Gut expression and regulation of FAT/CD36: possible role in fatty acid transport in rat enterocytes

MIN CHEN,[1] YINGKUI YANG,[1] EVAN BRAUNSTEIN,[2]
KEITH E. GEORGESON,[1] AND CARROLL M. HARMON[1]
[1]*Department of Surgery, University of Alabama at Birmingham, Birmingham, Alabama 35233;*
and [2]*Department of Surgery, The University of Michigan Medical School,*
and the C. S. Mott Children's Hospital, Ann Arbor, Michigan 48109

Chen, Min, Yingkui Yang, Evan Braunstein, Keith E. Georgeson, and Carroll M. Harmon. Gut expression and regulation of FAT/CD36: possible role in fatty acid transport in rat enterocytes. *Am J Physiol Endocrinol Metab* 281: E916–E923, 2001.—Fatty acid translocase (FAT)/CD36 is one of several putative plasma membrane long-chain fatty acid (LCFA) transport proteins; however, its role in intestinal absorption of LCFA is unknown. We hypothesized that FAT/CD36 would be differentially expressed along the longitudinal axis of the gut and during intestinal development, suggesting specificity of function. We found that intestinal mucosal FAT/CD36 mRNA levels varied by anatomic location along the longitudinal gut axis: stomach 45 ± 7, duodenum 173 ± 29, jejunum 238 ± 17, ileum 117 ± 14, and colon $9 \pm 1\%$ (means $\pm$ SE with 18S mRNA as control). FAT/CD36 protein levels were also higher in proximal compared with distal intestinal mucosa. Mucosal FAT/CD36 mRNA was also regulated during intestinal maturation, with a fourfold increase from neonatal to adult animals. In addition, FAT/CD36 mRNA levels and enterocyte LCFA uptake were rapidly downregulated by intraduodenal oleate infusion. These findings suggest that FAT/CD36 plays a role in the uptake of LCFA by small intestinal enterocytes. This may have important implications in understanding fatty acid absorption in human physiological and pathophysiological conditions.

fatty acid translocase/CD36; intestine; transport of fatty acids

AFTER HYDROLYSIS OF DIETARY LIPIDS, long-chain fatty acids (LCFA) are readily absorbed in the mammalian small intestine. In addition to serving as a major energy source for most tissues in the body, LCFA function as building blocks for phospholipids (26), signaling molecules (49), protein modulators (11, 37), and regulators of genes involved in lipid metabolism and cell differentiation (9, 23, 31). As a result of their multiple functions, LCFA have been implicated in conditions such as inflammation (15, 16), atherosclerosis (18), immune responses (17, 20), cancer, and cell differentiation (6, 7, 36). Therefore, the process by which LCFA move from the intestinal lumen into the enterocyte is a critical first step in delivering these important biological molecules to their in vivo sites of action.

The mechanism(s) responsible for the movement of LCFA across cellular plasma membranes, including that of enterocytes, is a subject of controversy (2, 32). Because of their lipophilic nature, LCFA have been expected to diffuse freely through the plasma membrane. However, given the general physiological importance of LCFA, it should be expected that cells would be able to regulate LCFA uptake to adapt to changes in utilization demands. Over the past 15 years, a wealth of information has been obtained arguing for the existence of a protein-facilitated component in membrane transport of LCFA. Several proteins have been proposed as candidates for plasma membrane LCFA transporter proteins. Three of these candidate LCFA transporters are expressed in the small intestine: plasma membrane fatty acid binding protein (FABPpm), fatty acid transport protein (FATP), and fatty acid translocase (FAT), the rat homologue of human CD36. FABPpm was isolated from rat hepatocytes (47) and is expressed in rat enterocytes (46). Several lines of investigation suggest a role for FAPBpm in transport of LCFA (44, 46, 50); however, there are also studies that cast considerable doubt regarding this function (13, 24). FATP is another promising candidate for a cellular LCFA transporter (41). FAT/CD36 is an 88-kDa integral membrane protein isolated from rat adipocytes (33, 34), which has been shown to inhibit LCFA transport when bound by synthetic reactive LCFA derivatives (34) and to increase cellular uptake of LCFA when expressed in fibroblasts (35). A recent report suggests that FAT/CD36 deficiency may underlie the defective fatty acid metabolism in the insulin-resistant syndrome, such as in type 2 diabetes (8).

The role that FAT/CD36 might play in the intestinal absorption of LCFA is unknown; however, FAT/CD36 has been shown to be expressed in the rat small bowel mucosa. In addition, intestinal FAT/CD36 mRNA levels were reported to be increased in rats on a high-fat diet (38). To further investigate the possible role of FAT/CD36 in the intestinal absorption of LCFA, this study investigates the expression of FAT/CD36 mRNA

---

and protein along the rat gut longitudinal axis as well as during intestinal maturation. Also, the rapid effects of intraduodenal oleate infusion on FAT/CD36 expression and on LCFA uptake by isolated jejunal enterocytes are reported.

MATERIALS AND METHODS

Materials. Oleate, bovine serum albumin (BSA, essentially fatty acid free), HEPES, and other chemicals were purchased from Sigma Chemical (St. Louis, MO). [³H]oleate was obtained from Du Pont-NEN. SDS-PAGE molecular weight standards were obtained from Bio-Rad Laboratories (Hercules, CA). Enhanced chemiluminescence (ECL) reagents, [α-³²P]dCTP, and random-primer cDNA labeling kits were from Amersham (Arlington Heights, IL). Horseradish peroxidase-conjugated anti-mouse IgG and anti-human CD36 mouse monoclonal antibodies were obtained from Transduction Laboratories (Lexington, KY). Glass fiber filters (type A/E) were from Gelman Sciences (Ann Arbor, MI). NucTrap Probe purification columns were purchased from Stratagene (La Jolla, CA). Normal mouse IgG was purchased from Santa Cruz Biotechnology (Santa Cruz, CA).

