ELSEVIER

Effect of Δ^{22}-5β-taurocholenic acid and dietary fat on hepatic cholesterol and fatty acid in hyperglycemic-obese mice

G. Richard Jansen

Department of Food Science and Human Nutrition, Colorado State University, Fort Collins, CO USA

*Previous work demonstrated that hyperglycemic obese mice (*obob*) fed a 1% corn oil diet accumulated cholesterol and fat in the liver when compared with nonobese littermates. The present experiment was carried out to test the hypothesis that feeding the bile acid analog Δ^{22}-5β-taurocholenic acid would correct this metabolic defect. Liver total fatty acid was greatly elevated in* obob *mice fed 1% corn oil compared with nonobese controls (21.04 ± 1.23% versus 4.41 ± 0.27%). Incorporation of [U-^{14}C]glucose into liver fatty acid was inhibited by taurocholenic acid in both obese and nonobese mice fed 1% corn oil. Taurocholenic acid reduced liver fatty acid in* obob *mice fed 1% corn oil to the level seen in nonobese mice (3.07 ± 0.22% versus 4.41 ± 0.27%). Cholesterol greatly accumulated in the livers of* obob *mice fed 1% corn oil compared with nonobese mice (0.97 ± 0.08% versus 0.28 ± 0.02%) and was reduced to normal by feeding taurocholenic acid (0.27 ± 0.05% versus 0.28 ± 0.01%) despite greatly elevating rates of incorporation of [U-^{14}C]glucose into liver cholesterol. Taurocholenic acid significantly reduced liver fat in obese mice fed 40% lard (11.62 ± 1.41% versus 5.87 ± 1.03%) but had no significant effect on the percentage of liver cholesterol (0.68 ± 0.05% versus 0.56 ± 0.04%). It is suggested that the elevation of liver cholesterol in* obob *mice fed 1% corn oil comes about through a defect in cholesterol removal from the liver, a defect corrected by taurocholenic acid via increasing the excretion of cholesterol and bile acids in the bile by an as yet unknown mechanism. The cholesterol accumulating in* obob *mice fed 40% lard occurs by a different mechanism than when these mice are fed 1% corn oil since it is unaffected by taurocholenic acid.* (J. Nutr. Biochem. 7:106–112, 1996.)

Keywords: obesity; cholesterol; bile acids; taurocholenic acid; linoleic acid; mice

Introduction

Considerable work has been carried out in order to understand the obesity that develops in the hyperglycemic obese (*obob*) mouse that was described over 45 years ago by Ingalls et al.[1] The mutant gene is designated *ob* and is an autosomal Mendelian recessive. Metabolic abnormalities in this animal and in other experimental obesities have been well-summarized by Bray and associates.[2–4] More recently, genetic aspects of this and other animal models of obesity have been explored by Johnson et al.[5] In this recent review, specific candidates for the primary genetic lesion related to central nervous system or peripheral tissue abnormalities were assessed. The evidence did not allow any firm conclusions to be drawn, nor was it possible to propose a unitarian hypothesis that would explain all the metabolic and other abnormalities that have been observed.

Johnson and colleagues listed over 20 metabolic abnormalities that have been observed in the hyperglycemic obese mouse. However, abnormalities in cholesterol metabolism are not mentioned in this review. In previously published work, we described an abnormality in cholesterol metabolism in the livers of these mice that would appear to be at least closely associated with the *ob* gene when expressed in C57BL/6J mice.[6] We reported that when 7-week-old *obob* mice were fed a glucose-based diet containing 1%

Present address for Dr. Jansen is Department of Food Science and Human Nutrition, Colorado State University, Fort Collins, CO 80523 USA.
Address reprint requests to Dr. G. Richard Jansen at the Department Food Science and Human Nutrition, Colorado State University, Fort Collins, CO 80523 USA.
Received March 13, 1995; accepted September 13, 1995.

655 Avenue of the Americas, New York, NY 10010

0955-2863/96/$15.00
SSDI 0955-2863(95)00184-0

corn oil for 12 weeks, they accumulated 1.5% cholesterol in their livers compared with 0.29% in the livers of nonobese littermates. The defect was substantially if not completely reversed by feeding 20% corn oil. Cholesterol synthesis from [U-^{14}C]glucose was half as high in obese as in nonobese mice when a 250 mg meal of [U-^{14}C]glucose was given by stomach tube. However, if the [U-^{14}C]glucose were added to the diet and fed over a 24 hr period, there was a 10-fold increase in the accumulation of labeled cholesterol in the liver. This suggests the possibility that hyperglycemic obese mice fed a 1% corn oil diet adequate in essential fatty acids for their nonobese littermates have difficulty removing cholesterol from the liver rather than exhibiting increased rates of cholesterol synthesis.

