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Effect of Δ^{22}-5β-taurocholenic acid on hepatic cholesterol and fatty acid in gold thioglucose obese mice fed low- or high-fat diets

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We previously demonstrated that hyperglycemic-obese (obob) mice fed a 1% corn oil diet accumulated 10 times as much hepatic cholesterol as did their non-obese (+/?) littermates fed this diet because of difficulty in removal of cholesterol from the liver rather than from increased synthesis. Furthermore, feeding the bile acid analog Δ^{22}-5β-taurocholenic acid completely prevented the accumulation of hepatic cholesterol in obob mice fed the 1% corn oil diet. The hypothesis to be tested in the current study is that these aspects of cholesterol metabolism in the obob mouse do not occur in the hyperinsulinemic and insulin-resistant gold thioglucose obese mouse. Gold thioglucose obese (gtgo) and non-obese (ngtgo) mice were fed diets containing either 1% corn oil or 40% lard each with or without added taurocholenic acid for 6 weeks and then given a 250 mg meal of [U-^{14}C]-glucose with incorporation of label into hepatic cholesterol and fatty acid measured 2 hours later. Consistent with earlier results in the obob model, incorporation of labeled glucose was significantly increased in obese compared with non-obese mice fed 1% corn oil and significantly reduced either by feeding 40% lard or by adding taurocholenic acid to the diet. In addition, taurocholenic acid greatly increased incorporation of labeled glucose into hepatic cholesterol in obese or non-obese mice fed either diet. In contrast to obob mice, the percentage of fat in the liver of gtgo mice was increased only 50% compared with ngtgo mice. The comparable increase in obob mice was 480%. Hepatic cholesterol did not increase significantly in the liver of gtgo mice fed 1% corn oil when compared with the ngtgo controls. The comparable increase in obob mice fed 1% corn oil was 350%. Also in marked contrast to obob mice, feeding taurocholenic acid increased hepatic cholesterol compared with non-obese controls fed either diet. The results are discussed in the light of the presence of circulating leptin in gtgo but not in obob mice. (J. Nutr. Biochem. 10:638–643, 1999)

Keywords: obesity; cholesterol; fatty acids; lipogenesis; essential fatty acids; taurocholenic acid

Introduction

The genetic obesity of hyperglycemic mice is known to be caused by the absence of leptin, the product of the obesity gene.[1] Leptin is produced in adipose tissue and acts on obesity receptors in the brain and elsewhere.[1] Gold thioglucose obesity is a result of a necrotic lesion in the ventromedial hypothalamus resulting from the deposition of gold.[2] In both models insulin resistance and hyperinsulinemia result, as does increased hepatic lipogenesis,[3–5] conditions consistent with non-insulin dependent diabetes mellitus (NIDDM). Hyperglycemic obese (obob) mice fed a glucose based diet containing 1% corn oil accumulated over 10 times as much fat and cholesterol in the liver as did their non-obese (+/?) littermates fed this diet.[6] This did not occur when obob mice were fed diets containing either 20% corn oil[7] or 40% lard.[6] In this report we compare our previously reported results for obob mice with the effect of feeding the same 1% corn oil or 40% lard diet to gold thioglucose obese and non-obese mice for an identical period of time.

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Received May 24, 1999; accepted July 27, 1999.

Materials and methods

Gold thioglucose obesity was induced in male mice of the ICR strain weighing 25 to 30 g, by intraperitoneal injection of gold

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PII S0955-2863(99)00054-6

Table 1 Composition of experimental diets

	Composition (g/100 g diet)				
	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Casein (Labco)	20.0	20.0	31.0	31.0	26.0
Salts no 2 USP XIII	4.0	4.0	4.0	4.0	4.0
Glucose	70.0	70.0	20.0	20.0	45.0
Celluflour	5.0	5.0	5.0	5.0	5.0
Corn oil	1.0	1.0	—	—	20.0
Lard	—	—	40.0	40.0	—
Potassium Δ^{22}-5β-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 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.

thioglucose (Schering-Plough, Madison, NJ USA) at a dose of 800 mg/kg body weight. The survivors were maintained on a diet containing 20% corn oil (*Table 1*, diet 5) for 8 weeks, at which time they were placed on the experimental diets. An equal number of control male ICR mice at the same weight were injected with saline and maintained an equal length of time on the 20% corn oil diet.