Animal preparation. Male Sprague-Dawley rats (Harlan Industries, Indianapolis, IN), weighing 250–300 g, were housed in an animal care facility and fed standard rat chow. For the intraduodenal oleate infusion experiments, rats were fasted overnight and anesthetized with a mixture of xylazine (13 mg/kg) and ketamine (87 mg/kg). Supplemental doses were used every 2 h as needed to maintain adequate anesthesia. Through a midline incision, a polyethylene catheter was placed in the duodenum, slightly above the ampulla. The abdominal wound was covered with a saline gauze, and the animal body temperature was monitored by a rectal thermal probe and maintained at 37°C with a heating pad. After 1 h of stabilization, the rats received an intraduodenal infusion of 10% oleate at the rate of 3 ml/h for 1 h. Fresh oleic acid emulsion was prepared by emulsifying oleic acid (Fisher, Pittsburgh, PA) with 3% polysorbate 80 (Sigma) and diluting it with 0.15 M NaCl to a concentration of 10% (vol/vol) with a final pH of 6.

Tissue sampling and preparation. In the adult rats, 5-cm intestinal segments were harvested from the duodenum, proximal jejunum (5 cm beyond the ligament of Treitz), and distal ileum (8 cm proximal to the cecum) and immediately rinsed twice with phosphate-buffered saline solution (PBS). The distal half of the gastric mucosa and the proximal colonic mucosa were harvested in certain experiments. The mucosa was scraped from the underlying submucosa. One-half of the mucosal scrapings was frozen in liquid nitrogen and stored at −80°C for subsequent RNA analyses. The remaining one-half of the mucosal scrapings was homogenized and assayed for protein content as described by Bradford (14). In the neonatal rat experiments, the entire small intestine was used for RNA isolation.

Preparation of intestinal cells. Fasted Sprague-Dawley male rats were killed by cervical dislocation. Intestinal cells were isolated according to a modification of the method of Gore and Honiara (28). Briefly, the jejunum was removed, rinsed thoroughly with 0.154 M NaCl and 1 mM dithiothreitol, filled with *solution A* (in mM: 1.5 KCl, 96 NaCl, 27 sodium citrate, 8 KH₂PO₄, and 5.6 Na₂HPO, pH 7.3), and incubated for 15 min at 37°C. After *solution A* had been discarded, the intestine was filled with *solution B* (PBS without Ca²⁺ or Mg²⁺ and with 1.5 mM EDTA and 0.5 mM dithiothreitol), incubated for 3 min at 37°C, and then gently palpated with the fingers for 2 min. The buffer containing mucosal cells was filtered through nylon gauze (70 μm pore size). After centrifugation (765 *g*), the isolated cells were rinsed twice and resuspended in Krebs-Ringer-Henseleit (KRH) buffer (2 × 10⁶ cells/ml or 3 mg protein/ml). Light microscopy of the cell suspension showed that ~90% of the cells displayed typical features of villus-tip cells. Viability was assessed by trypan blue exclusion capacity (12).

Immunoprecipitation. Immunoprecipitations were carried out using the method described by Courtneidge and Smith (21). Briefly, intestinal mucosal tissue was homogenized in lysis buffer [0.15 mM MgCl₂, 0.05 M HEPES, 0.15 M NaCl, 0.02 M EDTA, 1 mM Na-orthovanadate, 10 mM Na-pyrophosphate, 10 mM Na-fluoride, 1 mM 4-(2-aminoethyl)-benzenesulfonyl fluoride hydrochloride (AEBSF; ICN, Costa Mesa, CA)], 10% glycerol, 1% Triton X-100, and 10 μg/ml of aprotinin and leupeptin, with temperature maintained at 4°C throughout all procedures. The homogenate was centrifuged at 15,000 *g* for 20 min at 4°C. The concentration of supernatant protein was analyzed spectrophotometrically at 595λ Vis light. Supernatant protein (3 mg) was incubated with 12.5 μg of anti-human CD36 mouse monoclonal antibody overnight at 4°C while it was gently rotating. On the next day, 40 μl of 50× protein A-agarose was added and incubated at 4°C for 2 h while the mixture was gently rotating. Immunoprecipitates were collected by centrifugation at 7,000 rpm at 4°C, and the pellets were washed four times with 0.5 ml fresh lysis buffer for 10 min at 4°C. After the supernatant had been carefully aspirated and discarded, the pellets were resuspended in 20 μl of electrophoresis sample buffer. The samples were boiled for 5 min before analysis by 7.5% SDS-PAGE and then electrotransferred to an Immobilon-P transfer membrane (Millipore Intertech, Bedford, MA) according to the manufacturer's protocol. The membrane was blocked for 2 h with 5% nonfat dry milk (Bio-Rad), incubated overnight at 4°C with a 1:500 dilution of anti-CD36 antibody, washed, and then incubated for 1 h with a 1:1,500 dilution of horseradish peroxidase-conjugated anti-mouse IgG. The immune complexes were detected by following the ECL manufacturer's protocol.

