Effect of Bile Acid Composition and Manipulation of Enterohepatic Circulation on Leptin Gene Regulation

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In the rat, the ob gene product, leptin, putatively regulates energy balance via appetite control and energy expenditure. Bile acids in the intestinal lumen are necessary for efficient absorption of dietary lipids and may trigger the release of regulatory peptides. To investigate whether bile acids play a role in leptin gene expression, we altered the bile acid pool and then measured leptin mRNA levels in adipose tissue. Rats fed cholic acid (1% of chow wt/wt) for 2 weeks did not gain weight as rapidly as pair-fed control animals. Despite the lower weight, normalized leptin mRNA levels were 24% greater in cholic acid–fed rats compared with controls. Conversely, cholestyramine, a bile acid sequestrant, in chow (5% wt/wt) resulted in a 26% decline in leptin mRNA. Ligation of the common bile duct or chronic biliary diversion, experimental manipulations that decreased the intestinal concentration of bile salts, decreased leptin gene expression by 30% and 50%, respectively. A fluid and electrolyte (F/E) solution with and without taurocholate (36 μmol/h · 100 g rat^{-1}) was then infused for 12 hours into the duodenum in animals with chronic biliary diversion. Taurocholate infusion resulted in a fourfold increase in steady-state adipocyte leptin mRNA levels compared with F/E infusion. Intravenous infusion of taurocholate or incubation of cultured adipocytes with taurocholate had no effect on leptin mRNA levels. We conclude that bile acids upregulate leptin gene expression indirectly, probably via effects on the absorption of dietary lipids or the release of neurohumoral mediators.
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CIRCULATING LEVELS OF LEPTIN, a hormone produced by the adipocyte ob gene, appear to be regulated by two mechanisms in rodents. First, under conditions of consistent and timely ingestion of food, serum leptin is an index of the amount of triglycerides stored in adipose tissue.[1-8] Second, and independently of the first mechanism, circulating leptin is acutely regulated by perturbations in the normal energy balance such as occur with feeding or fasting.[9-15] In the rat, leptin mRNA levels increase within 3 hours when fasted animals are fed a mixed meal.[10,14] Known regulators of leptin gene expression such as glucocorticoids[9,16] and catecholamines[16,17] have a minimal role in the activation of leptin gene expression in response to meals. On the other hand, insulin[10,18,19] stimulation of the leptin gene has a prominent role in energy balance, but the kinetics of the insulin response on leptin gene expression do not correlate well with the kinetics of the leptin gene response to mixed meals. Therefore, it is likely that other unidentified meal-induced factors acutely stimulate leptin gene expression.

Models suggesting that the gastrointestinal tract plays a direct role in the regulation of energy balance have been proposed.[20] According to these models, intestinal factors are produced in response to nutrient ingestion and absorbed into the bloodstream; once circulating, these factors could affect several energy-regulatory centers in the central nervous system or in the periphery such as in the adipocyte. Manipulations of the intestinal milieu, as by altering bile acid composition or quantity, may influence the production or absorption of these factors and therefore alter the signals for regulating acute energy balance. In the present study, we altered rat bile acid composition and concentration within the enterohepatic circulation and then measured leptin gene expression. We have shown that the bile acid composition and concentration are important for regulating leptin gene expression. Cholic acid feeding and intraduodenal infusion of taurocholic acid increases adipocyte leptin mRNA levels, whereas intestinal reduction of bile acids with cholestyramine, common duct ligation, or biliary fistula reduces leptin mRNA. The effect of bile acids is indirect, and may result from changes in the absorption of intestinal nutrients and/or the release of regulatory peptides that stimulate leptin gene expression.

MATERIALS AND METHODS

Feeding Experiments

Male Sprague-Dawley rats (Charles River Breeding Laboratories, Cambridge, MA) weighing between 160 and 220 g were housed in metabolic cages under controlled lighting conditions on a natural dark-light cycle (6 PM to 6 AM). Groups of age- and weight-matched animals were used in all experiments. Cholestyramine (fed as 5% of diet) and individual bile acids (cholic, 1%; chenodeoxycholic, 1%; and deoxycholic, 0.25%) were added to powdered laboratory chow (Ralston Purina, St Louis, MO; the powdered laboratory chow is identical in content to the standard chow in pellet form). The concentration of deoxycholic acid was chosen to minimize the known hepatotoxicity induced by this bile acid. Each animal was fed 20 g chow at the beginning of each light cycle (8 AM). All of the chow was ingested by the end of the light cycle, and therefore, the animals were fasted during the 12 hours of the dark cycle. Feeding animals during a light cycle when their normal feeding pattern is nocturnal may have had some influence on leptin mRNA measurements, which have been shown to have a diurnal variation. In addition, although the food was provided at a comparable time for both control and experimental animals, no data were collected to identify the rate of consumption, and therefore, some differences in the duration of fasting are still possible. Each animal ingested the entire sample and gained weight daily. After 14 days, after an overnight fast, the epididymal fat pads were extracted for preparation of RNA. Before killing the rats by decapitation, we anesthetized each animal briefly with methoxyflurane to permit blood and bile collection as previously described.[21] The biliary bile acid composition in rats fed different bile acids has been previously published.[21] Blood was drawn