The gold thioglucose obese (gtgo) mice and non-obese (ngtgo) controls were weighed, divided into groups of equivalent body weight within their obesity status ($n = 8$ mice per group), and assigned to diets 1, 2, 3, or 4 (*Table 1*). Diets were glucose based and contained either 1% corn oil or 40% lard, with or without 0.05% Δ^{22}-5β-potassium taurocholenate (TC).[8] The 1% corn oil diet supplied 3.7 kcal/g, 1.4 en% as linoleate and 2.4 en% as fat. The 40% lard diet supplied 5.6 kcal/g, 6.8 en% as linoleate and 66.2 en% as fat. Because protein was fed at the same percentage of calories in both diets (21%), the high fat diet was consequently reduced in carbohydrate. The starting body weights of mice in all treatment groups are listed in *Table 2*.

The experimental diets were fed for 6 weeks, and daily food consumption, accounting for spillage, was measured 1 day each week. Between 8:00 and 10:00 AM on the day of sacrifice, the mice were weighed, given 250 mg of D-glucose including 2.5 μCi [U-^{14}C] glucose by stomach tube (0.5 mL), and sacrificed 2 hours later by cervical dislocation. At this time the liver and one epididymal fat pad were quickly excised, immediately frozen, and stored along with the remaining carcass in a deep-freeze at −20°C. The numbers of mice completing the experiment are listed in *Table 2*. Completed group sizes were seven or eight for all groups. 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/mmole. Liver and carcass fat were measured as total fatty acid extractable after saponification. Results are presented as means ± standard error. A 3-×-2 factorial design was used with the main effects being obesity status (obese or non-obese), diet (1% corn oil or 40% lard), and the presence or absence of TC. Statistical analysis was carried out using Statistical Package for the Social Science (SPSS).[11]

Results

Food consumption, body weight, and body fat content data are summarized in *Table 2*. Food consumption was slightly but significantly higher for gtgo than ngtgo control mice fed the 40% lard but not the 1% corn oil diet. As expected, food consumption was significantly less in gtgo or ngtgo control mice when fed 40% lard than when fed 1% corn oil because of the higher calorie density of the high-fat diet. TC did not significantly affect food consumption for gtgo or ngtgo control mice fed either diet. Compared with results in obob mice reported previously,[6] these gtgo mice were somewhat larger and less obese. TC reduced weight gain and dramatically reduced body fat in gtgo and ngtgo control mice fed either diet. The differences in body fat related to obesity, dietary fat, and feeding taurocholenic acid were all significant at a *P*-value of 0.000 across the other two main effects with no two-way or three-way interactions.

The reductions in body fat associated with feeding TC were as follows: gtgo mice fed 1% corn oil, 32%; ngtgo controls fed 1% corn oil, 46%; gtgo mice fed 40% lard, 30%; and ngtgo controls fed 40% lard, 51%.

As shown in *Table 3*, liver size was significantly increased by TC in gtgo and ngtgo control mice fed either 1% corn oil or 40% lard. Liver fat increased significantly in gtgo mice fed either diet compared with ngtgo control mice and was significantly lowered by TC. Incorporation of [U-^{14}C]-glucose into liver fatty acid was significantly elevated in gtgo compared with ngtgo control mice fed 1% corn oil and greatly reduced in both gtgo and ngtgo control mice fed 40% lard. TC significantly reduced incorporation of labeled glucose into liver fatty acids in obese and non-obese mice fed 1% corn oil, but significantly increased incorporation in obese or non-obese mice fed 40% lard.

Corresponding data for cholesterol also are shown in *Table 3*. Hepatic cholesterol was increased only slightly in gtgo mice fed either diet compared with ngtgo control mice. TC increased liver cholesterol significantly in gtgo and ngtgo control mice fed either diet. Feeding TC increased incorporation of labeled glucose fivefold in both gtgo and ngtgo mice fed 1% corn oil. When fed with the 40% lard diet, TC increased the incorporation 13-fold in the gtgo mice but only threefold in the ngtgo mice.

Discussion

The large reductions in body fat in mice fed TC in the diet were unexpected and are unexplained at this time, especially