Fatty acid transport assay. Membrane permeation of labeled oleate was measured as previously described in detail (3, 4). Briefly, KRH media containing labeled and unlabeled oleate at a final concentration of 100 μM (3,000 cpm/μl), complexed to BSA (50–400 μM), were pipetted into the bottom of polystyrene tubes. The assay was performed as previously described in adipocyte and jejunal cells (3, 46). Briefly, 125 μl of the isolated mucosal cells suspended in KRH (2 × 10⁶ cells/ml) were added to the isotope solution and swirled gently to ensure mixing. At desired times (2–60 s), 5 ml of ice-cold KRH "stop solution" containing 0.5% albumin were pipetted rapidly into the reaction tubes to stop cellular influx and efflux of LCFA. Cells were immediately recovered by quickly pipetting the cell solution onto the center of glass fiber filters (type A/E, 25 mm) at a rate equal to the rate of filtration under 50 mmHg vacuum pressure by use of filtration apparatus (model 7H, Hoefer Scientific, San Francisco, CA). The cells were washed with an additional 5 ml of stop solution. Next, the filters were placed in scintillation fluid (Ecolite, ICN, Costa Mesa, CA). Cell-associated radioactivity was quantified using a Beckman LS-3300 Liquid Scintillation Counter. Nonspecific association of radioactivity adhering to the filters and cells was determined in each experiment by adding the cold stop solution before addition of corresponding aliquots of cells and [³H]oleate working solutions. This value was typically 10–20% of the 12-s time point and was routinely subtracted from the uptake values.
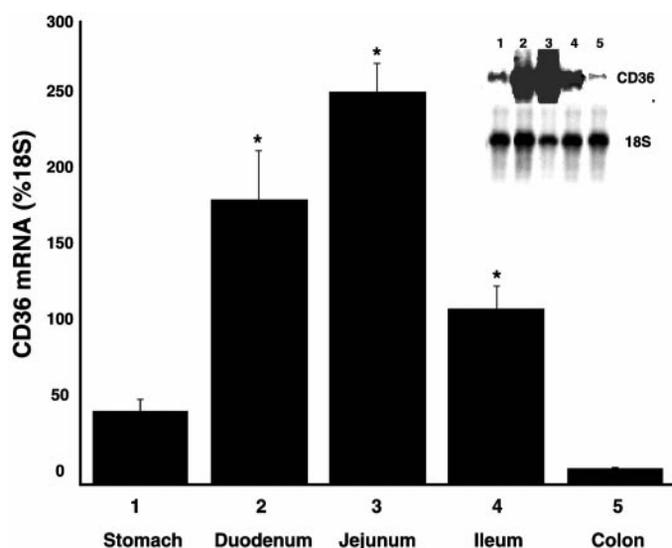
Fig. 1. Distribution of fatty acid translocase (FAT)/CD36 mRNA along the longitudinal intestinal axis of the adult rat. Northern blot analysis of total RNA (20 µg/lane). Membranes were probed with rat FAT cDNA and 18S rRNA as a control. FAT/CD36 mRNA levels were quantified by densitometry and standardized to an 18S rRNA signal. A representative Northern blot is also presented. Values are means ± SE ($n = 4$). Except for stomach vs. colon, all other results are statistically different from one another by ANOVA, $P < 0.05$.

Northern blot analysis. RNA was isolated as described by Chomczynski and Sacchi (19) by use of the Total RNA Isolation Reagent (GIBCO BRL, Grand Island, NY). Total RNA was electrophoresed on 1% agarose gels containing formaldehyde and transferred to nylon membranes by the Rapid Downward Transfer System (Schleicher & Schuell, Keene, NH). The membranes were prehybridized for ≥4 h and then hybridized with randomly primed γ-^{32}P-labeled cDNA for rat FAT (8×10^6 cpm/ml) for 16 h at 42°C in a rotary hybridization incubator (Lab-Line Instruments, Melrose Park, IL). The membranes were washed twice in $2 \times$ standard sodium citrate (SSC) containing 0.05% SDS (30 min each) at 23°C and then washed once at 65°C in $0.1 \times$ SSC containing 0.1% SDS buffer. The membranes were then exposed to Hyperfilm at -80°C for 24–72 h. Messenger RNA for 18S ribosomal RNA was used as a control to ensure that equivalent amounts of RNA were loaded and transferred. Autoradiographs were quantified with an automatic densitometric scanner by use of NIH Imaging software.

Statistical analysis. Results of FAT/CD36 mRNA levels are expressed as a percentage (means ± SE) of ribosomal 18S mRNA levels determined as a control for each sample. ANOVA was used to test for significant differences between groups. Initial rates of oleate uptake were determined from the best-fit curve for time course data by utilization of nonlinear regression analysis (33). Significant differences for kinetic parameters [the Michaelis-Menten constant (K_m) and maximum velocity (V_{max})] were assessed by Student's t-test. P values < 0.05 were considered significant.

RESULTS

Expression of FAT/CD36 mRNA and protein along the gut axis. To investigate the tissue distribution of FAT/CD36 mRNA along the gastrointestinal tract, total RNA was isolated from rat stomach, duodenum, jejunum, ileum, and colon and then analyzed by North-

ern blot analysis. It was found that FAT/CD36 mRNA was differentially expressed along the gut axis, with the highest mRNA levels found in the jejunum and duodenum and lower levels noted in the ileum, consistent with the expected distribution of LCFA absorption (Fig. 1). These findings are consistent with those reported previously by Poirier et al. (38); however, unlike their study, our results indicate that FAT/CD36 mRNA is also expressed in the gastric and colonic mucosa (Fig. 1).

By utilizing an anti-human mouse monoclonal antibody to CD36, studies were done to determine FAT/CD36 protein expression in the rat intestine. The pattern and level of mucosal protein expression along the gut axis also appear to be site specific (Fig. 2).

In samples of total cellular protein from duodenal and jejunal villus-tip enterocytes, there appear to be three distinctly labeled protein bands ranging from ~57 kDa to the expected 88 kDa. In other cell types, it is known that FAT/CD36 is modified by posttranslational glycosylation from 57 kDa to an apparent molecular mass of 88 kDa. Because total cell protein was used in this experiment, it is possible that the lower bands seen in the duodenum and jejunum identify FAT/CD36 proteins at different states of posttranslational modification. In distinction from duodenum and jejunum, only a single, less dense protein band at 88 kDa is identified in mucosal protein samples from ileum and colon. Total cellular protein isolated from rat adipocytes was used as a known positive internal control. Densitometric analysis of the 88-kDa labeled protein bands from this Western blot indicates that, relative to the level of expression of the positive adipocyte control, the expression of FAT/CD36 protein is highest in the jejunum (72%), followed closely by duodenum (64%), with ileum and colon having similar, lower levels of protein expression (50% and 58%, respectively).