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for measurement of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase levels.

The animal protocol is reviewed and approved annually by the institutional animal care and use committee of the Medical College of Virginia, and is in compliance with the institution's guidelines on the care of laboratory animals as set forth in the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (publication no. 86-23, revised 1985).

Chronic Biliary-Diverted Rat Model

Male Sprague-Dawley rats weighing between 240 and 360 g were housed under controlled lighting conditions in a natural light-dark cycle (6 AM to 6 PM). Under brief methoxyflurane anesthesia, the common bile duct was isolated, a biliary fistula was created, and an intraduodenal feeding tube was placed as previously described.[22] In sham-operated animals, a biliary fistula was not created and a feeding tube was not placed. After surgery, animals were placed in individual metabolic cages with free access to water and laboratory chow (Purina chow 50-01). Animals with a biliary fistula received continuous intraduodenal fluid-electrolyte (F/E) replacement solution (100 Meq/L NaCl, 30 Meq/L Na acetate, 6 Meq/L KCl, and 50 g/L glucose) at a rate of 1 mL/h. Diverted bile was collected continuously in timed increments throughout the course of the experiment. After 72 hours of chronic biliary diversion, taurocholic acid was added at 8 AM to the intraduodenal infusate and infused at a rate of 36 μmol/h · 100 g rat^{-1} for 12 hours. At the conclusion of the experiment, epididymal fat was harvested and total RNA was extracted by a single-step isolation method as described by the supplier (TRIzol LS reagent; Life Technologies, Gaithersburg, MD). It should be noted that animals undergoing biliary diversion were killed at approximately 8 PM, whereas animals in the feeding experiments were killed at approximately 8 AM.

In a set of control experiments, animals were infused with taurocholate or the F/E solution intravenously. Under brief methoxyflurane anesthesia, a jugular vein catheter was placed as previously described.[22] The animals were allowed to recover for 3 days. By the second day, all animals were consuming at least 20 g chow per day. After the third day, food was withdrawn after 6 PM. The next morning (8 AM), the animals were infused with the F/E solution in the absence or presence of taurocholate at a rate of 36 μmol/h · 100 g rat^{-1} for 12 hours. At the conclusion of the experiment (8 PM), epididymal fat was harvested and total RNA extracted as already described.

Biliary Ligation

The animals were anesthetized, and the abdominal cavity was opened. In four animals, the common duct was isolated and ligated with suture. In the other two animals (sham), the common bile duct was isolated but not ligated. The abdominal cavity was then closed, and the animals were housed in cages and fed ad libitum.

Northern Blot Analysis

Total RNA (15 μg) was applied to a 1% agarose/2-mol/L formaldehyde denaturing gel according to standard procedure.[23] After electrophoresis, the RNA was transferred to a nylon membrane (Hybond-N; Amersham, Arlington Heights, IL) by capillary action and fixed by UV light cross-linking. The leptin or cyclophilin (control) cDNA probes were radiolabeled with [^{32}P]-dCTP by random primer extension according to the instructions of the supplier (Boehringer Mannheim, Indianapolis, IN). Hybridization was performed in a solution as described by the supplier. After 24 hours of incubation at 65°C, the filter was washed twice for 5 minutes at 65°C in 2X SSC (20X SSC is 2 mol/L NaCl and 0.3 mol/L sodium citrate, pH 7.0) and 0.1% sodium dodecyl sulfate (SDS), and subsequently one time in 1X SSC and 0.1% SDS for 15 minutes at 65°C. The filter was exposed to a phosphorimager (Phosphorimager SF; Molecular Dynamics, Sunnyvale, CA) cassette overnight, and photons from the appropriate bands were measured. Cyclophilin gene expression was a good control: cyclophilin mRNA levels did not vary between any of the experimental feeding groups and controls or between enterohepatic circulation manipulations and controls.

