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MAIN TOPIC

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Effect of bowel resection and high-fat diet on heart CD36/fatty-acid translocase expression in a rat model of short-bowel syndrome

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Abstract Long-chain fatty acids (LCFA) are the major energy substrates for the heart. In short-bowel syndrome (SBS), LCFA delivery to the myocardium decreases due to fat malabsorption. Fatty-acid translocase (FAT)/CD36 has recently been identified as a LCFA-binding protein in heart tissue. To determine the effects of bowel resection and a high-fat diet (HFD) on myocardial CD36 expression, male Sprague-Dawley rats were randomly assigned to one of three groups: sham rats fed normal chow (Sham-NC); SBS rats fed NC (SBS-NC), and SBS rats fed a HFD (SBS-HFD). Control rats underwent transection and anastomosis; SBS animals underwent 75% small-bowel resection. Rats were killed at 3 or 14 days. Total body weight, heart weight, heart-tissue total lipid, serum cholesterol, and triglycerides were determined at death. Total RNA from the myocardium was extracted using TRIZOL reagent. Northern-blot analysis was used to determine FAT/CD36 mRNA. Statistical significance was determined by Student's *t*-test with *P* values below 0.05 considered significant. SBS-NC and SBS-HFD rats had significantly lower body weights compared with Sham-NC animals. The heart weights and myocardial total lipid did not vary among experimental groups. Decreases in plasma triglycerides (38.2 ± 3.8 vs 58.8 ± 5.5 mg/dl, $P < 0.05$) and cholesterol (38.2 ± 6.9 vs 55.3 ± 8.2 mg/dl, $P < 0.05$) in SBS-NC compared to Sham-NC rats on day 3 was accompanied by a twofold increase ($P < 0.05$) in myocardial CD36/FAT mRNA levels. Early exposure to HFD led to increased (vs SBS-NC) plasma cholesterol (82.9 ± 5.7 vs 38.2 ± 6.9 mg/dl, $P < 0.05$) and triglycerides (62.5 ± 15.6 vs 38.2 ± 3.8 mg/dl, $P < 0.05$), and a concomitant decrease in CD36/FAT mRNA levels (45.1 ± 17.8 vs 86.6 ± 15%, respectively, $P < 0.05$). Plasma lipid concentration and myocardial CD36/FAT mRNA levels on day 14 were not significantly different among the experimental groups. In this rat model of SBS, the heart thus reacts to decreased LCFA delivery by increased tissue CD36/FAT mRNA levels and, consequently, active LCFA uptake. A HFD increased plasma lipid concentrations and decreased CD36/FAT levels.

Keywords Short-bowel syndrome · CD36 · Long-chain fatty acids · Diet

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Introduction

Short-bowel syndrome (SBS) is a disorder in which a loss of either intestinal length or competence significantly compromises the ability to digest and absorb a regular diet [17]. Decreased absorption of most nutrients frequently occurs following bowel resection, however, lipid absorption is generally considered the most vulnerable function [6]. Despite lipid malabsorption and problems associated with steatorrhea, patients with SBS do not typically present with clinical signs of fatty-acid deficiency [20]. The reason for this phenomenon is unclear. It is possible that tissues with a high metabolic capacity for long-chain fatty acids (LCFA) (intestine, heart, and muscle) may regulate LCFA uptake in clinical settings where their delivery is decreased.

LCFAs are the major energy substrate for the heart. In the normal physiologic state, the myocardium preferentially uses LCFAs to provide the energy required for contraction, with glucose utilization being minimal [21, 22]. Previous work has indicated that LCFA uptake by cardiac myocytes is at least in part a saturable process,

suggesting that a protein facilitates plasma-membrane permeation [12]. Fatty-acid translocase (FAT)/CD36 is an 88-kD functional membrane-type glycoprotein receptor first identified in rat adipocytes [1, 10] that was found to be highly expressed in heart and skeletal muscle. Although CD36 has been assigned many possible functions [3, 10], evidence that it plays a role in the cellular uptake of LCFAs is strong [10].

The object of the present study was to investigate the effects of bowel resection and a high-fat diet (HFD) on CD36/FAT expression in cardiac muscle.

Materials and methods

Male Sprague-Dawley rats (240–280 g) were individually housed in hanging stainless-steel cages and provided with an unrefined diet and water ad libitum. The rats were randomly assigned to one of three groups: (1) Sham: operated control rats fed normal chow (Sham-NC, n = 15); (2) rats with SBS fed NC (SBS-NC, n = 15); and (3) rats with SBS fed a HFD (SBS-HFD, n = 14).