FAT/CD36 mRNA expression during intestinal maturation. Additional studies were done to investigate FAT/CD36 mRNA expression during rat intestinal maturation. FAT/CD36 mRNA levels were measured from the total RNA harvested from the entire small intestine of 1-day-, 2-day-, and 5-day-old rats and com-
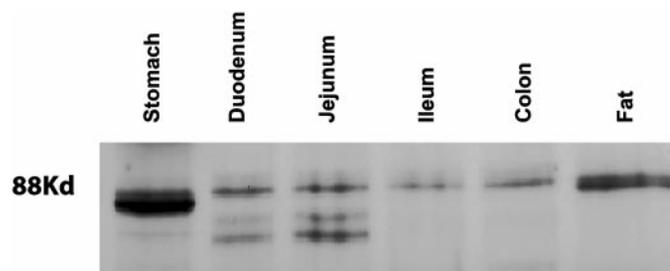

Fig. 2. Western blot analysis of FAT/CD36 along the longitudinal intestinal axis of the adult rat. After total protein isolation from intestinal mucosa and immunoprecipitation, solubilized protein was separated by SDS-PAGE, transferred to Immobilon-P membranes, labeled with anti-human CD36 mouse monoclonal antibody, and detected by conjugated anti-mouse IgG by use of enhanced chemiluminescence and autoradiography. Rat adipose tissue was used as a known positive control.

pared with FAT/CD36 mRNA levels of jejunum from adult rats (Fig. 3). Adipose tissue from adult rats was used as a positive internal control. In the adult rat, as noted for FAT/CD36 protein expression, adipose tissue has a higher level of mRNA expression than the intestine. In comparing FAT/CD36 mRNA levels in the neonatal intestine with those in the adult intestine, there is a significant increase in mRNA levels in the adult ($P < 0.05$). Although there appears to be a trend toward increasing FAT/CD36 mRNA levels over time in the intestine from the neonatal animals, the differences do not reach statistical significance (ANOVA).

Effect of intraduodenal oleate on FAT/CD36 mRNA expression in the small intestine. To test whether luminal LCFA might rapidly regulate intestinal FAT/CD36 expression in vivo, a well-described intraduodenal oleate infusion animal model was used (29, 43). This model has been extensively utilized to investigate the effects of enteral LCFA on pancreatic exocrine secretion, cholecystokinin release (45), and satiety (39). Using this model, we found that intraduodenal oleate infusion for 1 h rapidly and significantly decreased FAT/CD36 mRNA levels in the mucosa of the duodenum and jejunum compared with control rats infused with saline (Fig. 4). The ileum, however, showed no significant change in FAT/CD36 mRNA levels compared with controls. The effect of oleate infusion on FAT/CD36 expression did not appear to be related to enterocyte damage, in that trypan blue exclusion experiments indicated 85–90% cell viability in both the oleate-infused and saline-infused groups (data not shown).

Fig. 4. Effect of intraduodenal oleate infusion on small intestine levels of FAT/CD36 mRNA. Northern blot analysis of total RNA harvested from intestinal mucosa after 1 h of intraduodenal infusion of 10% oleate (or saline). FAT/CD36 mRNA levels were quantified by densitometry and standardized to an 18S rRNA signal. A representative blot is also shown, with the sequence of samples paralleling data in the histogram (D, duodenum; J, jejunum; I, ileum). Values are means $\pm$ SE ($n = 4$). **$P < 0.001$, *$P < 0.05$.

Effect of intraduodenal oleate infusion on LCFA transport in isolated enterocytes. Because of the rapid regulation of FAT/CD36 mRNA levels by enteral oleate infusion, experiments were conducted to determine whether LCFA uptake would be altered in isolated enterocytes after oleate infusion. Jejunal enterocytes were isolated for LCFA transport experiments after a 1-h intraduodenal infusion of 10% oleate. We found that oleate infusion resulted in a dramatic decrease in oleate uptake by isolated enterocytes as a function of time (Fig. 5). Initial rates of [3H]oleate uptake were
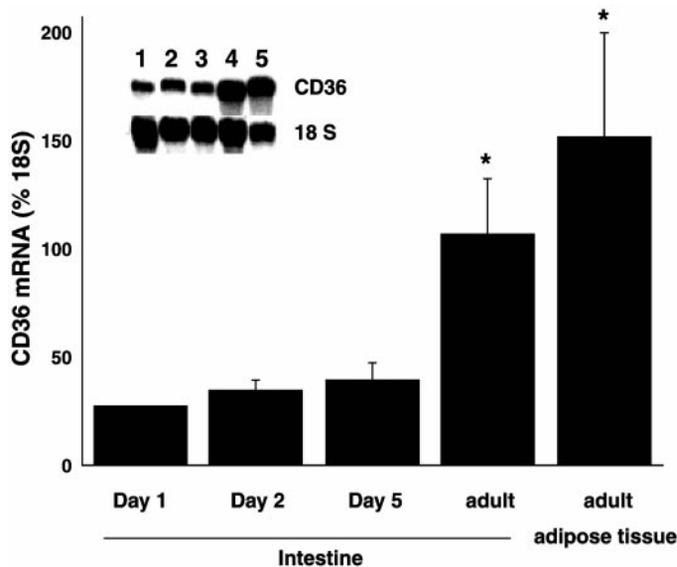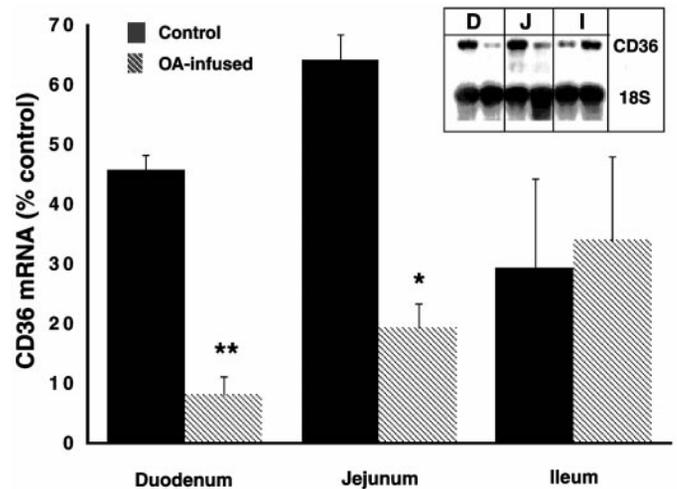

Fig. 3. Determination of FAT/CD36 mRNA levels during intestinal maturation. Northern blot analysis of total RNA samples from the entire small intestine of neonatal rats and from jejunal mucosa and adipose tissue of adult rats. FAT/CD36 mRNA levels were quantified by densitometry and standardized to an 18S rRNA signal. A representative blot is also shown. Values are means $\pm$ SE ($n = 3$). Values for adult rat intestinal mucosa and adipose tissue were statistically higher than the neonatal values. *$P < 0.05$.

