* **95:** 8625–8629.
11. Stahl, A., D. J. Hirsch, R. E. Gimeno, S. Punreddy, P. Ge, N. Watson, S. Patel, M. Kotler, A. Raimondi, L. A. Tartaglia, et al. 1999. Identification of the major intestinal fatty acid transport protein. *Mol. Cell.* **4:** 299–308.
12. Moulson, C. L., D. R. Martin, J. J. Lugus, J. E. Schaffer, A. C. Lind, and J. H. Miner. 2003. Cloning of wrinkle-free, a previously uncharacterized mouse mutation, reveals crucial roles for fatty acid transport protein 4 in skin and hair development. *Proc. Natl. Acad. Sci. USA.* **100:** 5274–5279.
13. 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.
14. Herrmann, T., F. van der Hoeven, H. J. Grone, A. F. Stewart, L. Langbein, I. Kaiser, G. Liebisch, I. Gosch, F. Buchkremer, W. Drobnik, et al. 2003. Mice with targeted disruption of the fatty acid transport protein 4 (Fatp 4, Slc27a4) gene show features of lethal restrictive dermopathy. *J. Cell Biol.* **161:** 1105–1115.
15. Moulson, C. L., M. H. Lin, J. M. White, E. P. Newberry, N. O. Davidson, and J. H. Miner. 2007. Keratinocyte-specific expression of fatty acid transport protein 4 rescues the wrinkle-free phenotype in Slc27a4/Fatp4 mutant mice. *J. Biol. Chem.* **282:** 15912–15920.
16. Milger, K., T. Herrmann, C. Becker, D. Gotthardt, J. Zickwolf, R. Ehehalt, P. A. Watkins, W. Stremmel, and J. Fullekrug. 2006. Cellular uptake of fatty acids driven by the ER-localized acyl-CoA synthetase FATP4. *J. Cell Sci.* **119:** 4678–4688.
17. Jia, Z., C. L. Moulson, Z. Pei, J. H. Miner, and P. A. Watkins. 2007. Fatty acid transport protein 4 is the principal very long chain fatty acyl-CoA synthetase in skin fibroblasts. *J. Biol. Chem.* **282:** 20573–20583.
18. Lobo, S., B. M. Wiczer, A. J. Smith, A. M. Hall, and D. A. Bernlohr. 2007. Fatty acid metabolism in adipocytes: functional analysis of fatty acid transport proteins 1 and 4. *J. Lipid Res.* **48:** 609–620.
19. Newberry, E. P., Y. Xie, S. Kennedy, X. Han, K. K. Buhman, J. Luo, R. W. Gross, and N. O. Davidson. 2003. Decreased hepatic triglyceride accumulation and altered fatty acid uptake in mice with deletion of the liver fatty acid-binding protein gene. *J. Biol. Chem.* **278:** 51664–51672.
20. Ninomiya, Y., M. Kagawa, K. Iyama, I. Naito, Y. Kishiro, J. M. Seyer, M. Sugimoto, T. Oohashi, and Y. Sado. 1995. Differential expression of two basement membrane collagen genes, COL4A6 and COL4A5, demonstrated by immunofluorescence staining using peptide-specific monoclonal antibodies. *J. Cell Biol.* **130:** 1219–1229.
21. Newberry, E. P., S. M. Kennedy, Y. Xie, B. T. Sternard, J. Luo, and N. O. Davidson. 2008. Diet-induced obesity and hepatic steatosis in L-Fabp / mice is abrogated with SF, but not PUFA, feeding and attenuated after cholesterol supplementation. *Am. J. Physiol.* **294:** G307–G314.
22. Newberry, E. P., Y. Xie, S. M. Kennedy, J. Luo, and N. O. Davidson. 2006. Protection against Western diet-induced obesity and hepatic steatosis in liver fatty acid-binding protein knockout mice. *Hepatology (Baltimore, MD).* **44:** 1191–1205.