Two cDNA probes were synthesized from rat adipocyte RNA by reverse transcriptase–polymerase chain reaction (RT-PCR). First-strand cDNA synthesis of total RNA was catalyzed by Superscript II as described by the supplier (Life Technologies). The cDNA was PCR-amplified with the following sense and antisense primers for each probe: leptin gene,[24] sense CCTATCCACAAAGTCCAGGA and antisense ATGTCCTGCAGAGAGCCCTG; rat ileal sodium-dependent bile acid transporter,[25] sense CAGTTTGGAATCATGCCTC and antisense AAGGGGCATCATTCCAAG. The PCR was "hot-started," and PCR parameters were as follows: denaturation at 95°C for 30 seconds, annealing at 55°C for 40 seconds, and extension at 72°C for 45 seconds for a total of 35 cycles.

Adipocyte Isolation and Culture

Isolated adipocytes were obtained from the epididymal fat pads of approximately five to six male Sprague-Dawley rats (160 to 220 g). The fat pads were minced and incubated with 4 mg/mL collagenase as described by Rodbell[26] and Marshall.[27] After filtering through a mesh and washing, the cells were pooled, divided into equal aliquots, and incubated in Dulbecco's modified Eagle's medium, 0.5% fetal bovine serum with and without 10^{-7} mol/L dexamethasone (positive control), or 1, 10, or 50 μmol/L taurocholic acid at 37°C and 5% CO_2 for 4 hours.

RESULTS

Every animal fully ingested 20 g chow per day in the absence or presence of cholestyramine and bile acids. Therefore, caloric intake was equal among treatment and control groups and every animal gained weight. The mean weight gain and final weight of rats fed chow plus cholic acid, deoxycholic acid, ursodeoxycholic acid, or cholestyramine (treatment groups) were lower than in rats fed chow alone (controls) (Table 1). This finding in the

Table 1. Effect of Feeding Bile Acids and Cholestyramine to Rats on Leptin mRNA and Body Weight

Treatment	Leptin mRNA (arbitrary units)	Body Weight (g)		Weight Gain
		Initial	Final	
Cholate (n = 8)	1.24 ± 0.11*	194 ± 8	256 ± 5	62 ± 4
Control (n = 9)	1.0 ± 0.04	193 ± 8	267 ± 3	73 ± 5
Deoxycholate (n = 9)	0.84 ± 0.12	204 ± 6	263 ± 4	59 ± 4
Control (n = 9)	1.0 ± 0.03	193 ± 8	267 ± 3	73 ± 5*
Ursodeoxycholate (n = 4)	1.02 ± 0.04	167 ± 3	240 ± 1.5	72 ± 3
Control (n = 3)	1.0 ± 0.05	165 ± 1	258 ± 3†	93 ± 4†
Cholestyramine (n = 9)	0.74 ± 0.08†	199 ± 6	252 ± 4	53 ± 4
Control (n = 9)	1.0 ± 0.02	193 ± 8	267 ± 3†	73 ± 5†

NOTE. Rats were weighed (mean ± SEM) and then fed chow without (control) or with the bile acids or cholestyramine at the following concentrations (wt/wt): cholate 1%, deoxycholate 0.25%, ursodeoxycholate 1%, and cholestyramine 5%. After 2 weeks, the final weight and weight gain were determined. The animals were killed and total RNA was extracted from the epididymal fat pads. Northern blot analysis was performed with the leptin and cyclophilin cDNA probes, and the bands were quantified by phosphorimaging. Leptin mRNA was normalized to cyclophilin mRNA and then expressed as a fraction of the mean for the control animals (mean ± SEM).

*$P < .05$.

†$P < .01$, treatment *v* control (Student's *t* test).

presence of equal caloric intake suggests that animals in the treatment group expended more energy. No diarrhea was observed in any treatment group. Serum levels of transaminases and alkaline phosphatase in rats fed chow containing cholic acid, ursodeoxycholic acid, or cholestyramine did not differ significantly from the levels in rats fed regular chow (data not shown). Feeding deoxycholic acid caused a modest but significant increase in transaminases, indicative of mild hepatotoxicity, but serum bilirubin and albumin were unchanged.