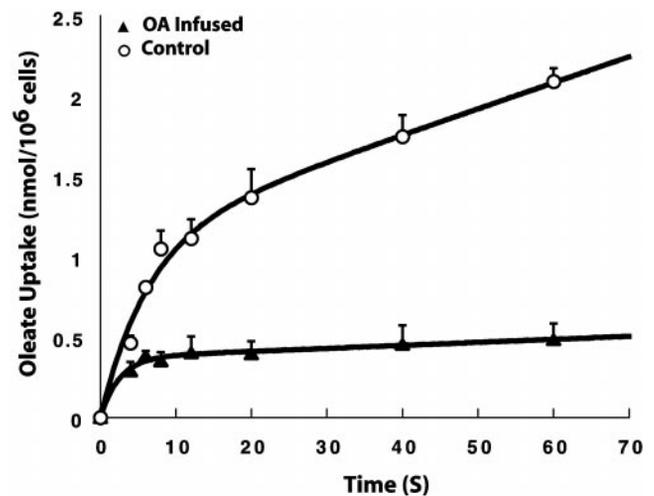Fig. 5. Effect of intraduodenal oleate infusion on the time course of [3H]oleate uptake by isolated enterocytes. After 1 h of intraduodenal oleate (or saline) infusion in anesthetized rats, jejunal enterocytes were isolated, and [3H]oleate uptake was measured as described in MATERIALS AND METHODS. [3H]oleate/albumin (OA, 100 μM) isotope was incubated with 125 μl (1×10^6 cells). Time courses for uptake were curve fitted using nonlinear regression. Values are means $\pm$ SE of 3 replicate experiments.

determined by the best curve fit method and nonlinear regression analysis, as described in MATERIALS AND METHODS. Oleate transport kinetics was determined by plotting initial uptake rates as a function of unbound oleate concentration (Fig. 6). Using a least squares fit of the data to the Michaelis-Menten equation, LCFA permeation of isolated enterocytes from both oleate-infused and control rats predominantly demonstrates nonlinear saturation kinetics as a function of LCFA concentration, consistent with protein-mediated transport. However, at the highest unbound LCFA concentrations tested, there appears to be a nonsaturable linear trend that may reflect a component of simple diffusion. This finding is consistent with LCFA transport data in other cell types, where it appears that facilitated LCFA transport occurs at low physiological substrate concentrations, with simple diffusion becoming increasingly significant at higher concentrations (2).

In vivo intraduodenal oleate infusion resulted in a significant inhibition of enterocyte [^{3}H]oleate uptake rates as a function of LCFA concentration, with a fivefold decrease in V_{max} (6.9 vs. 1.4 nmol·10^6 cells^{-1}·min^{-1}, $P < 0.05$). The drop in V_{max} suggests that the concentration of transport proteins in the plasma membrane is decreased in the oleate-infused group. The transport K_m, reflecting affinity of ligand for its receptor, is not statistically different in the two groups (25 vs. 45 nM). We believe that the inhibition of oleate uptake in isolated enterocytes harvested from this intraduodenal oleate-infused, anesthetized rat model is due directly to oleate. The oleate uptake kinetic parameters determined in these experiments are very similar to those reported in enterocytes har-

vested from otherwise normal rats (V_{max} = 2.1 nmol·min^{-1}·10^6 cells^{-1}, and K_m = 93 nM) (47).

DISCUSSION

The essential step in intestinal absorption of LCFA is the permeation of the jejunal microvillus plasma membrane. Previous work has suggested that LCFA uptake by isolated rat enterocytes (47), as well as by cultured Caco-2 (48) and IEC 6 enterocytes (M. Chen and C. M. Harmon, unpublished observations), is saturable, suggesting that a protein facilitates plasma membrane permeation. Three candidate LCFA transport proteins have been shown to be expressed in rat small intestine: FABPpm, FATP, and FAT/CD36. FABPpm has been reported to play a significant role in LCFA uptake in the rat intestine. However, the finding that the FABPpm appears to be identical to the mitochondrial glutamicoxaloacetic transaminase protein (13) and, more recently, conflicting reports regarding this protein's ability to facilitate LCFA transport in a Xenopus oocyte model (24, 50) have cast some doubt about its physiological role in LCFA uptake. Poirier et al. (38) have reported that FAT/CD36 mRNA is expressed predominantly in the rat proximal small intestine and that mRNA expression is increased by a LCFA, high-fat diet. In the present study, we have demonstrated that FAT/CD36 mRNA levels and the pattern and level of FAT/CD36 protein are differentially expressed along the longitudinal axis of the rat intestine. In addition, FAT/CD36 mRNA levels are developmentally regulated during rat intestinal maturation, with low levels in the newborn period and increased levels in adults. Furthermore, we have shown that enteral oleate rapidly regulates both FAT/CD36 mRNA levels and enterocyte uptake of radiolabeled LCFA.

