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Dietary fish oil increases fat absorption and fecal bile acid content without altering bile acid synthesis in 20-d-old weanling rats following massive ileocecal resection

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INTRODUCTION: Dietary fish oil (FO) was reported to lower fecal fat excretion in a weanling rat model of short bowel syndrome (SBS) after ileocecal resection (ICR), and to induce changes in secretion and synthesis of bile acid (BA) in adults. We hypothesized that dietary FO, as compared with corn oil (CO), increases intestinal fat absorption in weanling SBS rats in part due to increased hepatic BA synthesis and luminal BA concentrations.

METHODS: After undergoing ICR, 20-d-old rats were fed *ad lib* for 7 d with a CO or FO diet containing 5% sucrose polybehenate (SPB), a marker for dietary fat absorption. Fecal fatty acid, fecal and intestine luminal BA, liver mRNA expressions of cholesterol 7α-hydroxylase (Cyp7α1) and sterol-12α-hydroxylase (Cyp8β1), and serum 7α-hydroxy-4-cholesten-3-1 (7αC4) levels were determined.

RESULTS: As compared with CO-ICR rats, FO-ICR rats had higher intestinal absorption of total fat and most individual fatty acids. Although the BA content per gram of dry stool was increased in FO-ICR rats, there were no differences between groups for the BA content in remnant jejunum, liver mRNA expression of BA biosynthetic enzymes, Cyp7α1 and Cyp8β1, or serum 7αC4, a marker for BA synthesis.

CONCLUSION: Dietary FO increases dietary fat absorption without increasing hepatic BA synthesis in weanling SBS rats.

Dietary long-chain polyunsaturated fatty acids (LCPUFAs) have been shown to be effective in stimulating postresection intestinal adaptation in adult (1,2) and weanling (3) rat models of short bowel syndrome (SBS). Previously, we reported that feeding 20-d-old SBS rats with a high-fat diet enriched in n3 LCPUFAs from fish oil (FO) reduced fecal fat excretion as compared with littermates fed a high-fat diet enriched in n6 polyunsaturated fatty acids (PUFAs) from corn oil (CO; ref. 3). Although total dietary fat content is known to affect fat absorption in SBS rats (4), the effect of dietary FO (or n3 LCPUFA) intake on fat absorption after massive bowel resection has not been investigated in weanling rats. Bile acids (BAs) play a well-established role in the digestion and intestinal absorption of fat. Ileal resection impairs fat absorption due to compromised enterohepatic BA circulation and depletion of the BA pool. Dietary FO was found to increase BA synthesis in humans (5) and to increase the BA pool size in adult rats (6). However, the effect of dietary FO on BA metabolism in weanling rats with SBS has not been studied. Therefore, this study was designed to investigate the effect of dietary FO on intestinal fat absorption and BA metabolism in 20-d-old SBS rats following ileocecal resection (ICR). We hypothesized that 20-d-old SBS rats fed an n3 LCPUFA–enriched diet would exhibit an increased intestinal fractional fat absorption in part due to increased hepatic BA synthesis as compared with those fed an n6 PUFA–enriched diet.

In this study, sucrose polybehenate (SPB) was used as an intestinal marker to measure dietary fat absorption. SPB is a nonabsorbable dietary fat that can be readily measured by gas chromatography analysis of its hydrolysis product, behenic acid, a natural fatty acid not present in most dietary fats. The absorption of total and individual fatty acid is calculated from the ratios of behenic acid to other fatty acids in diets and feces (7). BA metabolism was examined by measuring fecal and intestine luminal BA contents, and markers of hepatic BA synthesis, including hepatic mRNA expression of cholesterol 7α-hydroxylase (Cyp7α1), the rate-limiting enzyme in BA synthesis, and sterol 12α-hydroxylase (Cyp8β1), the determinant of cholic acid biosynthesis, and serum levels of 7α-hydroxy-4-cholesten-3-1 (7αC4), a BA biosynthetic pathway intermediate whose levels correlate with hepatic Cyp7α1 activity and hepatic BA synthesis (8).

