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MAIN TOPIC

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Effect of low fat diet on lipid absorption and fatty-acid transport following bowel resection

Abstract Low-fat diets (LFD) are used extensively in many different clinical conditions. However, the effect of this diet on lipid absorption and cellular long-chain fatty-acid (LCFA) transport is unknown. Fatty-acid translocase (FAT), the rat homologue of human CD36, is one of several LCFA plasma-membrane transport proteins that may play an important role in intestinal lipid uptake. The purpose of this study was to investigate the effects of a LFD on intestinal expression of FAT/CD36, enterocyte fatty-acid transport, and in-vivo lipid absorption in rats following bowel resection. Adult male Sprague-Dawley rats were divided into five experimental groups: normal rats fed normal chow(NR-NC) (10 kcal% fat), normal rats fed a LFD (NR-LFD) (3 kcal% fat), sham rats fed normal chow (Sham-NC), short-bowel syndrome rats fed normal chow (SBS-NC), and SBS rats fed a LFD (SBS-LFD). SBS rats underwent 75% small-bowel resection, while sham animals underwent bowel transection and re-anastomosis. Food intake, fecal mass, and fecal fat were measured over the last 3 days before death on day 14. Final body weight, plasma lipids and protein, and tissue total lipids in liver, adipose tissue, and intestine were determined at death. Total RNA from the mucosa of the duodenum, jejunum, and ileum was extracted for Northern blot analysis to determine fatty-acid translocase (FAT)/CD36 mRNA levels. An established cellular LCFA transport assay was used to determine isolated enterocyte [^{3}H]-oleate uptake. Students's *t*-test was used to determine statistical significance ($P < 0.05$). NR-LFD rats demonstrated a small increase in overall food absorption and no change in fat absorption compared to NR-NC animals. A significant decrease in FAT/CD36 mRNA levels was seen in the duodenum and jejunum in NF-LFD rats (vs NR-NC) and was accompanied by reduced LCFA transport by isolated enterocytes from the jejunum and ileum. SBS-LFD rats demonstrated decreased FAT/CD36 mRNA levels in all three segments and a concomitant decrease in LCFA uptake enterocytes compared to the SBS-NC group. In addition, SBS-LFD rats showed significantly lower final body weight and plasma lipids compared to SBS-NC animals.

Keywords Fatty-acid translocase (FAT) · Lipid absorption · Short-bowel syndrome

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Introduction

Low-fat diets (LFD) have been considered to have a benefit in the overall nutrition of patients with different clinical conditions accompanied by impaired lipid absorption [1]. In short-bowel syndrome (SBS), the combination of the loss of absorptive surface area with a compromised enterohepatic circulation results in inefficient absorption of fat and steatorrhea [2]. Therefore, patients with SBS have historically been thought to benefit from a LFD early in the course of therapy [1, 2]. High-fat diets in these patients have been shown to increase both steatorrhea and gastrointestinal fluid losses [3], however, not all studies have supported this popular concept [4].

Optimal diet composition may play a major role in patients with impaired digestion and might contribute to optimal conditions for nutrient absorption [5]. Although it is generally believed that a LFD results in less diarrhea, less steatorrhea, and better absorption of nutrients [3–5], it is unknown whether the low lipid composition of the diet influences molecular and cellular mechanisms of long-chain fatty-acid (LCFA) intestinal absorption. The present study was undertaken to evaluate the effect of a LFD on intestinal expression of the LCFA plasma-membrane transport protein fatty-acid translocase (FAT)/CD36, fatty-acid transport in isolated enterocytes, and in-vivo lipid absorbability in rats with an intact intestinal tract and following bowel resection.

Materials and methods

Male Sprague-Dawley rats weighing 250–320 g were housed in individual cages and maintained in a controlled environment (22 °C with 12-h light/dark cycle). In experiment 1, the rats were randomly assigned to one of two groups: normal rats fed normal chow (NR-NC) or normal rats fed a LFD (NR-LFD). In experiment 2, the animals were divided into three groups: (1) sham-operated control rats fed normal chow (Sham-NC); (2) rats with SBS fed normal chow (SBS-NC); and (3) rats with SBS fed a LFD (SBS-LFD).