23. Jandacek, R. J., J. E. Heubi, and P. Tso. 2004. A novel, noninvasive method for the measurement of intestinal fat absorption. *Gastroenterology.* **127:** 139–144.
24. Wang, D. Q., and M. C. Carey. 2003. Measurement of intestinal cholesterol absorption by plasma and fecal dual-isotope ratio, mass balance, and lymph fistula methods in the mouse: an analysis of direct versus indirect methodologies. *J. Lipid Res.* **44:** 1042–1059.
25. Wang, D. Q., B. Paigen, and M. C. Carey. 2001. Genetic factors at the enterocyte level account for variations in intestinal cholesterol absorption efficiency among inbred strains of mice. *J. Lipid Res.* **42:** 1820–1830.
26. Garcia-Calvo, M., J. Lisnock, H. G. Bull, B. E. Hawes, D. A. Burnett, M. P. Braun, J. H. Crona, H. R. Davis, Jr., D. C. Dean, P. A. Detmers, et al. 2005. The target of ezetimibe is Niemann-Pick C1-Like 1 (NPC1L1). *Proceedings of the National Academy of Sciences of the United States of America.* **102:** 8132–8137.
27. Hall, A. M., B. M. Wiczer, T. Herrmann, W. Stremmel, and D. A. Bernlohr. 2005. Enzymatic properties of purified murine fatty acid transport protein 4 and analysis of acyl-CoA synthetase activities in tissues from FATP4 null mice. *J. Biol. Chem.* **280:** 11948–11954.
28. Chow, S. L., and D. Hollander. 1979. A dual, concentration-dependent absorption mechanism of linoleic acid by rat jejunum in vitro. *J. Lipid Res.* **20:** 349–356.
29. Murota, K., and J. Storch. 2005. Uptake of micellar long-chain fatty acid and sn-2-monoacylglycerol into human intestinal Caco-2 cells exhibits characteristics of protein-mediated transport. *J. Nutr.* **135:** 1626–1630.

30. Prieto, R. M., W. Stremmel, C. Sales, and J. A. Tur. 1996. Effect of dietary fatty acids on jejunal and ileal oleic acid uptake by rat brush border membrane vesicles. *Eur. J. Med. Res.* **1:** 355–360.
31. Prieto, R. M., W. Stremmel, C. Sales, and J. A. Tur. 1996. Oleic acid uptake by jejunal and ileal rat brush border membrane vesicles. *Eur. J. Med. Res.* **1:** 199–203.
32. Herrmann, T., H. J. Grone, L. Langbein, I. Kaiser, I. Gosch, U. Bennemann, D. Metzger, P. Chambon, A. F. Stewart, and W. Stremmel. 2005. Disturbed epidermal structure in mice with temporally controlled fatp4 deficiency. *J. Invest. Dermatol.* **125:** 1228–1235.
33. Hajri, T., A. M. Hall, D. R. Jensen, T. A. Pietka, V. A. Drover, H. Tao, R. Eckel, and N. A. Abumrad. 2007. CD36-facilitated fatty acid uptake inhibits leptin production and signaling in adipose tissue. *Diabetes.* **56:** 1872–1880.
34. Doege, H., R. A. Baillie, A. M. Ortegon, B. Tsang, Q. Wu, S. Punreddy, D. Hirsch, N. Watson, R. E. Gimeno, and A. Stahl. 2006. Targeted deletion of FATP5 reveals multiple functions in liver metabolism: alterations in hepatic lipid homeostasis. *Gastroenterology.* **130:** 1245–1258.
35. Doege, H., D. Grimm, A. Falcon, B. Tsang, T. A. Storm, H. Xu, A. M. Ortegon, M. Kazantzis, M. A. Kay, and A. Stahl. 2008. Silencing of hepatic fatty acid transporter protein 5 in vivo reverses diet-induced non-alcoholic fatty liver disease and improves hyperglycemia. *J. Biol. Chem.* **283:** 22186–22192.