CD36 is a member of a gene superfamily that includes scavenger receptor B1 (SR-B1), a protein that binds anionic phospholipids (1, 40) and transports high-density lipoprotein cholesteryl esters across the plasma membrane (5), and croquemort, a Drosophila macrophage receptor for apoptotic cells (25). Although CD36 has been assigned many possible functions, evidence that it plays a role in the cellular uptake of LCFA is strong. In an effort to identify potential LCFA transport proteins in rat adipocytes, covalent labeling with monofunctional esters of radiolabeled LCFA identified FAT. These reactive compounds irreversibly inhibited subsequent LCFA uptake by 80% (34). The protein was isolated, and a full-coding clone from an adipocyte cell cDNA library was obtained on the basis of the amino-terminal sequence of the protein (1, 33). The clone coded for a protein with 85% homology to human platelet CD36 (1). In general, CD36 expression distribution favors tissues with high metabolic capacity for LCFA, such as intestine, adipose, heart, and muscle, whereas it is absent from tissues like brain that do not utilize LCFA (1). CD36 mRNA is induced during preadipocyte differentiation (10), and when expressed in fibroblasts lacking the protein, saturable
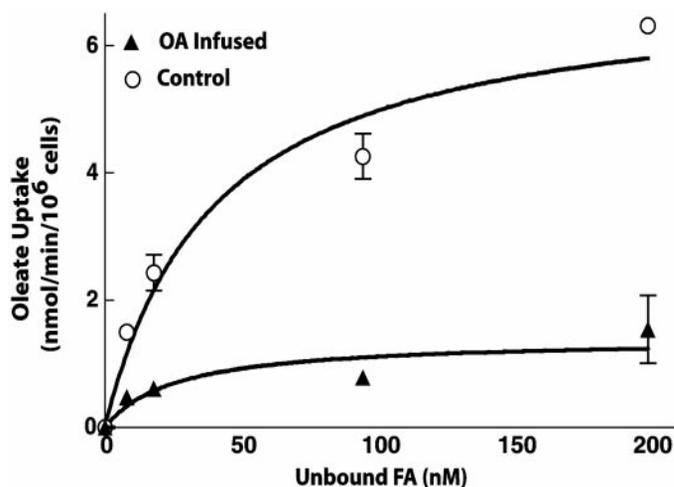

Fig. 6. Effect of intraduodenal oleate infusion on initial [^{3}H]oleate uptake rates as a function of unbound oleate. After 1 h of intraduodenal oleate (or saline) infusion in anesthetized rats, jejunal enterocytes were isolated, and [^{3}H]oleate uptake was measured, as described in MATERIALS AND METHODS. Isolated enterocytes of 125 μl (1 × 10^6 cells) in suspension were incubated with a fixed concentration of 100 μM [^{3}H]oleate bound to various concentrations of albumin (50–400 μM). The unbound oleate concentration, initial uptake rates, and kinetic parameters were calculated as described in MATERIALS AND METHODS. Values are means ± SE of 4 replicate experiments.

LCFA transport is induced (35). Regulation of FAT/CD36 expression in vivo is also consistent with a role in LCFA transport with increased expression in the muscle of diabetic animals (30), and a recent report demonstrates that CD36 deficiency underlies insulin resistance, defective fatty acid metabolism, and hypertriglyceridemia in a hypertensive rat model of insulin resistance (8).

The results of the present study add to the body of evidence that supports a role for FAT/CD36 in LCFA transport and/or metabolism. The finding that FAT/CD36 is differentially expressed, both during intestinal development and along the intestinal axis, argues for function specificity. In addition to high FAT/CD36 mRNA and protein expression in the jejunum, where most LCFA absorption is believed to occur, we confirmed the finding by Poirier et al. (38) that high expression is also found in the duodenum. FAT mRNA was constitutively expressed in the epithelial cells located in the upper two-thirds of villi, whereas it was undetectable in the crypt cells and submucosal cells. Immunochemical studies showed that FAT protein was limited to the brush border of enterocytes, and no fluorescence was found in the goblet cells (38). Our study also indicates that protein expression is moderately high in the duodenum compared with ileum and colon. These findings might suggest that significant LCFA absorption occurs in the duodenum as well as the jejunum, or they may suggest that FAT/CD36 serves as an LCFA receptor or transporter that is perhaps involved in other events, such as lipid-induced gut-hormonal signaling as in stimulation of pancreatic secretion or cholecystokinin release.

Our study also identifies FAT/CD36 expression in the stomach and colon, unlike the previous report by Poirier et al. (38). This discrepancy may relate to differences in the male Wistar rat model that was used in their study compared with the Spague-Dawley rat model in our experiments. However, our study also suggests that FAT/CD36 protein is significantly expressed in the colon and highly expressed in the stomach. The functional significance of these findings is unknown. The mouse monoclonal antibody utilized for Western blots in this study was raised to a peptide fragment of human CD36 (Transduction Laboratories), and therefore the specificity of binding could be questioned. Attempts to raise specific antibodies to rat FAT have been problematic. A variety of human anti-CD36 antibodies have been tested and appear to have poor cross-reactivity with proteins from rat tissues. This is not surprising, given that the peptide sequence to which most anti-CD36 antibodies bind is more heterogeneous between the rat and the human than the remainder of the protein (22). Additional experiments are required to verify our finding suggesting high FAT/CD36 expression in the gastric mucosa. If this finding is confirmed, then, as suggested above, FAT/CD36 function in the stomach, and possibly duodenum, may relate to LCFA transport or binding involved in signaling mechanisms and not necessarily lipid absorption or metabolism.

Our study shows a rapid and dramatic decrease in duodenal and jejunal FAT/CD36 mRNA levels after intraduodenal oleate infusion. This is in contrast to the finding that rats fed a high LCFA diet for 3 days upregulate small intestinal levels of FAT/CD36 mRNA (38). An explanation for the latter finding could be that, in response to a high-fat diet, the enterocyte is able to upregulate expression of genes and proteins that would be involved in uptake and metabolism of the dietary substrate. Consistent with this are findings that FAT/CD36 mRNA is induced by LCFA in preadipocytes (1, 42) and neonatal cardiomyocytes (27). The explanation for small bowel downregulation of FAT/CD36 mRNA noted in the oleate infusion model is uncertain. One possible mechanism is that the large load of LCFA delivered to the mucosa results in the rapid downregulation of mechanisms specifically involved in the cellular uptake of LCFA.

Additional experiments are required to evaluate whether oleate infusion alters FAT/CD36 protein expression. However, the rapid inhibition in LCFA uptake by isolated enterocytes after oleate infusion suggests that FAT/CD36 protein levels in the plasma membrane may be decreased or functionally altered. The finding that the LCFA uptake kinetic parameter, V_{max}, is decreased fivefold in cells from the oleate-infused rats compared with controls supports the suggestion that the concentration or number of plasma membrane LCFA transport proteins may be decreased by enteral oleate infusion. The one possible mechanism might be that LCFA binds to FAT/CD36, resulting in ligand/receptor internalization, thereby acutely lowering the plasma membrane availability of FAT/CD36 for subsequently delivered LCFA. In addition, decreased FAT/CD36 mRNA levels may lead to decreased FAT/CD36 protein levels.