After an overnight fast, animals were anesthetized with sodium pentobarbital 45 mg/kg a intraperitoneally. Under sterile conditions, the abdomen was opened by a midline incision. For SBS, a 75% resection was performed, preserving 5 cm proximal jejunum and 10 cm distal ileum. For sham animals, the intestine was divided and reanastomosed without resection. For all operations, the abdominal cavity was closed in two layers with a running 3/0 suture. Antimicrobial cream with pramoxine HC1 (Mycitracin Plus, Johnson & Johnson, Skillman, NJ) was applied to the incision for topical pain relief. Following laparotomy, all animals had access to water for the first 24 h.

In both experiments, the rats were given normal (10% kcal fat) or low-fat (3% kcal fat) diets (Research Diets, New Brunswick, NJ) and water ad lib for 14 days until they were killed. Food intake and clearance (dry fecal mass) were measured over the last 3 days and expressed as g/day. Fat intake was determined from the amount of food consumed. Fecal lipid was extracted using petroleum ether and alcohol, dried, and residue was weighed [6]. Food and fat absorbability was calculated as intake minus clearance and expressed as percent of intake as previously described [7]. The rats were killed on day 14. Final body weight, plasma cholesterol and triglycerides (Sigma, Kits #352 and #339), and total tissue lipid in liver, ileum, and white adipose tissue of the epididymis (method of Bligh and Dyer [8]) were determined at death. The remnant small intestine was removed and divided into three segments: duodenum, jejunum to anastomosis, and terminal ileum. The intestinal mucosa was harvested, weighed, and frozen in liquid nitrogen.

RNA was isolated from the mucosa using the TRIzol reagent (GIBCO BRL) as described by Chomcynski and Sacchi [9]. Total RNA was separated on 1% agarose gel, transferred to a nylon membrane using the Rapid Downward Transfer System (Schleicher & Schuell), and cross-linked to the membrane by exposure to ultra violet (UV) light for 20 min. After overnight pre-hybridization, the membrane was hybridized with ^{32}P-labelled FAT cDNA probe (Rediprime labeling kit, Amersham Life Science) and exposed to X-ray film (Kodak). For controls the membrane was stripped and re-probed with ^{32}P-labelled 18s cDNA probe. Autoradiographs were analyzed by densitometry using a Hewlett-Packard ScanJet 4c/T in conjunction with NIH Image software (version 1.60). Statistical analysis was by ANOVA with a P value below 0.05 considered significant.

The rats were killed by cervical dislocation and isolated intestinal cells were prepared according to a modification of the method employed by Gore and Hoinard [10]. Briefly, intestinal segments were removed, rinsed thoroughly with a solution of 0.154 M NaCl and 1 mM dithiothreitol, and then filled with Solution A (1.5 mM KCl, 96 mM NaCl, 27 mM sodium citrate, 8 mM KH_2PO_4, pH 7.3) and incubated for 15 min at 37 °C. The Solution A was discarded and the intestine filled with Solution B (phosphate-buffered saline without Ca^{2+} or Mg^{2+} with 1.5 mM dithiothreitol), incubated for 3 min at 37 °C, and then gently digitally palpated for 2 min. The buffer solution containing mucosal cells was then filtered through nylon gauze (70 μm pore size). After centrifugation (765 × G), the isolated cells were washed twice and re-suspended in KRH buffer (final concentration 2×10^6 cells/ml). Light microscopy demonstrated that roughly 90% of the cells displayed typical features of villus-tip cells.