RESULTS

Body Weight Changes

Figure 1 shows the daily percentage of preoperative body weight in ICR rats during the 7-d feeding period after surgery. Preoperative body weights were similar in the two groups (47.26 ± 4.20 g in the ICR rats fed a CO diet (CO-ICR group) and 47.70 ± 5.44 in the ICR rats fed a FO diet (FO-ICR group)). The bowel segments resected from the two ICR groups were similar in length (26.2 ± 2.7 cm in the CO-ICR group and 26.5 ± 3.6 cm in the FO-ICR group). Postoperative body weights declined about 13% in

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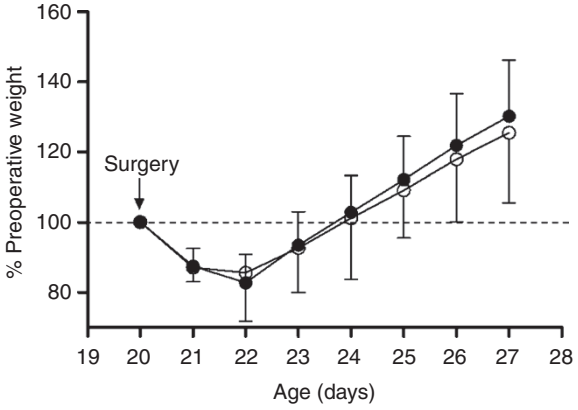

Figure 1. Daily body weight. Surgery was performed at 20 d of age. The daily postoperative body weight is expressed as % of preoperative weight (dotted line at 100%). Open circles: corn oil–ileocecal resection group ($n = 7$); filled circles: fish oil–ileocecal resection group ($n = 7$) rats.

both groups 1 d after surgery. Both CO-ICR and FO-ICR rats regained their preoperative weight by the 4th postoperative day, and the weights at the 7th postoperative day were 58.76 ± 7.11 g in the CO-ICR group and 61.51 ± 5.35 g in the FO-ICR group.

Absorption of Total Dietary Fat and Individual Fatty Acids

The total dietary fat absorption (%) was higher in six of seven FO-ICR rats than their matched CO-ICR counterparts, such that the total dietary fat absorption was ~12% higher in the FO-ICR group as compared with the CO-ICR group (37.89 ± 12.47 vs. 25.38 ± 8.31, $P < 0.05$; **Figure 2a**). **Table 1** displays the fatty acid compositions of diets and feces, and dietary fatty acid absorption from CO-ICR and FO-ICR rats. As compared with CO-ICR rats, FO-ICR rats had higher absorption for 16:0, 16:1, 18:1, 18:2 n6, and 18:2 n3 (all $P < 0.05$), lower absorption for 18:4 n3 ($P < 0.05$), and similar absorption for 14:0 and 18:0 fatty acids. The percentages of absorption for LCPUFA (FO diet only) are also listed in **Table 1**.

Fecal BA and Intestine Luminal BA Contents

All FO-ICR rats had higher fecal BA content (μmol/g dry feces) than their matched CO-ICR counterparts (FO-ICR group: 32.49 ± 5.95, as compared with the CO-ICR group: 24.78 ± 5.74; $P < 0.05$; **Figure 2b**). Fecal BA content was positively correlated with total dietary fat absorption in both FO-ICR and CO-ICR rats ($r^2 = 0.6926$, $P < 0.001$; **Figure 2c**). However, there were no differences between the two groups for the intestine luminal BA content in the four segments of remnant jejunum (**Figure 3**).

Liver mRNA Expressions of Cyp7α1 and Cyp8β1 and Serum 7αC4 Levels

Hepatic BA synthesis was evaluated by measuring liver mRNA expressions for Cyp7α1 and Cyp8β1 and serum levels of 7αC4. As shown in **Table 2**, neither liver mRNA expressions of Cyp7α1 and Cyp8β1, nor serum 7αC4 levels were different between CO-ICR and FO-ICR rats.