In conclusion, differential expression of FAT/CD36 along the longitudinal axis of the intestine and during intestinal development suggests that it has specificity of function. That in vivo intraduodenal oleate infusion rapidly downregulates FAT/CD36 mRNA levels and LCFA uptake in enterocytes adds support to the hypothesis that FAT/CD36 plays a role in intestinal absorption of LCFA. Therapeutic strategies designed to alter or manipulate intestinal FAT/CD36 expression or function may have important implications in treating a variety of pathological conditions, such as morbid obesity, short bowel syndrome, and other lipid malabsorption conditions.

REFERENCES

1. **Abumrad NA, el-Maghrabi MR, Amri EZ, Lopez E, and Grimaldi PA.** Cloning of rat adipocyte membrane protein implicated in binding or transport of long-chain fatty acids that is induced during preadipocyte differentiation. Homology with human CD36. *J Biol Chem* 268: 17665–17668, 1993.
2. **Abumrad NA, Harmon CM, and Ibrahimi A.** Membrane transport of long-chain fatty acids: evidence for a facilitated process. *J Lipid Res* 39: 2309–2318, 1998.
3. **Abumrad NA, Park JH, and Park CR.** Permeation of long-chain fatty acid into adipocytes. Kinetics, specificity, and evidence for involvement of a membrane protein. *J Biol Chem* 259: 8945–8953, 1984.

4. **Abumrad NA, Perkins RC, Park JH, and Park CR.** Mechanism of long chain fatty acid permeation in the isolated adipocyte. *J Biol Chem* 256: 9183–9191, 1981.

5. **Acton S, Rigotti A, Landschulz KT, Xu S, Hobbs HH, and Krieger M.** Identification of scavenger receptor SR-BI as a high density lipoprotein receptor. *Science* 271: 518–520, 1996.

6. **Ailhaud G.** Regulation of gene expression by fatty acids in the adipose cell. *Prostaglandins Leukotrienes Essent Fatty Acids* 48: 89–90, 1993.

7. **Ailhaud G, Amri EZ, and Grimaldi PA.** Fatty acids and adipose cell differentiation. *Prostaglandins Leukotrienes Essent Fatty Acids* 52: 113–115, 1995.

8. **Aitman TJ, Glazier AM, Wallace CA, Cooper LD, Norsworthy PJ, Wahid FN, Al-Majoli KM, Trembling PM, Mann CJ, Shoulders CC, Graf D, St. Lezin E, Kurtz TW, Kren V, Provenec M, Ibrahimi A, Abumrad NA, Stanton LW, and Scott J.** Identification of CD36 (fat) as an insulin-resistance gene causing defective fatty acid and glucose metabolism in hypertensive rats. *Nature Genet* 21: 76–83, 1999.

9. **Amri EZ, Ailhaud G, and Grimaldi PA.** Regulation of adipose cell differentiation. I. Fatty acids are inducers of the aP2 gene expression. *J Lipid Res* 32: 1457–1463, 1991.

10. **Amri EZ, Bonino F, Ailhaud G, Abumrad NA, and Grimaldi PA.** Cloning of a protein that mediates transcriptional effects of fatty acids in preadipocytes. Homology to peroxisome proliferator-activated receptors. *J Biol Chem* 270: 2367–2371, 1995.

11. **Anderson MP and Welsh MJ.** Fatty acids inhibit apical membrane chloride channels in airway epithelia. *Proc Natl Acad Sci USA* 87: 7334–7338, 1990.

12. **Bergmeyer HU, Bernt E, and Hess B.** Lactic dehydrogenase. In: *Methods of Enzymatic Analysis,* edited by HU Bergmeyer. New York: Academic, 1963, p. 736–741.

13. **Berk PD, Wada H, Horio Y, and Potter B.** Plasma membrane fatty acid-binding protein and mitochondrial glutamicoxaloacetic transaminase of rat liver are related. *Proc Natl Acad Sci USA* 87: 3481–3488, 1990.

14. **Bradford MM.** A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biol Chem* 72: 248–254, 1976.

15. **Calder PC.** n-3 Polyunsaturated fatty acids and cytokine production in health and disease. *Ann Nutr Metab* 41: 203–234, 1997.

16. **Calder PC.** Dietary fatty acids and the immune system. *Nutr Rev* 56: S70–S83, 1998.

17. **Calder PC, Bevan SJ, and Newsholme EA.** The inhibition of T-lymphocyte proliferation by fatty acids is via an eicosanoid-independent mechanism. *Immunology* 75: 108–115, 1992.

18. **Calder PC, Bond JA, Harvey DJ, Gordon S, and Newsholme EA.** Uptake and incorporation of saturated and unsaturated fatty acids into macrophage lipids and their effect upon macrophage adhesion and phagocytosis. *Biochem J* 269: 807–814, 1990.

19. **Chomczynski P and Sacchi N.** Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biol Chem* 162: 156–159, 1987.

20. **Corey SJ and Rosoff PM.** Unsaturated fatty acids and lipoxygenase products regulate phagocytic NADPH oxidase activity by a non-detergent mechanism. *J Lab Clin Med* 118: 343–351, 1991.

21. **Courtneidge SA and Smith AE.** Polyma virus transforming protein associates with the product of the c-src cellular gene. *Nature* 303: 435–439, 1983.

22. **Daviet L, Buckland R, Puente Navazo MD, and McGregor JL.** Identification of an immunodominant functional domain on human cD36 antigen using human-mouse chimaeric protein and homologue-replacement mutagenesis. *Biochem J* 305: 221–224, 1995.

23. **Distel RJ, Robinson GS, and Spiegelman BM.** Fatty acid regulation of gene expression. Transcriptional and post-transcriptional mechanisms. *J Biol Chem* 267: 5937–5941, 1992.

24. **Fitscher BA, Klaaben-Schluter CM, and Stremmel W.** Evidence for a hepatocyte membrane fatty acid transport protein using rat liver mRNA expression in Xenopus laevis oocytes. *Biochim Biophys Acta* 1256: 47–51, 1995.