Table 1 Average final body weight, food and fat intake, clearance, and absorbability in normal and operated rats fed normal chow or low-fat diet for 2 weeks. Values are mean ± SEM (*NR* normal rats, *SBS* short-bowel syndrome, *NC* normal chow, *LFD* low-fat diet)

	Experiment 1		Experiment 2		
	NR-NC	NR-LFD	Sham-NC	SBS-NC	SBS-LFD
Final body weight (% of preoperative)	146 ± 3	142 ± 2	131 ± 4	118 ± 3[a]	100 ± 4[a,b]
Food intake (g/day)	26.5 ± 1.2	24.6 ± 0.8	25.3 ± 1.7	21.7 ± 1.2[a]	21.3 ± 1.1[a]
Food clearance (g/day)	6.2 ± 0.5	2.6 ± 0.2[c]	6.2 ± 0.5	6.0 ± 0.4	2.0 ± 0.2[a,b]
Food absorbability (%)	76.8 ± 1.0	89.4 ± 0.6[c]	75.6 ± 0.6	72.4 ± 1.5[a]	90.6 ± 0.7[a,b]
Fat intake (g/day)	1.1 ± 0.05	0.3 ± 0.01[c]	1.1 ± 0.07	1.0 ± 0.09	0.3 ± 0.01[a,b]
Fat clearance (g/day)	0.1 ± 0.02	0.02 ± 0.005[c]	0.1 ± 0.01	0.2 ± 0.02[a]	0.05 ± 0.01[a,b]
Fat absorbability (%)	87.4 ± 0.8	85.3 ± 0.9	87.9 ± 1	79.4 ± 2.5[a]	81.7 ± 2.4[a]

[a] $P < 0.05$ SBS versus Sham-NC
[b] $P < 0.05$ SBS-LFD vs SBS-NC
[c] NR-NC vs NR-LFD rats

Table 2 Plasma cholesterol, triglyceride, albumin, and tissue total lipid composition of liver, intestine, and epididymal adipose tissue in rats with intact intestinal tract and following bowel resection fed normal chow (*NC*) or low-fat diet (*LFD*). Values are mean ± SEM (*NR* normal rats, *SBS* short-bowel syndrome)

	Experiment 1		Experiment 2		
	NR-NC	NR-LFD	Sham-NC	SBS-NC	SBS-LFD
Plasma (mg/dl)					
Cholesterol	62.2 ± 6.0	77.4 ± 7.9	60.5 ± 3.3	62.3 ± 6.6	41.6 ± 7.2[a,b]
Triglyceride	75.7 ± 8.1	76.1 ± 4.8	82.9 ± 8.2	74.0 ± 4.2	55.3 ± 5.5[a,b]
Albumin	62.4 ± 4.9	64.7 ± 7.9	75.5 ± 2.8	44.0 ± 3.5[a]	61.4 ± 8.4[a]
Tissue Lipid (mg/g)					
Liver	43.6 ± 6.4	46.5 ± 2.0	37.0 ± 2.1	38.3 ± 1.9	42.2 ± 2.0
Intestine	36.2 ± 6.9	44.8 ± 3.6	26.9 ± 2.6	25.2 ± 3.1	31.4 ± 1.5
Adipose tissue	18.8 ± 4.9	20.9 ± 5.0	29.1 ± 3.0	17.9 ± 3.3[a]	17.2 ± 3.2[a]

[a] $P < 0.05$ SBS versus Sham-NC
[b] $P < 0.05$ SBS-LFD vs SBS-NC

Uptake of [^{3}H] oleic acid (Sigma) was measured by a rapid vacuum filtration assay [11]: 125 µl cellular stock suspension was mixed with 125 µl [^{3}H] oleic acid/taurocholate solution (final free oleic acid concentrations ranging from 0.1 to 1.0 µM) in polypropylene tubes. At various time points (4, 8, 15, 30, 60, 90, and 120 s), 5 ml ice-cold KRH stop solution containing 0.5% albumin was pipetted into the tubes to stop cellular influx and efflux and the samples were pipetted onto the center of a glass-fiber filter (type A/E 25 mm) at a rate equal to the rate of filtration under 50 mmHg vacuum pressure using a filtration apparatus (model 7H Hoefer Scientific, San Francisco). The cells and filter were washed twice with 5 ml ice-cold 0.5% albumin KRH stop solution. The filters were then placed in Ecolite scintillation fluid (ICN, Costa Mesa, CA) and cell-associated radioactivity was quantified using a Beckmann LS-3300 liquid scintillation counter. Nonspecific association of radioactivity to filter and cells was determined in each experiment by adding the cold stop solution before the addition of the corresponding aliquots of cells and [^{3}H] oleate working solutions. This value was typically 10%–20% of the 12-s time point and was routinely subtracted from the uptake values.