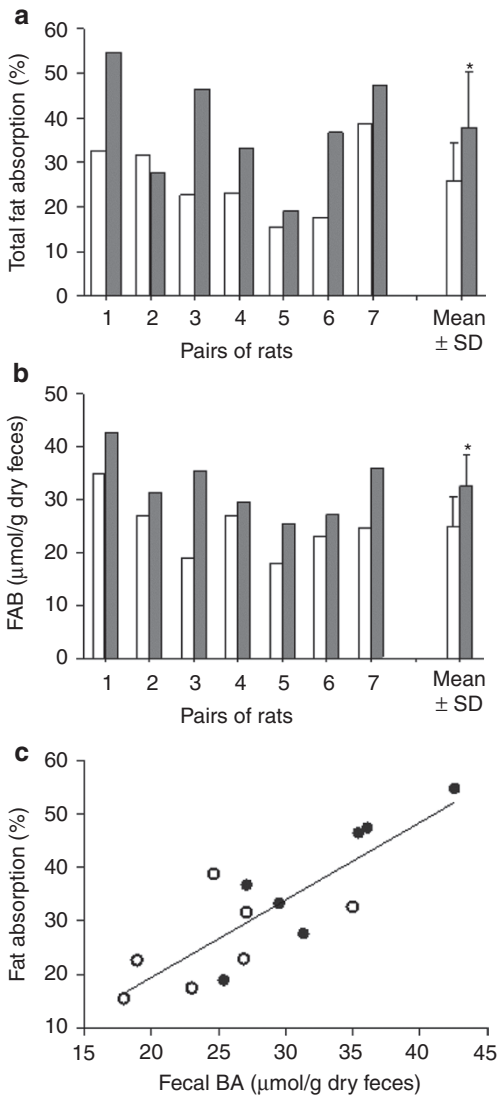

Figure 2. Intestinal absorption of total dietary fat and FBA content in 20-d-old ileocecal resection (ICR) rats fed with a corn oil (CO) or fish oil (FO) diet containing 5% sucrose polybehenate. (**a**) Total fat absorption (%) is displayed for each pair of rats (without SD) and as the two groups (with SD). ICR rats in the CO ($n = 7$, white bars) and FO groups ($n = 7$, gray bars) were matched by body weight and length of the resected small bowel. *$P < 0.05$, CO-ICR vs. FO-ICR rats. (**b**) FBA contents are displayed for each pair of rats (without SD) and as the two groups (with SD). ICR rats in the CO ($n = 7$, white bars) and FO groups ($n = 7$, gray bars) were matched by body weight and length of resected small bowel. *$P < 0.05$, CO-ICR vs. FO-ICR rats. (**c**) FBA content was positively correlated with total fat absorption in CO-ICR ($n = 7$, open circles) and FO-ICR ($n = 7$, filled circles) rats ($r^2 = 0.6926$, $P < 0.001$). FBA, fecal bile acid.

DISCUSSION

The intestine's ability to digest and absorb enteral nutrients is impaired in SBS. In particular, with ileal resection fat absorption is generally considered the most vulnerable because of the combined loss of absorptive surface area and compromised enterohepatic circulation and decreased BA pool. However, dietary fats (2,9,10), especially the dietary LCPUFAs (1–3), have been shown to be effective in stimulating postresection mucosal adaptation and increasing the remnant intestine's

Table 1 Dietary/fecal fatty acid compositions and dietary fatty acid absorption

	Dietary fatty acids (%)		Fecal fatty acid (%)		Dietary fatty acid absorption (%)	
Fatty acid	CO diet	FO diet	CO-ICR	FO-ICR	CO-ICR	FO-ICR
14:0	0.15	1.68	0.10 ± 0.02	1.18 ± 0.16	48.67 ± 13.09	53.19 ± 13.09
16:0	11.67	13.49	11.81 ± 0.19	14.49 ± 0.95	23.22 ± 8.77	29.86 ± 12.63[a]
16:1	0.49	2.62	0.37 ± 0.04	1.93 ± 0.24	43.68 ± 10.83	50.75 ± 14.14[a]
18:0	2.72	3.08	3.28 ± 0.21	4.17 ± 0.41	9.38 ± 4.82	12.44 ± 8.83
18:1 n9	48.85	47.17	48.65 ± 0.73	47.07 ± 2.60	24.33 ± 8.29	34.31 ± 13.21[a]
18:1 n7	1.38	1.86	1.43 ± 0.08	0.11 ± 0.02	21.04 ± 10.01	96.16 ± 0.06[a]
18:2 n6	29.18	20.21	26.42 ± 0.84	16.83 ± 2.44	30.91 ± 9.59	44.79 ± 15.70[a]
18:3 n3	0.65	0.79	0.51 ± 0.04	0.51 ± 0.13	39.30 ± 11.31	56.35 ± 16.99[a]
18:4 n3	0.25	0.40	0.03 ± 0.02	0.32 ± 0.05	87.62 ± 1.27	46.41 ± 17.08[a]
20:4 n6	n.d.	0.35	n.d.	0.27 ± 0.06	—	44.82 ± 12.49
20:5 n3	n.d.	2.20	n.d.	1.65 ± 0.49	—	45.91 ± 14.53
22:0 (behenate)	4.00	4.41	5.26 ± 0.57	6.92 ± 1.30	—	—
22:5 n3	n.d.	0.45	n.d.	0.40 ± 0.11	—	40.39 ± 16.43
22:6 n3	n.d.	1.72	n.d.	1.53 ± 0.49	—	35.95 ± 14.19

Values are means ± SD; $n = 7$ in CO-ICR or FO-ICR group.