Table 2 Food consumption, weight gain, body weight, and body fat*

	Obese		Non-obese	
Taurocholenic acid	−	+	−	+
	Diet-1% corn oil			
n	7	7	7	8
Initial body weight (g)	60.3 ± 2.8	60.6 ± 3.5	41.7 ± 0.8	40.9 ± 1.8
Food consumption (g/d)	6.3 ± 0.4	5.9 ± 0.2	5.6 ± 0.3	6.5 ± 0.5
Weight gain (g)	−1.7 ± 2.0	−5.2 ± 1.6	0.7 ± 1.2	0.1 ± 0.8
Final body weight (g)	58.6 ± 2.8	55.4 ± 2.8	42.4 ± 0.8	40.5 ± 1.5
Final body fat (%)	30.6 ± 0.9	20.9 ± 2.6	14.0 ± 0.9	7.6 ± 1.2
	Diet-40% lard			
n	7	8	8	8
Initial body weight (g)	60.3 ± 2.8	59.6 ± 1.8	40.5 ± 1.0	40.6 ± 0.6
Food consumption (g/d)	4.8 ± 0.3	4.8 ± 0.1	3.9 ± 0.2	4.0 ± 0.1
Weight gain (g)	14.2 ± 1.4	8.6 ± 2.5	8.5 ± 1.6	4.1 ± 0.6
Final body weight (g)	74.9 ± 3.7	68.2 ± 2.7	49.2 ± 2.2	44.7 ± 0.6
Final body fat (%)	38.4 ± 0.9	26.9 ± 3.9	22.4 ± 3.4	10.9 ± 1.4

Significant differences between treatments (*P*)†

	Main effects				2- and 3-way interactions		
	Obesity (O)	Fat (F)	TC	OxF	OxTC	FxTC	OxFxTC
Initial body weight (g)	**0.000**‡	0.778	0.721	0.958	0.944	0.991	0.854
Food consumption (g/d)	0.046	**0.000**‡	0.415	0.055	0.132	0.680	0.122
Weight gain (g)	0.565	**0.000**‡	**0.003**‡	**0.000**‡	0.375	0.196	0.722
Final body weight (g)	**0.000**‡	**0.000**‡	0.017	**0.007**‡	0.576	0.372	0.854
Final body fat (%)	**0.000**‡	**0.000**‡	**0.000**‡	0.739	0.598	0.270	0.592

*Results are expressed as means ± SE. Body fat is measured as total fatty acid in the carcass (minus liver and one epididymal fat pad) after saponification.
†Taurocholenic acid.
‡Values are significant at the 1% level or lower.

because food consumption did not appear to be reduced by TC. The fact that these reductions in body fat occurred in both gtgo and ngtgo control mice fed either a very low fat or a very high fat diet make them worthy of further investigation.

Considering size and concentration, obob mice fed a 1% corn oil diet accumulated over 10 times as much fat and cholesterol in the liver as did +/? mice fed this diet, and feeding 0.05% TC in the diet entirely prevented this accumulation.[6] In the present report, we demonstrate that such marked accumulations of hepatic fat and cholesterol do not occur in gtgo mice fed 1% corn oil, and TC does not reduce hepatic cholesterol in these mice. However, hepatic lipogenesis was elevated in gtgo and ngtgo control mice fed 1% corn oil and was greatly reduced in both by feeding 40% lard, results that are comparable to our previous results with the obob mice.[6] They also are consistent with the results of Blair et al.,[12] who reported that hepatic lipogenesis in vivo from ^3H_2O in gold thioglucose obese mice was elevated fourfold compared with non-obese controls as early as 2 weeks post-gold thioglucose injection.

The percentage of cholesterol in the livers of obob mice fed 1% corn oil was increased nearly fourfold compared with +/? mice fed this diet (0.97 vs. 0.28%),[6] and also in contrast to the current study, TC reduced hepatic cholesterol by over two thirds in obob mice fed 1% corn oil (0.27 vs. 0.97%).[6] Incorporation of [U-^{14}C]-glucose into hepatic cholesterol was increased in gtgo and ngtgo control mice fed either diet ranging from threefold in ngtgo control mice fed 40% lard to 13-fold in gtgo mice fed this diet. These results parallel our previously reported results with obob and +/? mice.[6]

As stated above, both gtgo and obob models are hyperglycemic, hyperinsulinemic, and insulin resistant. There does not appear to be any quantitative comparison of the degree of insulin resistance in the two models. However, Freychet et al.[13] suggested that the insulin resistance in both of these animal models is characterized by a common set of metabolic abnormalities. Even more relevant to the present discussion is a recent report that gold thioglucose, in addition to its well known effects on food intake, also damages the leptin receptor Ob-Rb in the hypothalamus, thus rendering gtgo mice leptin resistant as well as insulin resistant.[14]

The differences we observed in lipogenesis between the two models may relate to insulin-leptin relationships. Leptin has been reported to inhibit insulin secretion in mice in vivo,[15] in the isolated perfused rat pancreas,[16] and in rat pancreatic islets in vitro in response to glucose.[15,17] In contrast, in incubations with rat pancreatic islets, Poitout et al.[18] observed that leptin did not alter either basal or glucose stimulated insulin secretion. Presumably insulin secretion in gtgo but not in obob mice potentially would be restrained by circulating leptin. It is of interest that circulating levels of