Statistical significance for mean value ± SEM of food and fat intake and clearance, FAT/CD36 mRNA levels, and kinetic parameters (K_m and V_{max}) was determined using ANOVA. *P* values less than 0.05 were considered significant.

Results

Effect of LFD on lipid parameters in normal rats: experiment 1

Final body weight, expressed as percent of preoperative weight, was not significantly different among the experimental groups (Table 1). Likewise, food intake was not affected by dietary treatment. However, food clearance (dry fecal mass) was significantly decreased in NR-LFD rats compared to NR-NC animals. Consequently, calculated total food absorbability was higher in rats fed LFD compared to animals fed NC, suggesting that the LFD was more readily absorbed than normal chow.

As expected, NR-LFD rats demonstrated significantly decreased fat intake and clearance compared to their NR-NC counterparts. Therefore, calculated fat absorbability did not change significantly. Plasma lipids, albumin, and tissue total lipid were also unaffected by LFD compared to normal chow in normal rats (Table 2).

Significant alterations in [^{3}H] oleate uptake by isolated enterocytes were observed in all three intestinal segments in NR-LFD rats compared to NR-NC animals (Fig. 1). However, alterations in the kinetics of oleate uptake were different depending on the anatomic location along the small-bowel axis. In duodenal enterocytes from LFD rats, the K_m (oleate concentration at which the velocity of uptake is one-half of the maximum, i.e., V_{max}) of oleate uptake decreased (0.03 ± 0.01 vs 0.16 ± 0.07 µM), suggesting increased affinity for the oleate transport system. There was no effect on V_{max} in duodenal enterocytes. Jejunal enterocytes demonstrated a decreased K_m (0.03 ± 0.01 vs 0.2 ± 0.03) and decreased (V_{max} (130 ± 12 vs 243 ± 10 nmol/10^6 cells/min) in LFD rats compared to normal-chow controls. Finally, ileal enterocytes demonstrated no change in K_m, but a decrease in V_{max} (120 ± 10 vs 250 ± 35 nmol/10^6 cells/min) in rats fed a LFD compared to normal-chow controls.