We have previously demonstrated[21] that the predominant bile acids in chow-fed control rats are cholic acid (29%) and α- and β-muricholic acids (58%). Cholic acid feeding results in a predominance of cholic acid (74.3%) within the bile acid pool. Deoxycholic acid and ursodeoxycholic acid feeding results in a fractional increase in the deoxycholic acid and ursodeoxycholic acid pools and a proportionate decrease in the muricholic acid pool. The effect of cholic acid feeding on leptin gene expression is shown in Fig 1. Ten to 40 μg total RNA from a control rat was applied to the gels (lanes 1 to 3) and compared with an identical quantity of RNA from a rat fed cholic acid (lanes 4 to 6). After transfer, nylon filters probed with the constitutively active cyclophilin cDNA showed a similar intensity of the bands between regular and cholic acid–fed rats at equal RNA concentrations. In contrast, leptin gene expression was significantly higher in cholic acid–fed animals at each RNA concentration. The effect of feeding cholic acid, other bile acids, and cholestyramine on leptin gene expression is shown in Table 1. Normalized to cyclophilin, leptin mRNA levels increased 24% ($P = .048$) in animals fed chow plus cholic acid compared with animals fed chow alone. The increase in leptin gene expression with cholic acid feeding occurred in animals that weighed less than the controls, although this did not reach statistical significance ($P = .07$). There were no significant differences in leptin gene expression in animals fed deoxycholic acid or ursodeoxycholic acid. Rats fed chow containing the bile acid binding resin, cholestyramine, had a 26% decrease in leptin gene expression ($P < .01$).

In an additional series of studies, we reduced intestinal bile acids by ligating the common bile duct or by creating a chronic bile acid fistula. Before the common duct ligation, the mean ± SEM weight of experimental and sham-operated animals was 216 ± 8 and 211 ± 6 g, respectively. After 4 days of common duct ligation, the experimental animals (n = 4) did not gain as much weight as sham-operated animals (n = 2). The mean final weight of the rats with common bile duct ligation was 217 ± 23 g, compared with 239 ± 13 g for the sham-operated rats. As anticipated, common bile duct ligation resulted in an elevation of liver enzymes, with a 2.3- and 4.8-fold increase in liver ALT and AST. Common bile duct ligation led to a significant decline in adipocyte leptin mRNA compared with the levels in sham-operated animals (Fig 2). Densitometric analysis by phosphorimaging demonstrated that leptin mRNA levels were 30% lower in animals with a ligated common bile duct compared with sham-operated animals ($P = .0066$). A second experimental protocol for interruption of the enterohepatic circulation was performed. The animals were sham-operated or a chronic biliary fistula was created as described in the Methods. Before surgery, the starting weight of animals with a bile acid fistula

