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Regulatory enzymes of lipid metabolism in LA/N-cp rats

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Hepatic activities of rate limiting enzymes in fatty acid and cholesterol synthesis and cholesterol degradation were determined in lean and obese LA/N-cp rats. The hepatic activities of acetyl-CoA carboxylase and fatty acid synthetase, the key enzymes of fatty acid synthesis and 3-hydroxy-3-methylglutaryl coenzyme A reductase (the rate limiting enzyme in cholesterol synthesis), were increased 2-fold in the obese rats as compared with their lean littermates. In contrast, the activity of cholesterol 7α-hydroxylase, the rate limiting enzyme of cholesterol degradation to bile acids, was significantly decreased by 28% in the obese group as compared with the control group. Significantly, compared with the control group, the obese animals exhibited similar magnitudes of differences in the activities of the above enzymes even when they were pair-fed with the control animals. Thus these differences in the obese group are not due to hyperphagia but possibly to hypersecretion of the lipogenic hormone, insulin in this strain. These results indicate that the LA/N-cp obese rat has twice the capacity to synthesize body fat and cholesterol but has a reduced capacity to degrade the cholesterol, leading to increased accumulation of cholesterol and fat. (J. Nutr. Biochem. 6:348–352, 1995.)

Keywords: obesity; LA/N-cp obese rats; fatty acid synthetase; acetyl-CoA carboxylase; HMG-CoA reductase; cholesterol 7α-hydroxylase

Introduction

Obesity is characterized by excessive body fat deposits and is associated with increased morbidity and mortality and with detrimental effects on health, such as increased risks of cardiovascular disease, hypertension, diabetes, and hyperlipidemia.[1–5] Several genetically obese animal models have been developed in recent years.[6–9] Among them is the LA/N corpulent strain (LA/N-cp) of rat which is a normotensive strain exhibiting metabolic characteristics associated with human Type IV hyperlipoproteinemia.[8] Obesity is a multifactorial condition, confounded by underlying end organ diseases, endocrine problems, age, and assessment of lean body weight.[10] Earlier investigators have related the etiology of obesity in genetically obese animals to various lipogenic enzymes and hormonal factors.[11,12] Some of the enzymes studied were those involved in carbohydrate metabolism (gluconeogenesis).[13] Among the known biochemical changes that occur in obese conditions are changes in lipid, lipoprotein, and carbohydrate metabolism.[13–15]

Earlier metabolic studies on human subjects and animal models have shown increases in total body cholesterol synthesis, higher rates of LDL production and VLDL secretion, and lower concentrations of HDL cholesterol.[16,17] It is therefore logical to expect that such marked alterations in lipid metabolism will be reflected in corresponding changes in the activities of lipid synthesizing and degrading enzymes in the liver. Thus in order to explore the possible metabolic impact of obesity on lipid metabolism in mammalian systems, a study on the regulatory lipogenic enzymes in LA/N-cp obese rats was undertaken. Thus, using LA/N-cp rats as a model we have examined the activities of fatty acid synthetase (FAS), acetyl coenzyme A carboxylase (ACX), 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR), and cholesterol 7α-hydroxylase (7αH), which are the rate limiting enzymes for the synthesis of fatty acid, cholesterol, and degradation of cholesterol to bile acids, respectively.

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Methods and materials

3-Hydroxy[3-^{14}C]methylglutaryl coenzyme A [3-^{14}C](HMG-CoA) (specific activity 1861.1 MBq/mmol), and [^{14}C]$NaHCO_3$ (specific activity 9.25 MBq/mmol) were from New England Nuclear (Boston, MA USA). Enzyme substrates and all other chemicals and reagents were purchased from Sigma Chemicals (St. Louis, MO USA). The scintillation cocktail was purchased from National Diagnostics (Somerville, NJ USA).

Animals

The protocol described below was approved by the institutional animal care committee. Five adult male obese rats (LA/N-cp strain) weighing about 180 g and five lean control littermates, weighing approximately 140 g and 7 to 8 weeks old (USDA, Beltsville, MD USA), were studied. All the rats were housed in wire mesh cages and kept on a reverse light and dark cycle for 2 weeks. It is known that the activities of the lipogenic enzymes reach their peak around noon (mid-period of dark cycle).[18] These animals had free access to the normal Purina rat laboratory chow (Purina Co., St. Louis, MO USA). The obese group, on average, consumed 15 ± 2% more food than the control group. In addition, to rule out hyperphagia to be the cause for the differences between control and obese animals two obese rats were pair-fed with two lean control animals for 2 weeks.