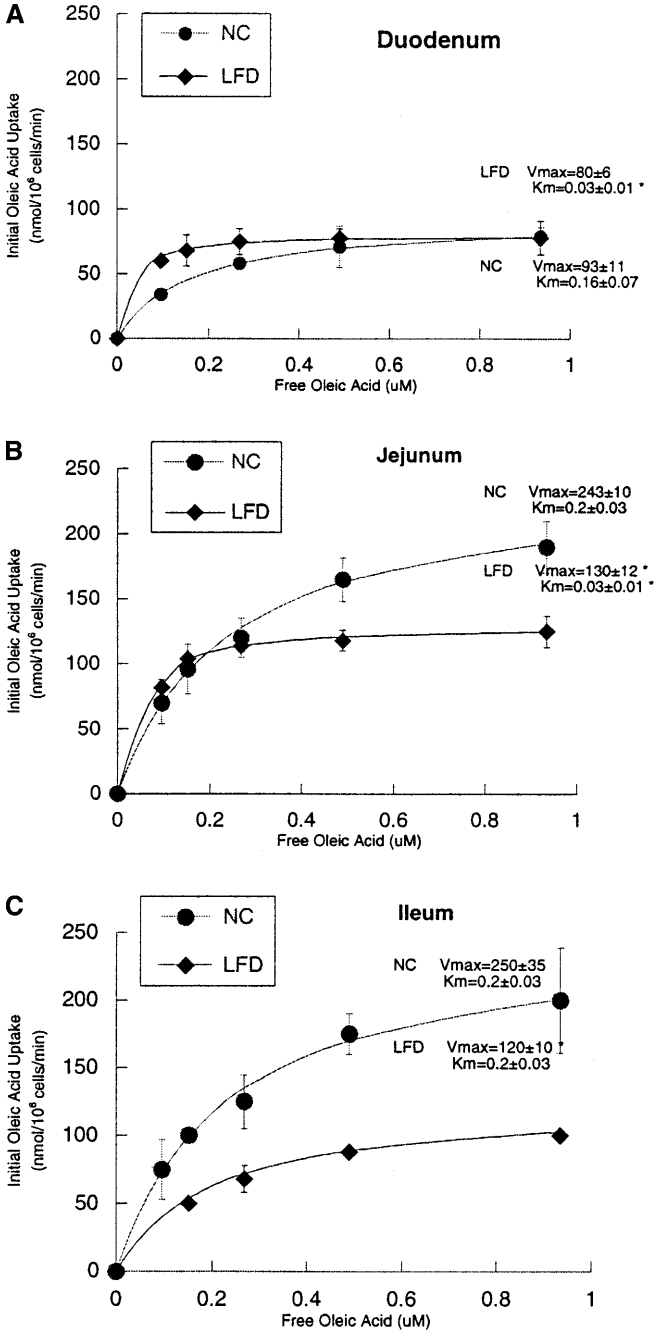

Fig. 1 Relationship between oleic-acid monomer concentration in incubation medium and initial uptake rate by isolated enterocytes in duodenum, jejunum, and ileum in rats with intact intestinal tract fed normal chow (NR-NC) or low-fat diet (NR-LFD). Value are mean ± SE. Kinetics parameters were generated from weighted least-square fits of individual data points from each experiment to a rectangular hyperbola (V_{max}) *$P < 0.05$ NR-NC vs NR-LFD

Following exposure to a LFD, the rats demonstrated significantly decreased FAT/CD36 mRNA levels in the mucosa of the duodenum (31.1 ± 2.0 vs 69.3 ± 3.3) and jejunum (59.6 ± 4.8 vs 91.0 ± 13.7) compared to control animals fed normal chow (Fig. 2). Assuming a