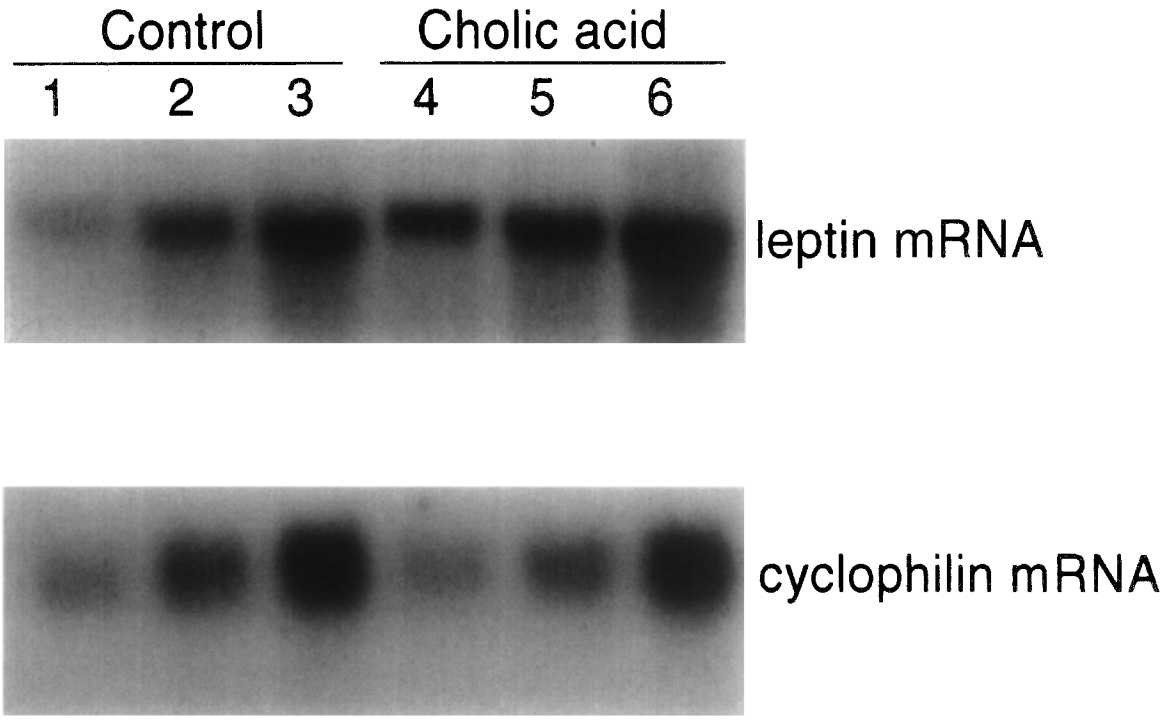

Fig 1. Effect of ingestion of cholic acid on leptin gene expression. Animals were fed a total of 20 g chow without (control) or with 1% (wt/wt) cholic acid. At the end of 2 weeks, the animals were killed and epididymal fat pads were removed and immediately frozen in liquid nitrogen. Total RNA was extracted, and 10 μg (lanes 1 and 4), 20 μg (lanes 2 and 5), and 40 μg (lanes 3 and 6) total RNA were applied to a gel. After electrophoresis and transfer to a nylon filter, the RNA was hybridized to the radiolabeled cDNA for the leptin gene or the housekeeping gene, cyclophilin.

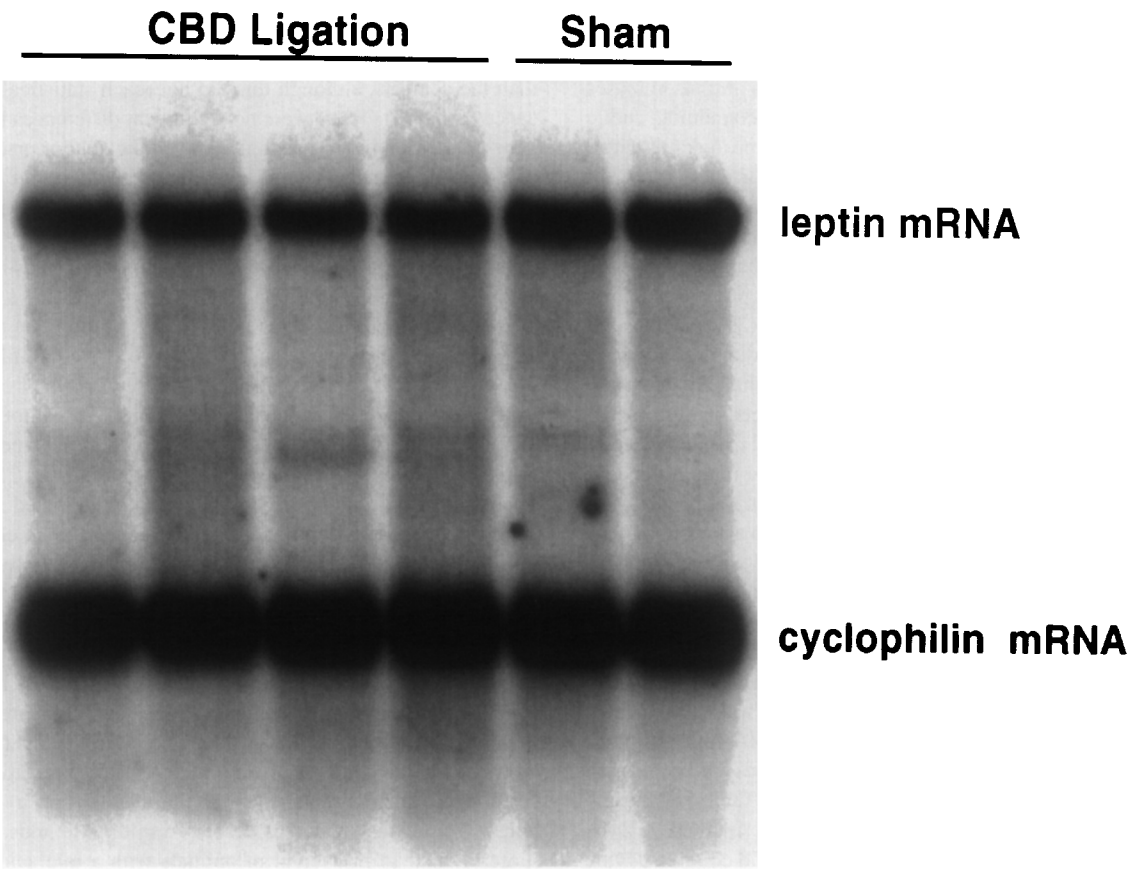

Fig 2. Effect of common bile duct (CBD) ligation on leptin gene expression. After the abdominal cavity was opened, CBD ligation was performed. After 4 days, animals were killed and epididymal fat pads removed. Northern analysis was performed with 15 μg total RNA extracted from 4 CBD animals and compared with similar quantities of RNA extracted from 2 sham-operated animals.