Experiment

On day 14, the rats were killed at noon by aortic exsanguination. Blood was collected into an EDTA-containing tube (1 mg/mL of blood), and the plasma was separated from the red cells by centrifugation at 500*g* for 10 min. The livers were quickly excised and weighed. The livers were homogenized in a Polytron homogenizer in two volumes of homogenizing buffer (250 mmol/L of sucrose in 10 mM potassium phosphate buffer, pH 7.4, containing 30 mmol/L of EDTA, 10 mmol/L of dithiothreitol, and 0.2 mmol/L of phenylmethylsulfonyl fluoride). The homogenate was centrifuged for 15 min at 16,000*g* and the postmitochondrial solution was recentrifuged at 105,000*g* for 45 min at 4°C.[19] The 105,000*g* supernatant (cytosolic fraction) was stored at −20°C for the assay of acetyl-CoA carboxylase and fatty acid synthetase activity, and the microsomal pellet was suspended in about 2 mL of the original homogenizing buffer and used for the assay of HMGR and 7αH activities.

Activities of the following enzymes were measured in the respective fractions according to published procedures. Enzyme activity of FAS (EC 2.3.1.85) was measured in the cytosolic fraction of the liver by the method of Nepokroeff et al.[20] Acetyl-CoA carboxylase (EC 6.4.1.2) activity in cytosolic fraction was measured as previously described by Inoue et al.[21] HMGR (EC 1.1.1.34) activity was measured in the microsomal fraction by the method of Shapiro et al.[22] Cholesterol 7α-hydroxylase (EC 1.14.13.17) activity was determined by a procedure described previously by Shefer et al.[23] in the microsomal fraction. For total hepatic cholesterol estimation, the method of Zlatkis et al.[24] was followed. The triglycerides were determined as to their glycerol content by the method of Van Handel and Zilversmit.[25] The data were statistically analyzed and significance was determined using the Students *t*-test.[26]

Results and discussion

Our results showed that the gain in body weights of the obese animals was significantly higher, by 84% ($P < 0.001$), than their lean counterparts, although the initial body weights of animals of the two groups were statistically not different (*Table 1*). Thus, the obese animals gained more weight than the lean ones, although both groups were fed the same isocaloric diet. The observed 15% increase in the food consumption by the obese group cannot fully account for their enhanced gain in body weight. It is possible that the corpulent rats are more efficient than lean rats in converting equivalent amounts of food (g/U of body weight) into body fat. A similar pattern of body weight gain was reported by Michaelis et al.[12] who showed that the La/N-cp rat is hyperphagic. It has been reported for the fatty Zucker rat that hyperinsulinemia contributes to hyperphagia, which enhances fat deposition.[6,27] High corticosterone levels combined with the high insulin levels in corpulent rats could lead to hyperphagia. This has also been shown in other genetically obese animals.[6,28] However, the liver weight/body weight ratio was not significantly different in the obese animals when compared with the controls (*Table 1*).

Our results showed that plasma cholesterol and triglycerides were greater in the obese rats by 71.2% ($P < 0.01$) and 912% ($P < 0.01$), respectively, than controls (*Table 2*). Hepatic cholesterol and triglyceride concentrations were greater in the obese rats by 111%, ($P < 0.01$) and 43.2% ($P < 0.05$), respectively (*Table 2*). Similar changes in plasma lipids were observed even when the obese rats were pair-fed with the control group. Michaelis et al.[12] have reported the serum triglyceride concentration to be eight times higher in corpulent than in lean rats, while Izpisua et al.[29] reported serum cholesterol concentrations to be greater in corpulent rats than lean ones initially, which increased 2-fold following cafeteria feeding. In another study by Dolphin et al.,[30] total cholesterol in serum was 2-fold higher and triglycerides were 40- to 75-fold higher in obese animals than their lean counterparts. Our results are thus in conformity with the ones already reported by other workers.

Table 1 Body weight gain and liver weights of obese and control LA/N-cp rats

Parameter	Control	Obese	% difference
Body weight (g)			
Initial	143.6 ± 34.3	177.3 ± 18.7	
Final	216.2 ± 14.9	311.2 ± 18.7	
Body weight gain (g)	72.6 ± 4.3	133.9 ± 9.7	84 ($P < 0.001$)
Relative liver weight (g/100 g of body wt)	2.6 ± 0.06	2.9 ± 0.53	11.5 (NS)

Each value is the mean ± SE from five rats in each group and the statistical significance of differences between the groups was determined by Students *t*-test.

Table 2 Hepatic and plasma cholesterol and triglycerides in the lean and obese LA/N-cp rats

Fraction	Control	Obese	% difference	*P* value
Plasma				
Cholesterol (mmol/L)	1.69 ± 0.07	2.90 ± 0.58	71.2	<0.01
Triglycerides (mmol/L)	0.23 ± 0.02	2.31 ± 0.78	912	<0.01
Liver				
Cholesterol (nmol/g tissue)	6.52 ± 2.19	13.8 ± 2.2	111	<0.01
Triglycerides (nmol/g tissue)	13.3 ± 2.61	19.06 ± 3.92	43.2	<0.05

The maintenance of animals and details of the experiment have been described in the Methods and materials section. Each value is the mean ± SE from five rats in each group. Statistical significance of the differences between the groups was determined by Students *t*-test.