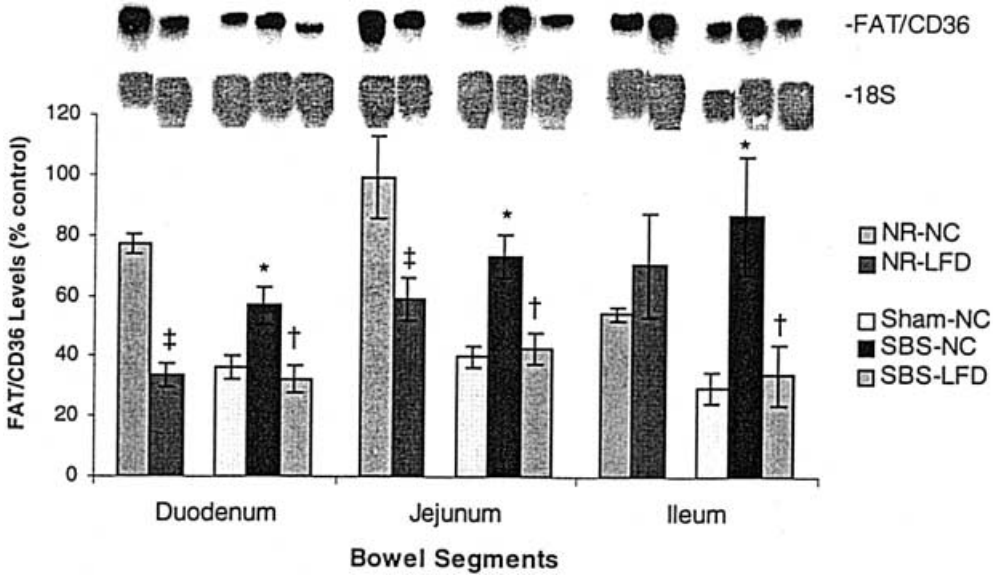

Fig. 2 Effects of low-fat diet (LFD) and bowel resection on FAT/CD36 mRNA expression (% of 18S) in duodenum, jejunum, and ileum on day 14 of dietary treatment. Values are mean ± SEM (*NR* normal rats, *SBS* short-bowel syndrome, *NC* normal chow diet) * $P < 0.05$ SBS vs Sham-NC † $P < 0.05$ SBS-LFD vs SBS-NC ‡ NR-NC vs NR-LFD

parallel decrease in FAT/CD36 protein (though not determined in this study), the decrease in V_{max} of oleate uptake by isolated enterocytes might correspond to a decrease in LCFA transporter numbers available in the plasma membrane.

Effect of LFD on lipid parameters in SBS rats: experiment 2

SBS-NC rats had significantly lower final body weight compared to Sham-NC animals (10% decrease) (Table 1). Exposure of SBS rats to a LFD led to an additional decrease in body weight compared to SBS-NC animals (15%). Food intake decreased in SBS-NC rats compared to their sham counterparts. However, food clearance (dry fecal mass) remained unchanged. This resulted in calculated decreased food absorbability in SBS-NC (vs Sham-NC) rats, suggesting some degree of total food malabsorption in the SBS-NC group. SBS-LFD animals also showed a decrease in food intake (vs Sham-NC) and a more marked decrease in food clearance compared to both SBS-NC and Sham-NC animals (Table 1). Calculated total food absorbability consequently increased in the SBS-LFD group compared to the other two groups. Fat intake remained unchanged in SBS-NC rats compared to sham animals, however, fat clearance was significantly increased. Therefore, calculated fat absorbability decreased in SBS-NC rats versus their sham counterparts, suggesting specific fat malabsorption. Following exposure to a LFD, SBS rats demonstrated a decrease in fat intake and clearance, however, calculated fat absorbability did not change compared to SBS-NC animals.

In SBS-NC rats, [^{3}H] oleate uptake V_{max} was increased (300%–400%) in enterocytes isolated from duodenum, jejunum, and ileum compared to sham controls fed NC (Fig. 3). The transport K_m, reflecting the affinity of oleate for the transport system, increased (decreased affinity) in SBS-NC (vs Sham-NC) rats in duodenum (20%) and ileum (50%) and showed no significant change in enterocytes from the jejunum. Enterocytes from the duodenum, jejunum, and ileum of SBS rats exposed to a LFD demonstrated significant decreases (30%, 30% and 75%, respectively) in the V_{max} of [^{3}H] oleate uptake compared to SBS rats on normal chow.

Enterocyte LCFA transport-system affinity was also altered differentially along the length of the bowel by a LFD in SBS. The [^{3}H] oleate uptake K_m decreased (increased affinity) in enterocytes from the duodenum (40%) and ileum (60%) in SBS-LFD animals compared to SBS-NC animals. The K_m for [^{3}H] oleate uptake in SBS-LFD enterocytes from the jejunum increased (decreased affinity) by 100% compared to SBS-NC. Taken together, these data suggest that at the enterocyte level, LCFA uptake capacity is diminished by in-vivo exposure to low dietary lipids.

Bowel resection in rats fed normal chow resulted in a significant increase in FAT/CD36 mRNA levels in the duodenum (56.9 ± 6.1 vs 31.3 ± 3.6%), jejunum (73.2 ± 6.9 vs 39.9 ± 3.7%), and ileum (87.1 ± 19.4 vs 30 ± 5.2%) compared to Sham-NC animals (Fig. 2). Early exposure to a LFD led to a significant decrease in FAT/CD36 mRNA levels in duodenum (32.3 ± 4.5 vs 56.9 ± 6.1%), jejunum (42.9 ± 4.9 vs 73.2 ± 6.9%), and ileum (29.7 ± 9.0 vs 87.1 ± 19.4%) compared to SBC-NC animals. As in experiment 1, there was a positive general correlation between changes in mucosal FAT/CD36 mRNA levels and