versus sham-operated animals was 271.3 ± 6.7 versus 258.6 ± 8.5 g, respectively. The animals with a chronic bile fistula ingested approximately 5 g/d less chow and tended to lose more weight than the sham-operated animals (a 12-g weight loss *v* a 9-g weight gain, respectively); however, the final weight of sham-operated versus bile-fistula animals was not statistically different. After 3 days, a F/E solution was infused with or without taurocholic acid into the duodenum for 12 hours. There were no differences in either the weight loss or the final weight of animals with a bile acid fistula who were infused with or without taurocholic acid. Cyclophilin mRNA levels were relatively constant in all groups. However, a 50% decline in leptin mRNA levels was observed in animals with a chronic biliary fistula (infused intraduodenally with F/E) compared with sham-operated animals (Fig 3). When the animals with a chronic biliary fistula were infused with taurocholic acid into the duodenum for 12 hours, leptin gene expression increased approximately fourfold compared with the level in animals infused with F/E alone ($P < .05$).

Since cholic acid feeding and intraduodenal taurocholate infusion increased leptin mRNA levels while intestinal reduction of bile acids with cholestyramine, common duct ligation, or biliary fistula reduced leptin gene expression, we concluded that bile acids within the enterohepatic circulation play a role in modulating the adipocyte leptin gene. To determine whether bile acids directly influence adipocyte leptin gene expression, three additional groups of experiments were performed. Firstly, we examined whether a bile acid transporter previously described in the ileum[25] is present in rat adipocytes. Total RNA from adipocytes was reverse-transcribed, and the cDNA was amplified with gene-specific primers for the rat ileal bile acid transporter. One band of the predicted size of the transporter was found on an ethidium bromide gel. The amplified product was then sequenced and found to be identical to the rat ileal bile acid transporter. However, a radiolabeled cDNA probe of the rat ileal bile acid transporter failed to hybridize to either 40 μg adipocyte total RNA or 10 μg adipocyte mRNA (data not shown). The ileal bile acid transporter appears to be present in adipocytes, but with a very low copy number. Secondly, after creating a chronic bile fistula, we infused taurocholate intravenously (jugular vein, n = 4) for 12 hours and measured leptin gene expression. Compared with sham-operated animals (n = 3) or animals infused with F/E solution (n = 3), no difference in leptin gene expression could be detected (data not shown). Finally, we incubated various concentrations of taurocholate (1 to 50 μmol/L) or 10^{-7} mol/L dexamethasone (as a positive control) with isolated rat adipocytes in primary culture. Dexamethasone increased leptin gene expression by approximately 300%, as previously shown.[28-30] In contrast, all concentrations of taurocholate had no effect on leptin gene expression.