After 48 h of acclimation to the environment and a 12-h fast, the rats were anesthetized with IP sodium pentobarbital 45 mg/kg; 44 underwent a 75% small-bowel resection, preserving the proximal jejunum 5 cm beyond the ligament of Treitz and the distal ileum 10 cm proximal to the ileocecal junction. An end-to-end anastomosis was performed using interrupted 6-0 silk sutures. In the sham-operated animals the bowel was transected and reanastomosed 10 cm proximal to the ileocecal valve. In all operations the abdomen was closed in two layers with a running 3/0 Dexon suture. Water was provided ad libitum for 24 h following the operation. Rats were then given a normal (10 kcal% fat) or HFD (50 kcal% fat) (Research Diets, Table 1) and water *ad libitum* until death. Both diets contained equal quantities of soybean oil, rich in (n-3) linolenic acid, but the HFD had a significantly greater amount of lard, which is rich in saturated fatty acids.

The animals were killed on day 3 or 14 after surgery after anesthesia with sodium phenobarbital IP (45 mg/kg) by open pneumothorax. Blood samples for cholesterol and triglycerides were obtained by direct cardiac puncture and transferred to a heparinized tube. The plasma concentrations of cholesterol and triglycerides were measured spectrophotometrically by enzymatic assays using commercially available kits (Sigma, Kits 352,339 and 16-UV). The heart was quickly removed, rinsed with cold saline to remove the remaining blood, and weighed. Total tissue lipid was extracted as described by the method of Bligh and Dyer [5].

Total RNA from heart tissue was isolated using TRIZOL reagent (GIBCO) as described by Chomczynski and Sacchi [7]. Total RNA (30 μg/lane) was separated on 1% agarose gel containing formaldehyde (18%) and transferred to a nylon membrane using the Rapid Downward Transfer System (Schleicher and Schuell) and cross-linked to the membrane by exposure to ultraviolet waves for 20 min followed by baking for 2 h at 80 °C. The membrane was pre-hybridized overnight at 42 °C and then hybridized with ^{32}P-labeled FAT cDNA probe (Rediprime labeling kit, Amersham Life Science) for 20 h at 42 °C. After three washes the membrane was exposed to X-ray film (Kodak) for 24–72 h; the ^{32}P-labeled FAT cDNA probe was removed from the membrane (boiled for 15 min) and the membrane was re-probed with ^{32}P-labeled 18S cDNA probe. Autoradiographs were analyzed by densometry using a Hewlett-Packard ScanJet 4c/T in conjunction with NIH Image software (version 1.60).

Table 1 Composition of experimental diets

Ingredient	Normal chow (10% kcal fat) Amount (g/kg)	High-fat diet (50% kcal fat) Amount (g/kg)
Fat	15	45
Casein	200	200
Sucrose	384	350
Starch	349	315
Vitamins[a]	12	12
Minerals[b]	45	45

[a]Provided (g%): Standard salt mix (S10001) 10.0, Calcium phosphate 13.0, Calcium carbonate 5.5, potassium citrate 16

[b]Supplied (mg%): Standard vitamin mix (V10001) 10.0, choline bitartrate 2.0

Results

Body and heart weight

Mean body weight did not differ significantly between SBS rats fed NC or a HFD (Table 2). Both groups lost weight 3 days after surgery and then gained weight at similar rates. Final body weight on day 14 was the same in both groups, and was significantly lower than in Sham-NC animals. The weight of the heart did not vary significantly among the three groups.

Serum lipids

SBS-NC rats had significantly lower (compared to Sham-NC) mean serum triglyceride (38.2 ± 3.8 vs 58.8 ± 5.5 mg/dl, $P < 0.05$) and cholesterol (38.2 ± 6.9 vs 55.3 ± 8.2 mg/dl, $P < 0.05$) levels on day 3 following bowel resection (Fig. 1). SBS-HFD rats demonstrated greater mean serum cholesterol (82.9 ± 5.7 vs 38.2 ± 6.9 mg/dl, $P < 0.05$) and triglyceride (62.5 ± 15.6 vs 38.2 ± 3.8 mg/dl, $P < 0.05$) levels on day 3 compared to the resected rats fed NC. Mean serum cholesterol and triglyceride levels on day 14 did not vary significantly among the three groups.