Table 3 Liver weight and incorporation of [U-^{14}]-glucose into fatty acids and cholesterol*

Taurocholenic acid	Obese		Non-obese	
	−	+	−	+
Diet-1% corn oil				
Liver weight (g)	3.20 ± 0.29	4.50 ± 0.34	2.24 ± 0.11	3.52 ± 0.18
% Fat	6.26 ± 0.56	3.52 ± 0.15	4.11 ± 0.38	3.52 ± 0.22
cpm/liver (× 10^{-3})	66.5 ± 14.1	12.6 ± 2.7	21.5 ± 3.9	14.8 ± 1.0
% Cholesterol	0.37 ± 0.05	0.40 ± 0.02	0.26 ± 0.02	0.35 ± 0.03
cpm/liver	190 ± 56	892 ± 189	140 ± 33	666 ± 185
Diet-40% lard				
Liver weight (g)	3.82 ± 0.34	4.94 ± 0.32	2.01 ± 0.09	3.59 ± 0.11
% Fat	12.2 ± 3.1	5.64 ± 0.68	5.44 ± 0.88	3.64 ± 0.24
cpm/liver (× 10^{-3})	2.67 ± 0.48	3.83 ± 0.65	1.32 ± 0.16	2.36 ± 0.32
% Cholesterol	0.40 ± 0.02	0.62 ± 0.06	0.26 ± 0.01	0.36 ± 0.02
cpm/liver	74.4 ± 24.4	972 ± 218	340 ± 88	1,057 ± 15

Significant differences between treatments (*P*)†

	Main effects				2- and 3-way interactions		
	Obesity (O)	Fat (F)	TC	OxF	OxTC	FxTC	OxFxTC
Liver weight	**0.000**‡	0.107	0.000	0.092	0.569	0.936	0.544
% Fat	**0.001**‡	**0.004**‡	**0.001**‡	0.044	0.035	0.121	0.421
cpm/liver	**0.003**‡	**0.000**‡	**0.000**‡	**0.008**‡	**0.002**‡	**0.000**‡	**0.002**‡
% Cholesterol	**0.000**‡	**0.010**‡	**0.000**‡	**0.010**‡	0.545	0.047	0.061
cpm/liver	0.854	0.176	**0.000**‡	0.128	0.381	0.345	0.991

*Results are expressed as means ± SE. Liver fat is measured as total fatty acid after saponification.
†Taurocholenic acid.
‡Values are significant at the 1% level or less.

leptin in gtgo mice are reported to be elevated in response to the adiposity.[19] The leptin-insulin picture is not yet fully understood.

Leptin also has been reported to have effects on glucose and lipid metabolism independent of insulin. Komohara et al.[20] reported that a 5-hour infusion of leptin in mice increased whole body glucose uptake and turnover and reduced hepatic glycogen content without affecting the plasma levels of glucose and insulin. Siegrist-Kaiser et al.[21] reported that intravenous infusion of leptin for 4 days in freely moving male rats increased glucose utilization 60% in brown adipose tissue. Increased lipolysis was observed in lean but not in obese Zucker (fafa) rats. However, Mick et al.[22] reported that leptin did not significantly affect basal or insulin stimulated glucose metabolism in cultured adipocytes from epididymal fat pads. Burcellin et al.[23] infused leptin in obob mice for 6 hours. Whole body glucose turnover increased. Glucose uptake increased in brown adipose tissue, heart, and brain but not in white adipose tissue or skeletal muscle. In addition, plasma insulin increased, as did hepatic glucose-6-phosphatase. The activity of phosphoenolpyruvate carboxykinase decreased substantially as a result of leptin infusion. Furnsinn et al.[24] reported that leptin did not increase basal or insulin stimulated in skeletal muscle in vitro. In contrast, Ceddia et al.[25] reported that leptin itself stimulated glucose uptake, glycogen synthesis, lactate formation, and glucose oxidation in incubated rat soleus muscle. Muoio et al.,[26] also with incubated rat soleus muscle, observed that leptin inhibited insulin stimulated lipogenesis.