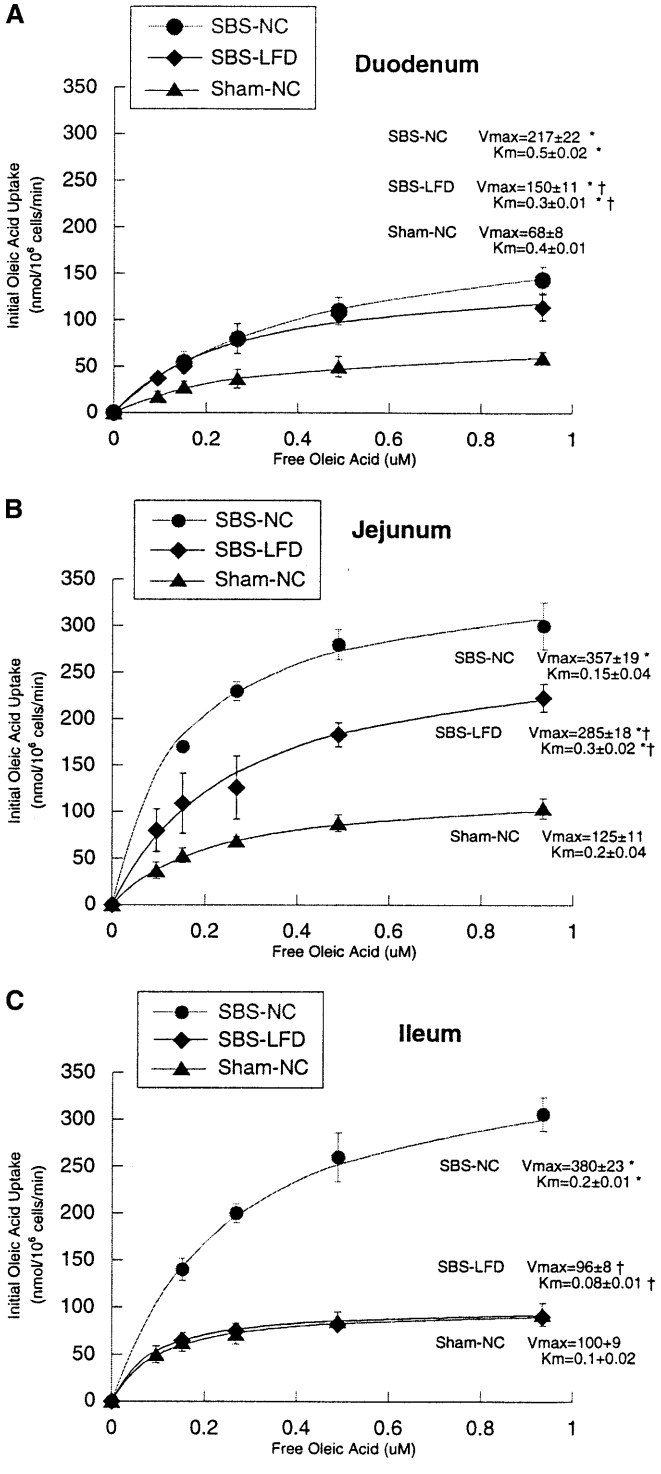

Fig. 3 Relationship between oleic-acid monomer concentration in incubation medium and initial uptake rate by isolated enterocytes in duodenum, jejunum, and ileum in rats with short-bowel syndrome fed normal chow (SBS-NC) or low-fat diet (SBS-LFD) vs Sham-NC animals. Values are mean ± SE * $P < 0.05$ SBS vs Sham-NC † $P < 0.05$ SBS-LFD vs SBS-NC

LCFA uptake V_{max}, suggesting the FAT/CD36 protein levels at the plasma membrane are also regulated by dietary lipids.