In addition to elevated levels of liver cholesterol, hyperglycemic obese mice fed the 1% corn oil diet exhibited a fatty liver (22.7% fatty acid) not observed in the nonobese littermates fed the same diet (4.81%).[6] The fatty liver was not prevented by feeding a purified diet containing 20% corn oil although it was when Purina Chow was fed.[6] We previously have reported that feeding 0.1% 5β-cholanic acid in the diet to mice from the Merck Sharp and Dohme colony reduced the percentage of cholesterol and fat in the liver and reduced fatty acid synthesis from [U-^{14}C]glucose in the liver.[7] In contrast, incorporation of [U-^{14}C]glucose into liver cholesterol was greatly elevated.[7] Because of these observations, we felt it would be of considerable interest to evaluate the effect of Δ^{22}-5β-taurocholenic acid,[8] a more potent analog of 5β-cholanic acid, on liver cholesterol and fatty acid in hyperglycemic obese and their nonobese littermates fed diets containing low or high amounts of fat. The results form the basis for this report.

Methods and materials

Adult male hyperglycemic obese mice and their male nonobese littermates (C57BL/6J) were purchased from the Jackson Memorial Laboratory (Bar Harbor, ME USA). Obese and nonobese littermates were maintained on Purina Laboratory Chow (Ralston Purina, St. Louis, MO USA) approximately 4 weeks then fed the experimental diets to be described. The hyperglycemic obese mice and nonobese littermates were weighed, blocked by weight into groups of equivalent body weight within their obesity status (eight/group), and assigned to diets 1 to 4 (*Table 1*). Diets were glucose-based and contained either 1% corn oil or 40% lard. One diet at each fat level contained in addition 0.05% potassium Δ^{22}-5β-taurocholenate (TC). Protein was fed at the same percentage of calories in all diets, and the high fat diets were therefore low in carbohydrate. After being fed the experimental diets for 6 weeks between 8:00 and 10:00 a.m., the mice were weighed and then given 250 mg of D-glucose including 2.5 μCi of D-[U-^{14}C]glucose by stomach tube (0.5 mL) and killed in 2 hr by cervical dislocation. At this time livers were quickly excised, immediately frozen, and stored in a deep freeze. The numbers of mice completing the experiment are listed in *Table 2*. The completed group size was seven to eight for the nonobese groups. Group sizes for the hyperglycemic obese mice were five to seven due to several unexplained deaths or extreme losses of body weight especially on the 1% corn oil diet during the 6-week feeding period. The techniques used in preparing and analyzing tissues for radioactivity have been described previously.[9,10] D-[U-^{14}C]glucose was obtained from New England Nuclear Corporation, (Boston, MA USA) and had a specific activity of 10 to 15 mCi/mmol. Liver and carcass fat were measured as total fatty acid extractable after saponification and cholesterol was isolated and counted as the digitonin precipitable sterol.[9,10] Results are presented as means ± standard error (SE), and data were analyzed by a three-way analysis of variance (ANOVA) with the main effects being obesity, fat level, and drug treatment using SPSS.[11]

Table 1 Composition of experimental diets

Ingredients	Composition (g/100 g of diet)			
	Diet 1	Diet 2	Diet 3	Diet 4
Casein (Labco)	20.0	20.0	31.0	31.0
Salts no. 2 USP XIII	4.0	4.0	4.0	4.0
Glucose	70.0	70.0	20.0	20.0
Celluflour	5.0	5.0	5.0	5.0
Corn oil	1.0	1.0	—	—
Lard	—	—	40.0	40.0
Potassium Δ^{22} Taurocholenate	—	0.05	—	0.05

Casein and Celluflour were obtained from the Borden Company (New York, NY, USA) and the Chicago Dietetic Supply House (Chicago, IL, USA). respectively. Glucose (Cerelose) and potassium Δ^{22} taurocholenate were obtained from Merck and Company, Inc. (Rahway, NJ, USA). The corn oil and lard were obtained locally. In addition, vitamins were added to all diets to supply the following nutrients per 100 g of diet: thiamin, 1.0 mg; riboflavin, 2.0 mg; pyridoxin, 1.0 mg; calcium pantothenate, 10.0 mg; niacinamide, 10.0 mg; inositol, 5.0 mg; choline, 100.0 mg; p-aminobenzoic acid, 30.0 mg; biotin, 0.05 mg; folic acid, 0.2 mg; α-tocopherol, 14.2 mg; menadione, 14.1 mg; B_{12} triturate (0.1% trituration with mannitol), 10.0 mg; ergocalciferol, 300 IU; vitamin A palmitate, 1,600 IU.

Results

Weight gain and liver weights are listed in *Table 2*. Hyperglycemic obese mice exhibited considerable liver hypertrophy when compared with their nonobese littermates. As shown by the significant interaction, taurocholenic acid reduced liver weight in hyperglycemic obese mice fed either fat level but not in their nonobese littermates. Weight gain was significantly less in obese mice fed 1% corn oil than in nonobese mice fed this diet. The reverse was true when the 40% lard diet was fed. Taurocholenic acid significantly decreased weight gain across diets and obesity status. The increased weight gain in response to dietary fat was significantly greater in obese than in nonobese mice as shown by the significance of the interaction.