Tissue lipid

SBS rats demonstrated a trend toward a decrease in myocardial tissue lipid on day 3, although this did not achieve statistical significance (Table 1). A significant three fold decrease in mean tissue lipid content was observed on day 14 in SBS-NC rats compared to Sham-NC animals. Exposure to a HFD attenuated this effect. SBS-HFD animals demonstrated a twofold increase in myocardial lipid content compared to SBS-NC rats.

CD36/FAT mRNA levels

A significant, two-fold increase in mean myocardial levels of FAT/CD36 mRNA was observed on day 3 in

Table 2 Final body weight, heart weight, and heart total lipid (*NC* normal chow. *SBS* short-bowel syndrome, *HFD* high-fat diet)

Experimental group	Feeding day	Final body weight (% pre-operative)	Heart weight (mg/100 g weight)	Heart total lipid (mg/g tissue)
Sham-NC	Day 3	99.7 ± 1.5	3.0 ± 0.2	48 ± 10
SBS-NC		91.3 ± 2.3[a]	3.0 ± 0.1	37 ± 8
SBS-HFD		90.9 ± 1.8[a]	3.0 ± 0.1	26 ± 4
Sham-NC	Day 14	130.6 ± 3.7	2.7 ± 0.1	56 ± 9
SBS-NC		118.1 ± 3.4[a]	2.8 ± 0.1	20 ± 5[a]
SBS-HFD		113.9 ± 2.7[a]	2.8 ± 0.1	46 ± 13[b]

[a] $P < 0.05$ SBS vs sham-NC
[b] $P < 0.05$ SBS-HFD vs SBS-NC

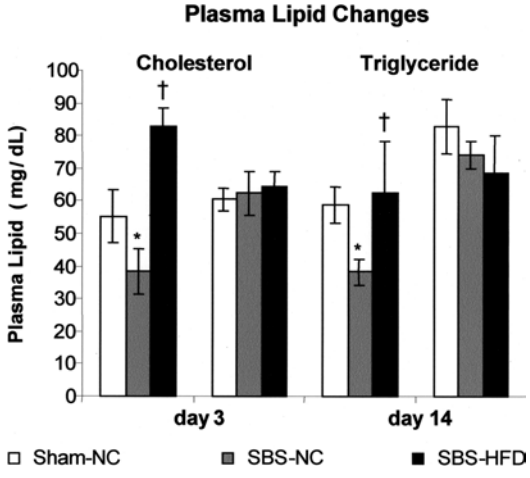

Fig. 1 Plasma lipid concentrations in Sham and SBS rats fed normal chow (NC) and high-fat diet (HFD). Values are mean ± SEM (*SBS* short-bowel syndrome, *$P < 0.05$ SBS vs Sham-NC rats, †$P < 0.05$ SBS-HFD vs SBS-NC rats)

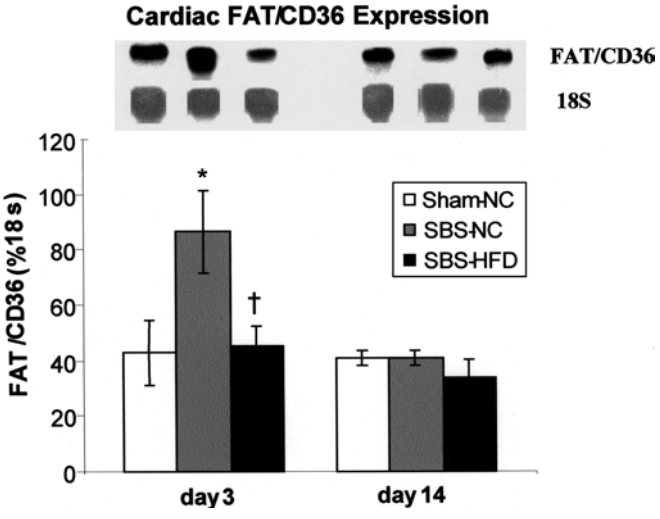

Fig. 2 Fatty-acid translocase (FAT)/CD36 mRNA expression in heart tissue in Sham-NC (normal chow) and short-bowel syndrom (SBS) rats fed NC or high-fat diet (HFD) on day 3 and 14 of treatment. Values are mean ± SEM. (*$P < 0.05$ SBS vs Sham-NC, †$P < 0.05$ SBS-HFD vs SBS-NC rats)

SBS-NC rats compared to Sham-NC animals (86.6 ± 15 vs 43 ± 11.7% control, respectively, $P < 0.05$) (Fig. 2). Exposure to a HFD for 3 days resulted in a two-fold decrease in FAT mRNA levels compared to SBS-NC rats (45.1 ± 17.8 vs 86.6 ± 15% control, respectively, $P < 0.05$), which reached values not different from Sham-NC rats.