Discussion

The use of a LFD is common in treating patients with SBS due to the belief that lipids are often poorly absorbed in this condition. However, evidence is accumulating that lipids in general and LCFA in particular are potent stimulators of intestinal adaptation. The purpose of this study was to evaluate the effect of a LFD on several in-vivo and in-vitro parameters of lipid absorption using a rodent model that included both normal and SBS rats fed a LFD or normal chow.

In our experiments comparing normal rats on a LFD versus normal chow, we found that the LFD did not results in dramatic in-vivo alterations of weight gain, total food intake, or serum and tissue lipid levels. These results suggest that a LFD alone in the setting of adequate total caloric intake does not drastically alter major in-vivo aspects of lipid metabolism. At the cellular level, a LFD results in a decrease in the maximum velocity of enterocyte LCFA uptake and downregulation of mRNA coding for the LCFA transport protein FAT/CD36. It is interesting to note that in jejunocytes, the affinity of oleate for the LCFA transport system increased. A decrease in transport capacity (V_{max}) and an increase in transport affinity in the region of the small intestine known to be most effective in lipid absorption is perhaps consistent with the finding that total lipid absorbability was not different between normal-chow and LFD animals.

In the SBS experiments reported here there is evidence that the model reflects a condition of malabsorption, with the SBS-NC animals not gaining weight in a comparable fashion to sham-operated rats fed normal chow and not absorbing food in general or fat in particular in comparable amounts. Nonetheless, serum cholesterol and triglyceride levels were similar in Sham-NC and SBS-NC rats, suggesting in-vivo compensation for the decreased lipid absorption. The finding that adipose-tissue lipid decreased in SBS-NC animals argues that serum lipid profiles were maintained at the expense of mobilizing peripheral lipid stores. The finding that SBS-NC animals demonstrated an increased cellular uptake capacity for LCFA compared to Sham-NC controls suggests that there is also a compensatory response at the intestinal level to maximize lipid delivery for metabolic requirements despite the loss of absorptive surface area. At the molecular level, increased mRNA levels of FAT/CD36 support this hypothesis and are consistent with the increased V_{max} of oleate uptake by isolated electrocytes.

The application of a LFD in the setting of SBS resulted in decreased weight gain compared to SBS-NC rats, arguing against any general in-vivo benefit from a LFD. The failure to gain weight was not due to the LFD being less palatable, since food intake was similar in the SBS-LFD and SBS-NC groups. However, food clearance (fecal mass) decreased and calculated food absorbability increased in the SBS-LFD animals,

suggesting some degree of compensation, perhaps related to the increased delivery of other adaptation-enhancing nutrients in the LFD. Significant decreases in serum cholesterol and triglycerides in the SBS-LFD compared to the SBS-NC group suggest that, despite mobilization of stored lipids from adipose tissue and some degree of intestinal adaptation, the animals could not maintain the normal serum lipid levels needed for optimal anabolic metabolism.

At the cellular level, a LFD in SBS resulted in diminished LCFA uptake by isolated enterocytes compared to enterocytes from SBS-NC animals. Consistent with its role in facilitating LCFA permeation of the plasma membrane, FAT/CD36 mRNA was also decreased in the small-intestinal mucosa of SBS-LFD animals compared to the SBS-NC group. These findings indicate that a LFD does not stimulate intestinal adaptation, at least in terms of lipid absorption, as well as a normal chow diet.

In summary, the present study demonstrates that in rats with an intact intestinal tract and following small-bowel resection, early exposure to a LFD inhibits molecular and cellular mechanisms of LCFA uptake. This is evidenced by a decrease in FAT/CD36 mRNA expression and LCFA uptake by isolated enterocytes. In normal rats, decreased cellular LCFA uptake did not result in lipid malabsorption because the small intestine had sufficient reserve capacity. However, in SBS animals impaired LCFA transport was associated with decreased body weight and low plasma lipid concentrations. The present data suggest that low-fat formulas should perhaps be avoided in patients with SBS unless severe symptoms of steatorrhea are manifest.

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