25. **Franc NC, Dimarcq JL, Lagueux M, Hoffman J, and Ezekowitz RA.** Croquemort, a novel Drosophila hemocyte/macrophage receptor that recognizes apoptotic cells. *Immunity* 4: 431–443, 1996.

26. **Galli C and Marangoni F.** Recent advances in the biology of n-6 fatty acid. *Nutrition* 13: 978–985, 1997.

27. **Glatz JF, van Nieuwenhoven FA, Luiken JJ, Schaap FG, and van der Vusse GJ.** Role of membrane-associated and cytoplasmic fatty acid-binding proteins in cellular fatty acid metabolism. *Prostaglandins Leukotrienes Essent Fatty Acids* 57: 17–21, 1997.

28. **Gore J and Honiara C.** Linolenic acid transport in hamster intestinal cells is carrier-mediated. *J Nutr* 123: 66–73, 1993.

29. **Green GM, Taguchi S, Friestman J, Chey WY, and Liddle RA.** Plasma secretin, CCK, and pancreatic secretion in response to dietary fat in the rat. *Am J Physiol Gastrointest Liver Physiol* 256: G1016–G1021, 1989.

30. **Greenwalt DE, Scheck SH, and Rhinehart-Jones T.** Heart CD36 expression is increased in murine models of diabetes and in mice fed a high fat diet. *J Clin Invest* 96: 1382–1388, 1995.

31. **Grimaldi PA, Knobel SM, Whitesell RR, and Abumrad NA.** Induction of aP2 gene expression by nonmetabolized long-chain fatty acids. *Proc Natl Acad Sci USA* 89: 10930–10934, 1992.

32. **Hamilton JA.** Fatty acid transport: difficult or easy? *J Lipid Res* 39: 467–481, 1998.

33. **Harmon CM and Abumrad NA.** Binding sulfosuccinimidyl fatty acids to adipocyte membrane proteins: isolation and amino-terminal sequence of an 88-kD protein implicated in transport of long-chain fatty acids. *J Membr Biol* 133: 43–49, 1993.

34. **Harmon CM, Luce P, Beth A, and Abumrad NA.** Labeling of adipocyte membranes by sulfo-N-succinimdyl derivatives of long chain fatty acids: inhibition of fatty acid transport. *J Membr Biol* 124: 261–268, 1991.

35. **Ibrahimi A, Sfeir Z, Magharaie H, Amri EZ, Grimaldi PA, and Abumrad NA.** Expression of the CD36 homolog (FAT) in fibroblast cells: effects on fatty acid transport. *Proc Natl Acad Sci USA* 93: 2646–2651, 1996.

36. **Imagawa W, Bandyopadhyay GK, Wallace D, and Nandi S.** Phospholipids containing polyunsaturated fatty acyl groups are mitogenic for normal mouse epithelial cells in serum-free cell culture. *Proc Natl Acad Sci USA* 86: 4122–4126, 1989.

37. **Ordway RN, Singer JJ, and Walsh JV.** Direct regulation of ion channels by fatty acids. *Trends Neurosci* 44: 96–100, 1991.

38. **Poirier H, Degrace P, Niot I, Bernard A, and Besnard P.** Localization and regulation of the putative membrane fatty-acid transporter (FAT) in the small intestine. Comparison with fatty acid-binding proteins (FABP). *Eur J Biochem* 238: 368–373, 1996.

39. **Ramirez I, Tordoff MG, and Friedman MI.** Satiety from fat? Adverse effects of intestinal infusion of sodium oleate. *Am J Physiol Regulatory Integrative Comp Physiol* 273: R1779–R1785, 1997.

40. **Rigotti A, Acton SL, and Krieger M.** The Class B scavenger receptors SR-BI and CD36 are receptors for anionic phospholipids. *J Biol Chem* 270: 16221–16224, 1995.

41. **Schaffer JE and Lodish HF.** Expression cloning and characterization of a novel adipocyte long chain fatty acid transport protein. *Cell* 79: 427–436, 1994.

42. **Sfeir A, Ibrahimi A, Amri EZ, Grimaldi PA, and Abumrad NA.** Regulation of FAT/CD36 gene expression: further evidence in support of a role of the protein in fatty acid binding/transport. *Prostaglandins Leukotrienes Essent Fatty Acids* 57: 17–21, 1997.

43. **Shiratori K, Watanabe S, and Takeuchi T.** Intestinal fat digestion plays a significant role in fat-induced suppression of gastric acid secretion and gastrin release in the rat. *Dig Dis Sci* 38: 2267–2272, 1993.

44. **Shoo SL, Stump D, Serentin D, Potter BJ, and Berk PD.** Adipocyte differentiation of 3T3–L1 cells involves augmented

expression of a 43-kDa plasma membrane fatty acid-binding protein. *J Biol Chem* 267: 14456–14461, 1992.

45. **Spannagel AW, Nakano I, Tawil T, Chey WY, Liddle RA, and Green GM.** Adaptation to fat markedly increases pancreatic secretory response to intraduodenal fat in rats. *Am J Physiol Gastrointest Liver Physiol* 270: G128–G135, 1996.

46. **Stremmel W.** Uptake of fatty acids by jejunal mucosal cells is mediated by a fatty acid binding membrane protein. *J Clin Invest* 82: 2001–2010, 1988.

47. **Stremmel W, Strohmeyer G, Borchard F, Kochwa S, and Berk PD.** Isolation and partial characterization of a fatty acid binding protein in rat liver plasma membranes. *Proc Natl Acad Sci USA* 82: 4–8, 1985.

48. **Trotter PJ, Ho SY, and Storch J.** Fatty acid uptake by Caco-2 human intestinal cells. *J Lipid Res* 37: 336–346, 1996.

49. **Yamashita A, Sugiura T, and Waku K.** Acyltransferases and transacylases involved in fatty acid remodeling of phospholipids and metabolism of bioactive lipids in mammalian cells. *J Biochem* 122: 1–16, 1997.

50. **Zhou SL, Stump D, Isola L, and Berk PD.** Constitutive expression of a saturable transport system for nonesterified fatty acids in Xenopus Laevis oocytes. *Biochem J* 297: 315–319, 1994.