Table 3 presents data showing the concentration of liver cholesterol and the incorporation of [U-^{14}C]glucose into cholesterol as affected by obesity, dietary fat, and taurocholenic acid. These results confirm and extend our previous findings.[6] The concentration of cholesterol in the liver was significantly elevated in *obob* mice fed either diet compared with the nonobese littermates. This was strikingly the case when mice were fed the 1% corn oil diet (obese mice 0.97%, nonobese mice 0.28%). When expressed per liver, the livers of *obob* mice fed 1% corn oil contained 51.7 mg of cholesterol compared with 5.0 mg in the nonobese, which is a 10 fold increase. It should be noted again that this accumulation occurred without feeding either cholesterol or bile acids. The effect of taurocholenic acid was most dra-

Table 2 Initial weight, weight gain, and liver size in hyperglycemic obese mice and nonobese littermates*

Taurocholenic acid	Obese		Nonobese	
	–	+	–	+
	Diet–1% corn oil			
n	5	5	7	8
Initial body wt (g)†	52.1 ± 1.9	51.6 ± 2.2	26.6 ± 0.6	26.7 ± 0.6
Weight gain (g)	–0.6 ± 1.4	–3.4 ± 1.3	4.0 ± 0.8	–1.8 ± 0.8
Liver weight (g)	5.33 ± 0.40	4.04 ± 0.28	1.80 ± 0.07	2.14 ± 0.16
	Diet–40% lard			
n	6	7	7	8
Initial body wt (g)	51.2 ± 1.5	52.7 ± 2.4	26.4 ± 0.6	26.6 ± 1.0
Weight gain (g)	11.1 ± 1.2	5.8 ± 2.2	6.8 ± 0.7	3.5 ± 0.2
Liver weight (g)	4.66 ± 0.42	3.86 ± 0.26	1.50 ± 0.04	2.18 ± 0.10

	Significant differences between treatments (*P*)						
	Main Effects			Interactions			
	Obesity (O)	Fat (F)	TC‡	O × F	O × TC	F × TC	O × F × TC
Initial body wt	0.000	0.965	0.689	0.881	0.843	0.661	0.623
Weight gain	0.684	0.000	0.000	0.000	0.843	0.814	0.138
Liver weight	0.000	0.097	0.409	0.350	0.000	0.202	0.804

*Results expressed as mean ± SE.
†Initial weights given are for the mice who completed the experiment.
‡Taurocholenic acid.

matic when fed to *obob* mice consuming the 1% corn oil diet. The large increase in liver cholesterol seen in these animals was totally prevented by taurocholenic acid (0.27% versus 0.28%) while having no significant effect on liver cholesterol in nonobese littermates. In contrast, taurocholenic acid had no significant effect on the concentration of cholesterol in the livers of *obob* mice fed the 40% lard diet and increased the liver cholesterol in nonobese littermates fed this diet. Taurocholenic acid greatly increased incorporation of [U-^{14}C]glucose into the liver cholesterol in hyperglycemic obese and nonobese mice fed either diet.

Total liver fatty acid was affected significantly by obesity, dietary fat, and taurocholenic acid, and all interactions were significant (*Table 4*). Liver fat was greatly elevated in *obob* mice fed 1% corn oil compared with nonobese littermates (21.04% versus 4.41%) but was completely reduced to the level seen in the nonobese littermates by taurocholenic acid (3.07% versus 4.41%). Liver fat in *obob* mice

Table 3 Incorporation of [U-^{14}C]glucose into liver cholesterol in hyperglycemic obese mice and nonobese littermates*

Taurocholenic acid	Obese		Nonobese	
	–	+	–	+
	Diet–1% corn oil			
% liver cholesterol	0.97 ± 0.08	0.27 ± 0.05	0.28 ± 0.02	0.28 ± 0.01
cpm/liver	158 ± 51	607 ± 192	396 ± 114	1,478 ± 325
cpm/g of liver	28 ± 8	160 ± 53	224 ± 66	714 ± 146
	Diet–40% lard			
% liver cholesterol	0.68 ± 0.05	0.56 ± 0.04	0.23 ± 0.01	0.34 ± 0.01
cpm/liver	48 ± 22	812 ± 278	278 ± 36	1,164 ± 268
cpm/g of liver	13 ± 8	247 ± 100	183 ± 26	521 ± 117

	Significant differences between treatments						
	Main effects			Interactions			
	Obesity (O)	Fat (F)	TC†	O × F	O × TC	F × TC	O × F × TC
% Cholesterol	0.000	0.928	0.000	0.988	0.000	0.000	0.000
cpm/liver	0.011	0.498	0.000	0.389	0.265	0.939	0.427
cpm/g of liver	0.000	0.414	0.000	0.245	0.109	0.753	0.353

*Results expressed as means ± SE. Cholesterol isolated and counted as the digitonide.
†Taurocholenic acid.