CO, corn oil; FO, fish oil; ICR, ileocecal resection; n.d., not detectable for a peak of gas chromatography with a signal:noise ratio <10, and/or <1,000 area units, and/or no peak within a retention time window for a component fatty acid.

[a]Different from CO-ICR, $P < 0.05$.

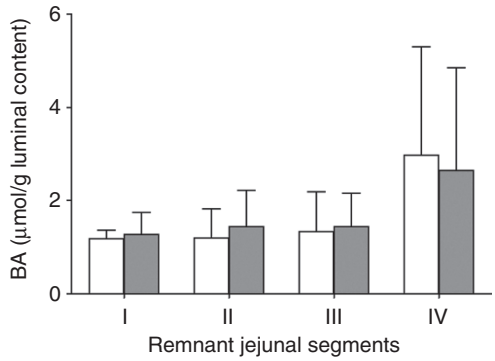

Figure 3. Intestine luminal BA contents in segments of remnant jejunum in 20-d-old corn oil-ileocecal resection ($n = 6$, white column) and fish oil–ileocecal resection ($n = 6$, gray column) rats. I, II, III, and IV on the *x* axis: the four quartiles of remnant jejunum from proximal to distal. BA, bile acid.

digestive and absorptive capacity. In this study, we used the SPB method (7) to investigate the impact of an FO diet on fat absorption in SBS weanling rats. SPB has been reported to have no effects on BA excretion and absorption (11). Although the traditional fat-balance method is a widely accepted approach for measuring dietary fat absorption (7,12), the technique depends on quantitative 24-h stool collections. However, in the case of liquid diet–fed weanling rats after ICR, the stools are very loose, making quantitative long-term stool collections difficult. The SPB method provides quantitative measures of total fat and individual fatty acid absorption using spot fecal samples (7). In our study, we found that ICR rats fed with an n3 LCPUFA-enriched FO diet had higher total dietary fat absorption relative to those fed with an n6 PUFA-enriched CO diet, and this was reflected by increased absorption for most individual species of dietary fatty acids. This result confirmed our previous finding that feeding a FO-enriched diet decreased fecal fat excretion in the same weanling rat model of SBS (3).

Subsequently, we found that FO-ICR rats excreted more BA per unit weight of dry feces than CO-ICR rats. This finding was consistent with previous reports that dietary FO increased biliary excretion of BA in adult rats (6) and fecal BA excretion in adult mice (13). Although previous studies demonstrated effects of dietary FO on bile flow, BA pool size, and fecal BA excretion in adult rodents (6,13–16) and humans (5), the underlying mechanisms remain unclear. For example, Smit *et al.* (6) showed that activity of liver Cyp7α and absolute amount of BA synthesis were not altered by FO feeding in rats; others found that dietary FO upregulated Cyp7α1 mRNA expression in mice (13) and increased BA synthesis in humans (5). To assess BA synthesis, hepatic mRNA expression of Cyp7α1 and Cyp8β1 and serum levels of 7αC4 (8,16,17) were measured in the ICR rats fed with CO and FO diets. Neither mRNA levels of Cyp7α1 and Cyp8β1, nor serum levels of 7αC4 were increased by an FO diet as compared with a CO diet, suggesting that the higher fecal BA content in FO-ICR rats was not due to increased hepatic BA synthesis. To further confirm this finding, we examined the BA concentration in the luminal contents from remnant jejunum and found that there were no differences in BA contents between the two groups. The apparently contradictory observations of no change in hepatic BA synthesis or luminal BA content and an increased fecal BA content can be explained by the increased intestinal and colonic adaptation in the FO-ICR relative to the CO-ICR rats (3) and the spot fecal collection method. The CO-ICR rats have increased diarrhea relative to the FO-ICR rats (3), and the

Table 2 Liver mRNA levels of Cyp7α1 and Cyp8β1 and serum 7αC4 in 20-d-old ICR rats

Liver mRNA[a]	CO-ICR ($n = 7$)	FO-ICR ($n = 7$)
Cyp7α1	24.0 ± 11.8	23.6 ± 4.2
Cyp8β1	8.6 ± 3.0	10.3 ± 4.5
Serum 7αC4 (ng/ml)	841.1 ± 534.7	961.4 ± 480.9

Values are mean ± SD.