As is the case for insulin-leptin relationships, a direct effect of leptin on peripheral tissues remains to be fully clarified. Perhaps more relevant to the present study is a recent report by Cohen et al.[27] in which the effect of leptin on hepatic intermediary metabolism was studied using [2-^{13}C]-pyruvate and nuclear magnetic resonance spectroscopy. The livers from C57BL6J obob and lean mice were perfused with or without the addition of leptin. Perfused livers from obob mice injected in vivo for 5 days without leptin added to the perfusate. The addition of leptin to the perfusate with livers from obob mice not injected with leptin increased glycogen synthesis and also the de novo synthesis of fatty acid from acetyl CoA. However, in the experiment with the perfused livers from obob mice injected with leptin, de novo fatty acid synthesis was greatly reduced although glycogen synthesis was tripled. This observation could partially explain the increased hepatic synthesis of fatty acid in obob mice because these animals are essentially devoid of circulating leptin.

The accumulation of cholesterol in the obob mice fed the 1% corn oil diet and the effect of TC in so dramatically preventing this accumulation may relate to insulin and essential fatty acid relationships combined with the regulation of hepatic cholesterol metabolism by bile acids, because taurocholenic acid is a bile acid analog without hydroxy groups. Because TC greatly increased cholesterol synthesis in both types of obese and non-obese mice regardless of the diet fed, it is difficult at this time to see how the lowering of the amount of cholesterol in the liver, which occurs only in obob mice fed 1% corn oil, relates to the broad effect of TC on cholesterol synthesis, which was

greatly increased in both types of obese and both types of non-obese mice fed either 1% corn oil or 40% lard.

Twisk et al.[28] studied the effect of insulin on bile acid synthesis in cultured rat hepatocytes. The rats had been maintained on standard rat chow and diet was not studied as a variable. The hepatocytes were incubated with and without physiologic concentrations of insulin for 48 hours. Bile acid synthesis, which was quantified by gas liquid chromatography (GLC) and also from [4-^{14}C] cholesterol, was decreased 33 to 53% by the insulin. Enzyme activities and transcription of cholesterol 7α-hydroxylase and sterol 27-hydroxylase were reduced in good agreement with the synthesis data. Levy et al.[29] evaluated the effect of essential fatty acid deficiency in young adult male rats fed either a stock diet or a commercial essential fatty acid-deficient diet. The diets were fed for 12 weeks and the common bile duct was cannulated. After the pool size of bile was depleted, 6-hour bile collections were made. There were significant reductions in the 6-hour excretion of cholesterol, bile acid, and phospholipid in the essential fatty acid-deficient rats. The enzyme activity of HMG-CoA reductase was decreased and the enzyme activity of cholesterol 7α-hydroxylase was increased, the latter effect presumably due to depletion of bile acids in the bile pool. Bjorkhem et al.[30] summarized the regulation of the expression of HMG-CoA-reductase and concluded that it generally is coordinately regulated with the expression of cholesterol 7α-hydroxylase. Transcriptional control of HMG-CoA reductase is via a single sterol regulatory element (SRE-1) in the promotor. Bile acids are able to suppress transcriptional and post-transcriptional mechanisms for both enzymes.

In summary, obob mice accumulate large amounts of hepatic cholesterol when fed a diet marginal in linoleic acid. This does not occur in gtgo mice or in the non-obese controls to obob or gtgo mice. Furthermore, feeding Δ^{22}-5β-taurocholenic acid completely prevented this accumulation in obob mice without a significant effect on the level of hepatic cholesterol in gtgo mice and in spite of a large increase in cholesterol synthesis in both obesities. The presence or absence of circulating leptin would appear to be the major physiologic difference that distinguishes obob from gtgo obesity.

The diet low in linoleic acid is also high in glucose and it cannot be totally excluded that the high glucose level is more important than the low level of linoleic acid. However, we have argued elsewhere that the defect is, in fact, related to the low level of linoleic acid and involves an interaction with bile acid metabolism such that the accumulation is secondary to diminished clearance of cholesterol from the liver and not enhanced synthesis.[6] We suggest that a role for leptin in hepatic cholesterol metabolism is worthy of further investigation. Supporting this suggestion is a recent report by Silver et al.[31] It has been known for a long time that the hypercholesterolemia in obob mice is characterized by greatly increased high density lipoprotein (HDL) cholesterol.[32] Silver et al.[31] reported that this increased level of HDL cholesterol in the obob mouse is accompanied by defective hepatic catabolism of ApoA-I and ApoA-II and decreased expression of ApoA-I mRNA. The leptin story gets more complicated but more intriguing by the day.

Acknowledgments

The author would like to acknowledge outstanding technical assistance from Mary Zanetti, Cameron Hutchison, and Frank Andriuli. Appreciation is expressed to the Merck Research Laboratories (Rahway, NJ) where this work was carried out.

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