Table 4 Incorporation of [U^{14}C]glucose into liver fatty acid in hyperglycemic obese mice and nonobese littermates*

Taurocholenic acid	Obese		Nonobese	
	–	+	–	+
	Diet–1% corn oil			
% liver fat	21.04 ± 1.23	3.07 ± 0.22	4.41 ± 0.27	3.53 ± 0.24
cpm/liver†	63.90 ± 29.16	26.80 ± 4.90	29.92 ± 4.70	16.02 ± 1.59
cpm/g of liver†	10.98 ± 4.39	6.62 ± 1.25	16.62 ± 2.35	7.58 ± 0.79
	Diet–40% lard			
% liver fat	11.62 ± 1.41	5.87 ± 1.03	3.96 ± 0.22	3.69 ± 0.14
cpm/liver†	7.30 ± 1.72	3.73 ± 0.51	2.70 ± 0.34	3.14 ± 0.51
cpm/g of liver†	1.52 ± 0.39	1.03 ± 0.20	1.79 ± 0.21	1.41 ± 0.21

	Significant differences between treatments						
	Main effects			Interactions			
	Obesity (O)	Fat (F)	TC†	O × F	O × TC	F × TC	O × F × TC
% liver fat	0.000	0.004	0.000	0.003	0.000	0.000	0.000
cpm/liver	0.036	0.000	0.036	0.083	0.248	0.046	0.390
cpm/g of liver	0.148	0.000	0.002	0.196	0.347	0.004	0.287

*Results expressed as means ± SE. Liver fat measured as total fatty acid after saponification.
†cpm × 10^3.
‡Taurocholenic acid.

fed the 40% lard diet was also elevated compared with the nonobese littermates (11.62% versus 3.96%) and was significantly lowered by taurocholenic acid but to a lesser extent than in *obob* mice fed 1% corn oil. Neither dietary fat nor taurocholenic acid significantly affected liver fat in the nonobese littermates. Incorporation of [U-^{14}C]glucose into liver fatty acid was not significantly different between obese and nonobese mice when expressed per gram of liver, although, because of liver hypertrophy, when expressed per liver it was doubled in the *obob* mice. Taurocholenic acid significantly reduced incorporation of [U-^{14}C]glucose into the liver fatty acid in obese and nonobese mice fed 1% corn oil but not when they were fed 40% lard. Consistent with the literature,[12,13] feeding dietary fat inhibited incorporation of the label into fatty acid in both obese and nonobese mice.

Effect of taurocholenic acid on body weight and body fat

Taurocholenic acid significantly reduced body weight and body fat for obese mice and their controls whether the diet fed contained 1% corn oil or 40% lard (*Table 5*). The percentage reductions in body fat were considerably greater than the reductions in body weight for nonobese mice on either diet but this was not the case for the *obob* mice. In contrast was the effect of the drug in the *obob* mice where, on either diet, the reductions in body weight and fat were closely comparable. It would be tempting to ascribe reductions in body fat to the effect of taurocholenic acid on fatty acid synthesis in the liver. However, this is unlikely to be the case since the reductions in body fat were as great when

Table 5 Effect of taurocholenic acid on final body weight and body fat*

Taurocholenic acid (TC)	Final body wt (g)		Body fat (%)	
	–	+	–	+
	Diet–1% corn oil			
Hyperglycemic obese	51.4 ± 2.1	48.2 ± 1.6	48.3 ± 1.1	44.8 ± 0.4
TC effect (% reduction)		6.2		7.2
Nonobese littermates	30.5 ± 2.0	24.9 ± 1.0	14.4 ± 1.3	10.0 ± 0.7
TC effect (% reduction)		18.4		30.6
	Diet–40% lard			
Hyperglycemic obese	62.3 ± 2.3	58.6 ± 2.2	50.1 ± 1.2	47.1 ± 0.6
TC effect (% reduction)		5.9		6.0
Nonobese littermates	33.2 ± 1.0	30.1 ± 1.0	20.1 ± 1.4	13.1 ± 0.9
TC effect (% reduction)		9.3		34.8

*Results expressed as means ± SE. Body fat measured as total fatty acid in the carcass following saponification.

40% lard was fed compared with 1% corn oil and under conditions where very little fatty acid was being synthesized in the liver and where taurocholenic acid had no effect on hepatic lipogenesis.

Discussion

An acknowledged potential problem associated with the use of glucose to measure lipogenesis is the question of recycling, i.e., the formation of three-carbon intermediates either in the liver or muscle that recycle back to the liver and are resynthesized into glucose-6-phosphate before fatty acids or glycogen is formed. This issue recently was discussed extensively by Shulman and Landau.[14] These authors emphasize that the direct pathway with little recycling predominates under conditions of feeding and glucose loading. The technique that we have used to compare lipogenesis in obese and nonobese mice is to give a 250 mg load of [U-^{14}C]glucose per os to ad libitum fed mice and measure the incorporation into fatty acids in liver and extra hepatic tissues 2 hr later. These conditions were designed to maximize insulin secretion and to minimize glucagon secretion, lipolysis, and gluconeogenesis. Plasma glucose rises to a maximum in 10 min.[9] The specific activity of plasma glucose is constant from 10 to 60 min with 25% of the label expired as CO_2 during this time period.[9] Baker et al.[15] defined this protocol as fed-refed and demonstrated that under these conditions over 80% of the fatty acids newly synthesized were derived from carbon fed in the glucose test meal[15] and, further, that essentially none of the fatty acids found in adipose tissue were derived from the liver.[16]