CO, corn oil; Cyp7α1, cholesterol 7α-hydroxylase; Cyp8β1, sterol-12α-hydroxylase; FO, fish oil; ICR, ileocecal resection; 7αC4, 7α-hydroxy-4-cholesten-3-1.

[a]mRNA levels are expressed as ($Ct_{Cyp7α1}$ or $Ct_{Cyp8β1}/Ct_{18S}$) × 1,000.

decreased BA content in CO-ICR rats may reflect dilution by increased fecal water loss. Of note, the fecal BA content was positively correlated with dietary fat absorption in both groups. The correlation between the fecal BA and the percentage of fat absorption may reflect a common relationship to the intestinal adaptation. In fact, trophic effects of the FO diet on mucosal adaptation were seen in an adult (1) and a weanling (3) rat model of SBS. With increases in intestinal adaptation, intestinal functions such as fat and water absorption would improve: the percentage of fat absorption would be increased and dilution of the BA content by fecal water loss would be diminished.

In conclusion, feeding 20-d-old SBS rats with a FO diet increased total dietary fat absorption as compared with a CO diet, as reflected by an increased absorption of most individual dietary fatty acids. Feeding the FO diet did not increase hepatic BA synthesis, as indicated by the lack of differences in liver mRNA levels of Cyp7α1 and Cyp8β1 and serum 7αC4 between the two groups. The results suggest that the beneficial effects of dietary FO for intestinal dietary fat absorption are not associated with an increase in BA synthesis and intestine luminal BA concentrations in weanling SBS rats. The underlying mechanisms responsible for the stimulation of intestinal adaptation and function by FO in SBS-ICR rats remain to be identified. The consistent observation of improved fat absorption in FO-ICR rats raises the question of whether enteral administration of FO may also be of benefit for management of SBS infants. A FO-based fat emulsion (Omegaven) has been studied for the treatment and prevention of parental nutrition–associated liver disease in infants with SBS (18). However, Omegaven is currently not available in the United States and requires a central venous catheter, which increases the risk for infection and other catheter-related complications. Supplementing SBS infants with enteral FO may provide a new strategy to augment the postresection adaptive process of intestinal mucosa in SBS infants. In fact, we are conducting a clinical trial to test this hypothesis.

METHODS

Diets

The liquid diet, Lieber-DeCarli Regular Control Rat Diet, Dyets #710027 (Dyets, Bethlehem, PA), was used as a base diet. The SPB was a gift from Ryan Temel (Department of Pathology–Lipid Sciences, Wake Forest University Health Sciences). The FO and CO diets were made using 10.4 ml of FO (menhaden oil, Dyets #402940,) or CO plus 2.6 g of SPB mixed thoroughly with 210.69 g of the base diet before adding water up to 1 l. This mixture was then homogenized for 30 s using a Polytron-like mixer according to the manufacturer's instructions. Both diets contained ~5% (g fat/100 g diet, w/w) SPB. The FO diet contained 18.3% (w/w) FO and 16.7% (w/w) CO, whereas the CO diet had 35% (w/w) CO. The nutritional profiles of the two diets are listed in **Table 3**. Dietary fatty acids were extracted (19) and analyzed (20). The diets were stored at 4 °C and used within 48 h of preparation.

Table 3 Nutritional profiles of CO and FO diets

	CO diet	FO diet
g/l (% kcal)		
Protein	42.8 (16.6)	42.8 (16.6)
Carbohydrate	109.4 (42.5)	109.4 (42.5)
Fat	49.3 (40.8)	49.3 (40.8)
g fat/100 g diet		
Corn oil	35.0	16.7
Olive oil	54.8	54.8
Safflower oil	5.2	5.2
Fish oil[a]	—	18.3
SPB	5.0	5.0
Mineral Mix[b]	8.4	8.4
Vitamin Mix[c]	2.4	2.4
kcal/ml	1.0	1.0

CO, corn oil; FO, fish oil; SPB, sucrose polybehenate.

[a]Dyets #402940 (Dyets, Bethlehem, PA), menhaden oil. [b]Dyets #210011 (Dyets). [c]Dyets #310011 (Dyets).