Myocardial levels of FAT/CD36 mRNA did not vary significantly between the three groups on day 14.

Discussion

Heart has a high metabolic requirement for LCFAs. However, because the capacity of cardiac myocytes for de-novo synthesis of fatty acids is limited, the heart strongly relies on an exogenous source of needed LCFAs [21]. Heart cells are able to regulate LCFA uptake in order to adapt to changes in energy demands. The mechanisms responsible for the movement of LCFAs across the sarcolemma of cardiac myocytes are a subject of controversy. Due to their lipophilic nature, LCFAs tend to diffuse freely through the plasma membranes, including those of cardiac myocytes. Several investigators favor passive diffusion through the lipid bilayer as the central mechanism of transport of LCFAs into and out of the cells; this mechanism guarantees that LCFAs are supplied to the cell and that excess LCFAs not metabolized can be removed [9, 15].

Recent studies on the mechanism of LCFA uptake have suggested, however, that fatty-acid absorption by cardiac myocytes is, at least in part, a carrier-mediated process [18, 19]. Passive permeation and protein-mediated transport are not necessarily mutually-exclusive processes. Both may occur concurrently, and the relative importance of each may vary under different physiological conditions. Some investigators have suggested that LCFAs diffuse passively into cells under basal conditions and that transporters increase the uptake of LCFAs above this basal level [16], while others have suggested that transporters work at low concentrations of LCFAs while at high concentrations, LCFAs diffuse passively into the cell [1, 2].

CD36 is a member of a gene superfamily that includes scavenger receptor B1(SR-B1), a protein-binding anionic phospholipid [14] that transports high-density lipoprotein cholesterol esters across the plasma membrane [4]. Although CD36 has been assigned many possible functions, evidence suggests that it plays a role in the cellular uptake of LCFAs. In general, CD36 expression distribution favors tissues with a high metabolic capacity for LCFAs such as adipose tissue, intes-

tine, heart, and muscle, while it is absent from tissues like brain, which do not utilize LCFAs [3]. Many investigators [8, 19, 21, 22] have described the role of CD36 in the transmembrane transport of LCFAs by cardiac myocytes. It has been reported that patients with type I CD36 deficiency show impaired myocardial fatty-acid uptake [11, 13], which may result in some types of cardiac hypertrophy as well as other cardiac diseases [23]. It has also been shown that fatty-acid delivery to the heart may influence FAT/CD36 expression. Greenwalt et al. [8] observed that mice fed a HFD (40% kcal fat) expressed heart CD36 at a level 3.5-fold higher than those fed a 9% fat diet.

The aim of this study was to determine the expression of FAT/CD36 in rat heart after massive bowel resection followed by exposure to a HFD. Although the final body weight was significantly lower in SBS rats compared to their Sham counterparts, the heart weight did not vary significantly among experimental groups. An obvious inverse relationship between plasma lipids and FAT/CD36 mRNA levels was observed. Massive bowel resection led to a significant decrease in plasma triglyceride and glucose levels 3 days postoperatively. In an effort to compensate for the reduced LCFA delivery, the heart dramatically increased its FAT/CD36 mRNA levels.

Early exposure to HFD led to a significant increase in plasma lipid and a concomitant decrease in myocardial FAT/CD36 mRNA levels. 14 days after surgery, resection and a HFD had minimal effects on serum lipid levels. Correspondingly, cardiac FAT/CD36 mRNA levels were similar in the three experimental groups. Despite this compensatory process, resected rats showed a trend toward a decrease in tissue total lipid content that achieved statistical significance on day 14. The total lipid content in the tissues depends on in-situ synthesis, uptake from the plasma, catabolism, and release from the organ. The myocardium has a decreased capacity for LCFA synthesis. In addition, decreased uptake of fatty acids following their reduced delivery resulted in decreased tissue lipid, despite a compensatory increased transport process. Early exposure to a HFD prevented this dramatic decrease in myocardial total lipid composition.

In conclusion, in a rat model of SBS the heart reacted to decreased LCFA delivery by increasing tissue CD36/FAT mRNA levels and, consequently, active LCFA uptake. Early exposure to a HFD led to increased plasma lipid concentrations and a concomitant decrease in myocardial CD36/FAT mRNA levels. The failure of this compensatory mechanism may contribute to impaired myocardial lipid homeostasis and cellular integrity.

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