The most interesting and important finding in the current study is the considerable accumulation of cholesterol and fatty acid in the livers of *obob* mice fed the 1% corn oil diet and its complete prevention by the addition to the diet of 0.05% of the bile acid analog Δ^{22}-5β-taurocholenic acid. The linoleic acid requirement of the mouse is estimated as 0.3% of the diet.[17] As calculated from USDA Handbook 8 data, the 1% corn oil diet supplied 0.6 g of linoleic acid/100 g of diet, an amount equal to twice the estimated requirement. This dietary level resulted in no elevation of liver cholesterol in the nonobese littermates of the *obob* mice, nor in gold thioglucose obese mice and their nonobese controls (unpublished data). The 40% lard diet supplied 4.0 g of linoleic acid and 40 mg of cholesterol/100 g of diet. The complexity of the effect of taurocholenic acid on liver cholesterol and its interactions with the genetic obesity and dietary fat is shown by the fact that all three statistical interactions involving taurocholenic acid with these two variables were highly significant (*Table 3*). It would not appear likely that the 40 mg of cholesterol/100 g in the 40% lard diet played much of a role in this experiment.

We previously reported that when *obob* mice were fed the 1% corn oil diet for 12 weeks their livers contained 1.5% cholesterol.[6] In the current study, the livers of *obob* mice fed this diet for 6 weeks contained 0.97% cholesterol compared with 0.28% for nonobese littermates. Our previous report suggested that this accumulation of liver cholesterol resulted from a defect in removing cholesterol from the liver and not from an elevated rate of cholesterol synthesis in the liver.[6] This conclusion was based on our observations that (1) conversion of a single 250 mg meal of [U-^{14}C]glucose to hepatic cholesterol in *obob* mice fed a 1% corn oil diet was reduced compared with nonobese littermates, whereas (2) when the [U-^{14}C]glucose was fed in the diet over a 24 hr period, accumulation of labeled cholesterol in the liver was increased 10 fold.[6] Turley and Dietschy have pointed out that ^3H_2O is a better substrate for measuring absolute rates of cholesterol synthesis than are ^{14}C substrates.[18] However, whatever problems are associated with measuring absolute rates of cholesterol synthesis from ^{14}C substrates they do not invalidate our hypothesis that in our experiments the accumulation of cholesterol in the liver of *obob* mice fed a purified diet containing 1% corn oil and no cholesterol most likely comes about as a result of a defect in cholesterol removal from the liver rather than increased cholesterol synthesis. We chose [U-^{14}C]glucose in this experiment to be able to compare our present results with those we reported earlier. Our previously published observations, based on work with [U-^{14}C]glucose as a substrate, first demonstrated in vivo the importance of skin and other extra hepatic tissues besides the intestines as sites for de nove sterologenesis in the mouse,[19] observations that were later confirmed by Turley et al.[20] and Feingold et al.[21] using ^3H_2O in studies in the intact rat.

In our study using [U-^{14}C]glucose, we found of the labeled cholesterol found in the body 15%, 56%, 10%, and 19% was found in the liver, intestine, skin, and remaining carcass, respectively.[19] By feeding cholesterol and thereby effectively eliminating cholesterol synthesis in the liver, we showed that transport from the liver to the extrahepatic tissues was a negligible factor in the experiment. The corresponding data for Turley et al.[20] and Fiengold et al.[21] using ^3H_2O are 50%, 24%, 8%, 18%, and 15%, 10%, 24%, 58%, respectively. Since we administered the labeled glucose as a 250 mg load, it is not surprising that in our study a large amount of the labeled cholesterol was found in the intestines. Fiengold et al.,[21] based on measurements of thoracic duct drainage, concluded that the transport of newly synthesized cholesterol from the intestine to the liver would be very limited in a short-term experiment such as ours.

This defect in cholesterol metabolism would appear to be associated with the *ob* gene since it occurred in *obob* mice and not in +/? littermates. Also it did not occur in gold thioglucose obese mice or their nonobese controls (unpublished data). The abnormality, although related to the genetic state of *obob* mice, is not secondary to or related to the resulting obesity per se since feeding 20% corn oil almost completely prevented the accumulation of liver cholesterol while at the same time nearly doubling the weight gain.[6]

It has long been known that *obob* mice exhibited a fatty liver[22] thought to be secondary to hyperinsulinemia observed in these animals.[22,23] Although hypercholesterolemia was observed in *obob* mice nearly 40 years ago,[24] no defects in hepatic metabolism of cholesterol were noted in the four extensive reviews of this genetic obesity published over a 20 year period.[2–5] Hepatic cholesterol metabolism in the *obob* mouse does not appear to have been widely studied. We summarize our findings in this and a previous study related to the accumulation of cholesterol in *obob* mice in

Table 6. Although the defect is accentuated when a diet marginal in essential fatty acids is fed, it occurs in diets ranging in fat from 1% corn oil to 40% lard, with the smallest increases seen when the 20% corn oil diet, or Purina Chow, was fed. Further research needs to answer the following questions: (1) What is the mechanism by which cholesterol accumulates in the liver of *obob* mice and why does a diet low in linoleic acid accentuate the accumulation? (2) What is the mechanism by which feeding higher levels of linoleic acid (e.g., corn oil) greatly reduce the accumulation? (3) What is the mechanism by which taurocholenic acid totally prevents hepatic cholesterol accumulation in *obob* mice fed 1% corn oil but has no effect when the mice are fed 40% lard? and (4) What is the mechanism by which taurocholenic acid greatly increases liver cholesterol synthesis in both obese and nonobese mice whether they are fed 1% corn oil or 40% lard?