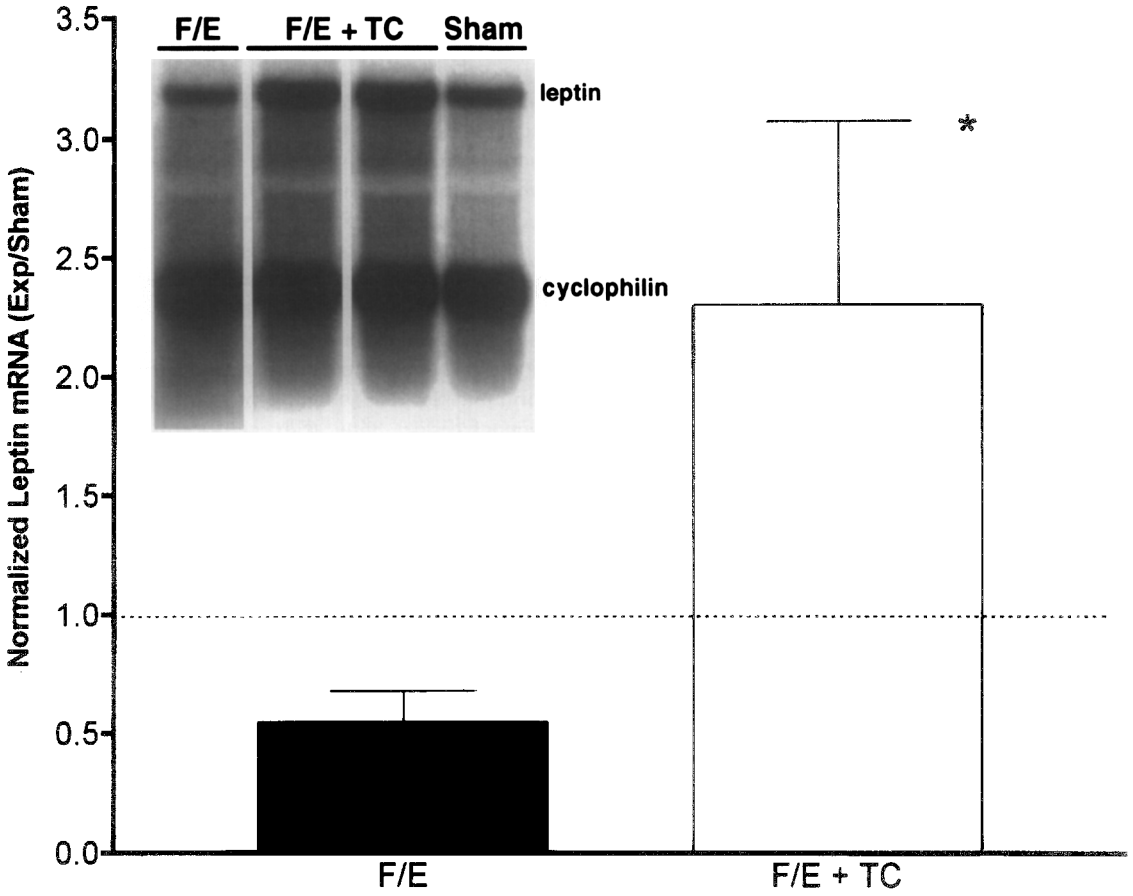

Fig 3. Effect of chronic biliary fistula and intraduodenal infusion of taurocholate on leptin gene expression. Seventy-two hours after creation of the biliary fistula and placement of the intraduodenal feeding tube, the feeding tube was infused at 8 AM with a F/E solution without (F/E) or with (F/E + TC) taurocholate. After 12 hours, the animals were killed, epididymal fat pads were removed, and Northern blot analysis was performed with 15 μg total RNA per lane. Insert, a representative Northern blot analysis. Quantitation of normalized leptin mRNA by phosphorimaging was performed in a total of 3 experiments. Each experiment included 1 sham-operated animal, 1 biliary-diversion animal that received F/E, and 2 biliary-diversion animals that received F/E plus bile salt. Each data point represents the mean ± SEM of normalized leptin mRNA in the experimental animals divided by the sham-operated animals (. . . . .). *$P < .05$, F/E + TC *v* F/E alone by Student's *t* test.

DISCUSSION

In addition to being a long-term, adiposity-related signal, a growing body of evidence in rodents suggests that leptin is acutely regulated and may function as a satiety signal.[31] In free-feeding mice, an apparent diurnal rhythm in leptin mRNA levels is the direct result of short-term feeding behavior and is not observed in food-restricted animals.[10] In rats, leptin gene expression increases approximately threefold after a meal and declines with a mRNA half-life of approximately 2 hours.[11] Several other studies have demonstrated acute effects on leptin gene expression with fasting and refeeding.[9,12-15] The mechanism of the increase in meal-induced leptin gene expression is incompletely characterized. It is known that with nutrient ingestion numerous gastroenteropancreatic hormones that coordinate digestive functions are secreted into the bloodstream; a subset of these hormones have been shown to serve as feedback regulators of meal size.[20] Bile acids in the intestinal lumen are necessary for efficient absorption of dietary lipids and may trigger the release of regulatory peptides. Therefore, in the present study, we investigated whether changes in bile acid composition or quantity within the enterohepatic circulation have an influence on leptin gene expression, a putative mediator of satiety.