Animal Model of SBS

The experimental protocol for this study was approved by the Institutional Animal Care and Use Committee at Wake Forest University Health Sciences. Fourteen male weanling Sprague-Dawley rats from several different litters were separated from their dams at 20 d of age. The ICR surgery was performed as previously described (21). Briefly, surgery was performed aseptically, using inhaled 2% isoflurane and oxygen for anesthesia throughout the procedure. The segment of intestine between the 5th and the 6th mesenteric vessel arcade proximal to the ileocecal junction and 1 cm distal to the cecum in the ascending colon was removed after ligation of the mesenteric vessels. This results in removal of an average of 26 cm of small intestine, which was ~50% of the small bowel. The residual of jejunum and proximal colon were end-to-end anastomosed. Following surgery, the rats were hydrated with 1 ml of warm saline by intraperitoneal instillation, and the abdomen was closed. The rats were held in an infant incubator at 35 °C for at least 30 min after surgery, until fully recovered from anesthesia, and then returned to conventional individual housing cages. The rats were divided into two groups by matching the body weight and length of resected small bowel, and fed a CO diet (CO-ICR) or a FO diet (FO-ICR) with both containing 5% SPB, for 7 d after surgery. Water was available only for the initial 24 h after surgery when intake of the liquid diet was minimal.

Analysis of Fecal Fatty Acid and Calculation of Fat Absorption

Fresh spot fecal samples were collected at the end of the 7th feeding day post-ICR. Approximately 160–200 mg of wet stool was dried under N_2 blow at 70 °C, resulting in ~40 to 80 mg of dry feces. Fatty acids were extracted from the dry feces (19), saponified, methylated, and analyzed by gas chromatography (20). The fractions of absorbed total fat and individual fatty acids were calculated from the ratios of behenic acid to other fatty acids in diets and feces as described by Jandacek et al. (7).

Fecal and Intestine Luminal BA Measurements

Fresh spot fecal samples were collected over the last 3 d of feeding and pooled. Two grams of pooled stool samples were dried in a vacuum oven at 70 °C overnight. Approximately 0.21–0.37 g of dry feces were

extracted as described by Dawson *et al.* (22) and used to determine the total BA content by an enzymatic method (23).

In a separate experiment, 12 ICR rats were killed after being fed the CO ($n = 6$) or FO diets ($n = 6$) for 7 d post-ICR. The remnant jejunum was isolated between 4 cm distal of the pylorus and 1 cm proximal of the reanastomosis site. The average length of remnant jejunum was 38.4 ± 6.6 cm in CO-ICR rats and 37.7 ± 4.6 cm in FO-ICR rats. The remnant jejunum was then divided equally into four segments I–IV from proximal to distal. The luminal contents in each segment were collected and BA concentrations in luminal contents were measured using an enzymatic assay (Colorimetric Total Bile Acids Assay Kit; Diazyme Laboratories, Poway, CA).

Measurements of Liver mRNA Expressions of Cyp7α1 and Cyp8β1 and Serum 7αC4

Total RNA was extracted from liver using TRIzol Reagent (Invitrogen, Grand Island, NY) as suggested by the manufacturer. The cDNA was synthesized from 1 μg of RNA using random hexamer primers and a Transcriptor First Strand cDNA Synthesis Kit (Roche Applied Science, Indianapolis, IN). For quantitation of mRNA expression, in each real-time PCR reaction, cDNA synthesized from 25 ng of RNA was mixed with 2× SYBR Green PCR Master Mix (Roche Applied Science) and 500 nmol/l of forward and reverse primers, and the reaction was analyzed using a 7500 Fast Real-Time PCR System (Applied Biosystems, Carlsbad, CA). The values presented are the means of triplicate determinations; expression was normalized using 18S ribosomal RNA. The primer sequences for the real-time PCR measurements were designed by the Roche Universal Probe Library (designed by Probe Finder) or obtained from the indicated references. The rat primer sequences used were: Cyp7α1: 5′-GGAGCTTATTTCAAATGATCAGG-3′ and 5′-CACTCTGTAAAGCTCCACTCACTT-3′; Cyp8β1 (ref. 24): 5′-GGCTGGCTTCCTGAGCTTATT-3′ and 5′-ACTTCCTGAACAGCTCATCGG-3′; and 18S ribosomal RNA (ref. 24): 5′-GTAACCCGTTGAACCCCATT-3′ and 5′-CCATCCAATCGGTAGTAGCG-3′.

Rats were decapitated at the end of 7th feeding day after ICR and serum were collected. The serum 7αC4 was measured using liquid chromatography–tandem mass spectrometry (8) by the Immunochemistry Core Laboratory (Mayo Clinic, Rochester, MN).

Statistical Analyses

All results are expressed as means ± SD. Mean values between the two groups were compared using the Student's *t*-test. Pearson correlation was used to analyze the relationships between two variables. A P value of <0.05 was considered statistically significant.

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