The answers to these questions remain to be determined but may involve interactions of linoleic acid or one or its metabolites with bile acid synthesis, bile formation and excretion of cholesterol, and bile acids in the bile. Pandek et al.[25] reported that there is a linkage between hydroxymethylglutaryl (HMG) CoA reductase activity and cholesterol 7-alpha-hydroxylase activity, i.e., between cholesterol and bile acid synthesis. A number of investigators have examined bile flow and biliary lipid secretion as affected by injection or infusion of a variety of bile salts. The effect of the bile salt appears to be related to the hydrophilic or hydrophobic nature of the bile salts along with its ability to form micelles.[26–29] None of these studies can explain the effect of taurocholenic acid in dramatically lowering liver cholesterol in the *obob* mice fed the 1% corn oil diet. For example, a single injection of taurolithocholate inhibited biliary excretion of bile acids, phospholipids, and cholesterol.[27] This bile salt differs from taurocholenic acid only in having a hydroxy group in the 3 position and no double bond in the 22 position. We have shown that neither the taurine conjugate nor the double bond is essential since cholanic acid has a similar pharmacological effect as taurocholenic acid.[7] Rioux et al.[30] reported that short-term feeding of a diet enriched in phospholipids increased bile formation and bile acid transport. A number of research groups have demonstrated that the activity of cholesterol-7-alpha-hydroxylase, the rate-limiting step in bile acid synthesis, is down regulated by both cholesterol and bile acids at the transcriptional level.[31–34]

Recently, three groups of investigators have described experiments in which injecting the gene product of the *ob* gene into *obob* and +/? mice has caused weight loss, reduced food intake, and increased energy expenditure.[35–37] The mechanism suggested is a hormone produced by adipose tissue acting on a satiety center in the brain. The results described in the present paper do not appear to be explainable by this mechanism. Likewise it is not clear how this proposed mechanism relates to earlier papers in which the genetic defect in *obob* mice is reported to be related to the expression of and regulator of beta-3-adrenergic receptors.[38,39]

Considerably more work will be required to answer the questions posed above. A following tentative and incomplete hypothesis is offered. The *obob* mouse has an increased requirement for linoleic acid. The nature of the defect is not clear but the result is a large accumulation of cholesterol and fat in the liver which occurs despite the observed and expected reduction in cholesterol synthesis resulting from the accumulation of newly synthesized cholesterol in the liver. It appears that cholesterol and bile acid synthesis are both reduced, but the net effect is the reduction of cholesterol and/or bile acid excretion in the bile. Taurocholenic acid may reduce liver cholesterol by increasing cholesterol and/or bile acid excretion in the bile. It seems possible that the effect of taurocholenic acid is at least in part the transcriptional regulation of cholesterol-7-alpha-hydroxylase via the bile acid responsive element of the gene. The elevation of liver cholesterol in *obob* mice fed 40% lard is not reduced by taurocholenic acid because the mechanism of liver cholesterol accumulation is quite different in this case. It would be tempting to speculate that cholesterol accumulation in the 40% lard diet is a result of an increase in cholesterol synthesis caused by feeding a high level of saturated fatty acids.[40] However, we observed a considerable reduction is incorporation of [U-^{14}C]glucose into liver cholesterol in *obob* mice fed 40% lard. The considerable increase in incorporation of [U-^{14}C]glucose into liver cholesterol in obese and nonobese mice fed either diet caused by feeding taurocholenic acid could be via an effect on the

Table 6 Effect of diet and duration of feeding on liver cholesterol in obese and nonobese mice

Diet	Time fed (weeks)	% liver cholesterol	
		Obese	Nonobese
20% casein, 71% glucose, 1% corn oil*	1	0.41 ± 0.02‡	0.31 ± 0.02
20% casein, 71% glucose, 1% corn oil*	4.5	1.09 ± 0.08	0.27 ± 0.01
20% casein, 71% glucose, 1% corn oil†	6	0.97 ± 0.08	0.27 ± 0.02
20% casein, 71% glucose, 1% corn oil*	12	1.54 ± 0.10	0.29 ± 0.01
20% casein, 45% glucose, 20% corn oil*	4.5	0.34 ± 0.01	0.23 ± 0.01
20% casein, 45% glucose, 1% corn oil, 19% lard*	4.5	0.45 ± 0.04	0.24 ± 0.01
31% casein, 20% glucose, 40% lard†	6	0.68 ± 0.05	0.23 ± 0.01
Purina chow*	1	0.31 ± 0.01	0.20 ± 0.01
Purina chow*	12	0.40 ± 0.03	0.22 ± 0.01

*Data from Ref. 6.
†Data from this paper.
‡Means ± SE.

expression of the gene for HMG CoA reductase. More research is clearly needed to explain the observations we have described in this paper.