We have shown that in the rat, the bile acid composition and concentration within the enterohepatic circulation regulates the adipocyte leptin gene expression. Feeding animals cholic acid for 2 weeks increased the steady-state concentration of cholic acid and the hydrophobicity of bile acids within the enterohepatic circulation; this change in the bile salt milieu resulted in increased leptin mRNA levels. The increase in leptin appeared specific: ingestion of ursodeoxycholic or deoxycholic acids had no demonstrable effect on leptin gene expression. Although the increases in leptin mRNA with cholic acid feeding were relatively modest, they occurred despite a reduced weight gain in bile acid–fed animals. The observed inverse correlation between body weight and leptin gene expression appears paradoxical, since the preponderance of studies to date have demonstrated a direct correlation between body weight and leptin gene expression.[31-33] However, it has recently been demonstrated that leptin has weight-reducing effects at physiologic levels.[34] Therefore, the long-term ingestion of bile acids

to increase leptin gene expression actually may have resulted in weight reduction in the bile acid–fed animals. Since animals were fed chow with and without bile acids at 8 AM, it is possible that the animals in this study had a differing duration of food restriction before fat harvesting and assessment of leptin gene expression; this may have influenced the results if one surmises that the control animals finished their ration of chow without bile salts before the animals that were fed chow with bile salts. Although not strictly measured, we did not observe gross differences in the rate of chow consumption. In addition, feeding animals bile salts other than cholic acid did not affect leptin gene expression. Finally, the cholic acid–mediated increase in leptin gene expression is supported by other experiments. Infusion of taurocholate into the duodenum depleted of bile acids by a chronic biliary fistula acutely increased leptin gene expression. In animals with a fistula, there were no differences in the weight of rats infused with either taurocholate or a F/E solution.

Reduction of intestinal bile acids by several methodologies decreased leptin gene expression. Feeding animals cholestyramine in chow resulted in a 25% decline in leptin mRNA levels. Ligation of the common bile duct or diversion of bile flow with a chronic biliary fistula decreased leptin expression by 30% and 50%, respectively. However, in these examples, the weight gain was less in the experimental group than in the respective controls, and it is difficult to quantify how much the loss of body fat contributed to the decrease in leptin mRNA levels. We believe that at least part of the decrease of leptin gene expression with reduced intestinal bile acids is independent of lower weight. This conclusion is based on a couple of observations. Both cholestyramine- and bile acid–fed animals had a similarly reduced weight at the end of the 2-week feeding period compared with animals fed chow alone, yet only cholestyramine-fed animals had a significant decrease in leptin mRNA levels. Short-term restoration of bile acids into the intestinal lumen in animals with a bile acid fistula increased leptin mRNA levels without a change in body weight. In all of the studies we performed, a consistent pattern emerged—bile acid feeding and infusion into the intestine increased leptin mRNA levels, while intestinal reduction of bile acids reduced leptin gene expression.

The lack of effect of bile acids added to cultured adipocytes in these studies indicates that bile acids modulate leptin gene expression indirectly. The mechanism appears to depend on the concentration of bile acids in the intestine, since external biliary drainage (which depletes circulating bile acids) and bile duct ligation (which causes bile acids to accumulate in the blood) both reduced leptin gene expression while eliminating bile acids from the intestine. Moreover, in bile fistula rats, replacement of taurocholate by intravenous infusion did not alter leptin mRNA, whereas intraduodenal taurocholate infusion led to a marked increase in leptin mRNA relative to other adipose tissue genes.

The indirect effects of intestinal bile acids on leptin gene expression could result from altered nutrient flux. Bile acids are detergents whose presence in the intestinal lumen is critical for fat digestion and absorption. Bile acids stabilize lipid emulsions, act as cofactors for pancreatic triglyceride lipase, and form micelles that solubilize products of fat digestion such as monoglycerides, fatty acids, cholesterol, and fat-soluble vitamins.[35] Exclusion of bile acids from the intestine leads to near-complete interruption of cholesterol absorption[36,37] and moderate steatorrhea.[38] In the current studies, the daily chow consumption was uniform and the weight gain in the different treatment groups differed only minimally. Thus, differences in gross caloric absorption cannot explain the differences in leptin gene expression with the different diets. However, more subtle effects related to variation in the absorption of specific nutrients cannot be excluded.

Alternatively, intestinal bile acids may alter adipose tissue gene expression through effects on neuroendocrine mediators. The precedent for this hypothesis comes from Pandak et al,[39] who found in bile fistula rats that the ability of taurocholate to downregulate hepatic bile acid synthesis was dependent on its being administered intraintestinally—intravenous infusions were ineffective. These studies suggested that a neuroendocrine signal from the intestine triggered by the presence of luminal bile acids could play a role in mediating the negative-feedback regulation of hepatic bile acid synthesis at the level of the liver. Luminal bile acids in the gastrointestinal tract are known to inhibit cholecystokinin release in the duodenum,[40,41] but they stimulate the release of neurotensin in the jejunum and ileum[42] and peptide YY and enteroglucagon in the colon.[43,44] The effects of these and other digestive hormones on expression of the leptin gene in adipose tissue remain to be explored.

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