Acknowledgments

The author would like to acknowledge outstanding technical assistance from Mary Zanetti, Cameron Hutchison, and Frank Andriuli. The research described in this paper was carried out in the Merck Research Laboratories, Rahway, N.J.

References

1 Ingalls, A.M., Dickie, M.M., and Snell, G.D. (1950). Obese, new mutation in the mouse. *Heredity* **41,** 317–318

2 Bray, G.A. and York, D.A. (1971). Genetically transmitted obesity in rodents. *Physiol. Rev.* **51,** 598–646

3 Bray, G.A. and York, D.A. (1979). Hypothalamic and genetic obesity in experimental animals: An autonomic and endocrine hypothesis. *Physiol. Rev.* **59,** 719–809

4 Bray, G.A., York, D.A., and Fister, J.S. (1989). Experimental obesity: a homeostatic failure due to defective nutrient stimulation of the sympathetic nervous system. *Vitamins Hormones* **45,** 1–125

5 Johnson, P.R., Greenwood, M.R.C., Horwitz, B.A., and Stern, J.S. (1991). Animal models of obesity: Genetic aspects. *Ann. Rev. Nutr.* **11,** 325–353

6 Jansen, G.R., Zanetti, M.E., and Hutchison, C.F. (1967). Studies in lipogenesis *in vivo:* Fatty acid and cholesterol synthesis in hyperglycaemic-obese mice. *Biochem. J.* **102,** 870–877

7 Jansen, G.R., Zanetti, M.E., Hutchison, C.F., Andriuli, F.J., and Howe, E.E. (1969). The effect of cholanic acid on sterol and fatty acid synthesis. *Arch. Biochem. Biophys.* **134,** 185–195

8 Patchett, A.A. and Hannah, J. (1971). 5β-taurocholenic acids and 5β-taurocholadienic acids. U.S. Patent No. 3,580,936

9 Jansen, G.R., Hutchison, C.F., and Zanetti, M.E. (1966). Studies on lipogenesis *in vivo:* Effect of dietary fat or starvation on conservation of [^{14}C]glucose into fat and turnover of newly synthesized fat. *Biochem. J.* **99,** 323–332

10 Jansen, G.R., Zanetti, M.E., and Hutchison, C.F. (1966). Studies on lipogenesis *in vivo:* Effects of starvation and refeeding, and studies on cholesterol synthesis. *Biochem. J.* **99,** 333–340

11 *SPSS-X Users Guide,* 2nd ed. (1986). SPSS Inc., 444 N. Michigan Ave., Chicago, IL 60611 USA

12 Clarke, S.D., Armstrong, M.K., and Jump, D.B. (1990). Dietary polyunsaturated fats uniquely suppress rat liver fatty acid synthase and S14 mRNA content. *J. Nutr.* **120,** 225–231

13 Herzberg, G.R. (1983). The influence of dietary fatty acid composition on lipigenesis. *Nutr. Res.* **5,** 221–231

14 Shulman, G.I. and Landau, B.R. (1992). Pathways of glycogen repletion. *Physiol. Rev.* **72,** 1019–1033

15 Baker, N., Learn, D.B., and Bruckdorder, K.R. (1978). Re-evaluation of lipogenesis from dietary glucose carbon in liver and carcass of mice. *J. Lipid. Res.* **19,** 879–893

16 Baker, N., Mead, J., Jr., and Kannan, R. (1981). Hepatic contribution to newly made fatty acids in adipose tissue in rats and inhibition of hepatic and extrahepatic lipogenesis from glucose by dietary corn oil. *Lipids* **16,** 568–576

17 Nutrient Requirements of Laboratory Animals, No. 10 3rd revised ed. (1978). National Academy of Sciences, Washington, DC USA

18 Turley, S.D. and Dietschy, J. M. (1988). The metabolism and excretion of cholesterol by the liver. In *The Liver: Biology and Pathobiology* (T.M. Arias, Jakoley, W.B., Popper, H., Schachter, D., and Shafritz, D.A., eds.), 617–642, Raven Press, New York, NY USA

19 Jansen, G.R., Zanetti, M.E., and Hutchison, C.F. (1970). Synthesis of cholesterol from glucose-U-^{14}C in the liver and extrahepatic tissues of the mouse. *Arch Biochem. Biophys.* **138,** 433–442

20 Turley, S.C., Anderson, J.M., and Dietschy, J.M. (1981). Rates of sterol synthesis and uptake in the major organs of the rat in vivo. *J. Lipid Res.* **22,** 551–569

21 Feingold, K.R., Wiley, M.H., MacRae, G., Lear, S., Moser, A.H., Zsigmond, G., and Siperstein, M.D. (1983). De novo sterologenesis in the intact rat. *Metabolism* **32,** 75–81

22 Assimacopoulos-Jeannet, F., Singh, A., LeMarchand, Y., Loten, E.G., and Jeanrenaud, B. (1974). Abnormalities in lipogenesis and triglyceride secretion by perfused livers of obese-hyperglycaemic (*ob/ob*) mice: Relationship with hyperinsulinaemia. *Diabetologia* **10,** 155–162

23 Jeanrenaud, B. (1978). Hyperinsulinemia in obesity syndromes: its metabolic consequences and possible etiology. *Metabolism* **27** (Suppl 2), 1881–1892

24 Zomzely, C. and Mayer, J. (1958). Levels of serum cholesterol in obese mice. *Nature* **182,** 1738–1739

25 Pandak, W.M., Heuman, D.M., Hylemon, P.B., and Vlahcenic, Z.R. (1990). Regulation of bile acid synthesis IV Interrelationships between cholesterol and bile acid biosynthesis pathways. *J. Lipid Res.* **31,** 79–90

26 Baumgartner, V., Scholmerich, J., Leible, P., and Farthmann, E.M. (1992). Cholestasis, metabolism and biliary lipid secretion during perfusion of rat liver with different bile salts. *Biochim Biophy. Acta* **1125,** 1429

27 Marinelli, R.A., Roma, M.G., Pellegrino, J.M., and Rodriguez Garay, E.A. (1992). Taurolithocholate-induced inhibition of biliary lipid and protein excretion in the rat. *Biochim. Biophys. Acta* **1125,** 44–48

28 Angelico, M., Barocchi, L., Nistri, A., Franchitto, A., Della-Guardia, P., and Gaudio, E. (1994). Effect of taurohyodeoxycholic acid, a hydrophilic bile salt on bile salt and biliary lipid secretion in the rat. *Dig. Dis. Sci.* **39,** 2389–2397

29 Zamashita, H. and Setchell, K.D. (1994). Metabolism and effect of 7-oxo-lithocholic acid 3-sulfate on bile flow and biliary lipid secretion in rats. *Hepatology* **20,** 663–671

30 Rioux, F., Perea, A., Yousef, I.M., Levy, E., Malli, L., Carrillo, M.C., and Tuchweber, B. (1994). Short-term feeding of a diet enriched in phospholipids increases bile formation and the bile acid transport maximum in rats. *Biochem. Biophys. Acta* **1214,** 193–202

31 Pandak, W.M., Li, Y.C., Chiang, Y.L., Strider, E.J., Gurley, E.C., Heuman, D.M., Vlahcevic, Z.R., and Hylemon, P.B. (1991). Regulation of cholesterol 7α-hydroxylase m-RNA and transcriptional activity by taurocholate and cholesterol in the chronic biliary diverted rat. *J. Biol. Chem.* **266,** 3416–3421

32 Ramirez, M.I., Karaoglu, D., Haro, D., Barillas, C., Bashirzadeh, R., and Gil, G. (1994). Cholesterol and bile acids regulate cholesterol-7-alpha-hydroxylase expression at the transcriptional level in culture and in transgenic mice. *Mol. Cell. Biol.* **14,** 2809–2821

33 Pandak, W.M., Vlakcenic, Z.R., Heuman, D.M., Redford, K.S., Chiang, J.Y., and Hylemon, P.B. (1994). Effects of different bile salts on steady-state in RNA levels. *Hepatology* **19,** 941–947

34 Stravitz, R.T., Hylemon, P.M., Heuman, D.M., Hagey, L.R., Schteingart, C.D., Ton-Nu, H.T., Hofmann, A.F., and Vlahcenic, Z.R. (1993). Transcriptional regulation of cholesterol-7-alph-hydroxylase in RNA by conjugated bile acids in primary cultures of rat hepatocytes. *J. Biol. Chem.* **268,** 13987–13993

35 Pelleymounter, M.A., Cullen, M.J., Baker, M.B., Hecht, R.H., Winters, D., Boone, T., and Collins, F. (1995). Effects of the obese gene product on body weight regulation in *ob/ob* mice. *Science* **269,** 540–543

36 Halaas, J.L., Gajiwala, K.S., Maffei, M., Cohen, S.L., Chait, B.T., Rabinowitz, D., Lallone, R.L., Burley, S.K., and Friedman, J.M. (1995). Weight-reducing effects of the plasma protein encoded by the *obese* gene. *Science* **269,** 543–546

37 Campfield, L.A., Smith, F.J., Guisez, Y., Devos, R., and Burn, P. (1995). Recombinant mouse ob protein: Evidence for a peripheral signal linking adiposity and central neural networks. *Science* **269,** 546–549

38 Arbeeny, C.M., Meyers, D.S., Hillyer, D.E., and and Bergquist, K.E. (1995). Metabolic alterations associated with the antidiabetic effect of beta 3-adrenergic receptors agonists in obese mice. *Am. J. Physiol.* **268,** E678–E684

39 Collins, S., Daniel, K.W., Rohlfs, E.M., Ramkumar, V., Taylor, I.L., and Gettys, T.W. (1994). Impaired expression and functional activity of the beta 3- and beta 1-adrenergic receptors in adipose tissue of congenitally obese (C57BL/6J *obob*) mice. *Mol. Endocrinol.* **8,** 518–527

40 Woollett, L.A., Spady, D.K., and Dietschy, J.M. (1992). Regulatory effects of the saturated fatty acids 6:0 through 18:0 on hepatic low density lipoprotein receptor activity in the hamster. *J. Clin. Invest.* **89,** 1133–1141