Comparative hepatic activities of ACX, FAS, HMGR, and 7αH in LA/N-cp obese rats and their lean counterparts are presented in *Table 3*. The rate-limiting enzymes studied here can be broadly divided into three categories: the key enzymes that are involved in fatty acid synthesis, namely, FAS and ACX; HMGR, which is the rate-limiting enzyme for cholesterol biosynthesis; and 7αH, which is the rate-limiting enzyme for cholesterol degradation to bile acids.

Fatty acid synthesis

With respect to fatty acid synthetic pathway, the activities of the two cytosolic enzymes, ACX and FAS were significantly elevated by 90% ($P < 0.05$) and 125% ($P < 0.01$), respectively, in the obese group as compared with controls when they were fed ad libitum. Significantly, even when the obese group was pair-fed with the lean control group, significant elevations of hepatic FAS by 75% and ACX by 105% were observed. Thus, the increased activities of lipogenic enzymes in the obese strain is not due to hyperphagia per se but to the increased secretion of the lipogenic hormone, insulin. FAS is a multienzyme complex responsible for the synthesis of long-chain fatty acids. These results indicate a higher rate of fatty acid synthesis in the obese group as compared with the lean group. Thus, from these data it is clear that these two rate-limiting enzymes may be working synergistically to synthesize more fatty acids in the obese strain of rats. This, in turn, may contribute to increased synthesis of triacylglycerols, the major storage form of fat. Increased triglyceride levels in plasma, liver, and adipose tissue, in obese rats have been reported by several workers.[12,29–31]

Cholesterol synthesis

With regard to cholesterol synthetic pathway, it was observed that there was a 2-fold higher activity of the enzyme HMGR ($P < 0.001$) in the obese as compared with the lean littermates (*Table 3*). The magnitude of the difference between the obese and lean-control group was 85% even when they were pair-fed. This again rules out hyperphagia to be the major cause for the increased activity of HMGR in the obese group. In fact, we have previously demonstrated that the administration of insulin to diabetic fasted lean Wistar rats promptly stimulated hepatic HMGR activity within 2 hr[32] implying that the primary regulatory factor for the increased lipogenic activities of the obese is possibly insulin since this strain is hyperinsulinimic.[33] The increased activity of HMGR in the liver implies that the rate of hepatic cholesterol synthesis is increased 2-fold in the obese group as compared with the lean group.

Cholesterol degradation

The major pathway of degradation of cholesterol in the mammalian system is its conversion to bile acids. The rate-limiting step for cholesterol degradation to bile acids is the conversion of cholesterol to 7α-hydroxycholesterol, catalyzed by 7αH.[34–36] Hepatic activity of this enzyme was decreased by 28% ($P < 0.001$) in the obese rats when compared with those in lean rats (*Table 3*). This could account for the decreased rate of elimination of cholesterol from the body of obese animals. The activity of 7αH in the obese group was decreased by 22% compared with the lean controls even when they were pair-fed. It is well known that hepatic 7αH activity exhibits diurnal variation with increased activity on feeding and decreased after starvation.[37,38] This again proves that hyperphagia is not responsible for the observed decrease in 7αH activity.

Based on these findings, it seems reasonable that an increased rate of cholesterol synthesis coupled with the decreased rate of cholesterol degradation in LA/N-cp obese rats as compared with their control counterparts may well be

Table 3 Hepatic activities of Acetyl CoA carboxylase, fatty acid synthetase, 3-hydroxy-3-methylglutaryl CoA reductase, and 7α-hydroxylase in LA/N-cp rats

Enzyme	Control	Obese	% Change	*P* value
ACX (μmol malonyl-CoA g^{-1} hr^{-1})	7.3 ± 1.7	14.0 ± 3.5	+90	<0.05
FAS (μmol acetyl units g^{-1} hr^{-1})	24.0 ± 4.0	54.0 ± 7.0	+125	<0.01
HMGR (nmol mevalonate g^{-1} hr^{-1})	130.0 ± 6.0	260.0 ± 9.5	+100	<0.001
7αH (nmol 7αOH cholesterol g^{-1} hr^{-1})	18.9 ± 0.5	13.7 ± 0.8	−28	<0.001

The experimental details are the same as in Table 2. The values are mean ± SE. Sample size is five.

responsible for increased accumulation of cholesterol in this strain.

Very little literature is available on lipogenic enzymes in genetically obese rats. Hormonal and enzymatic alterations and impairment in thermogenesis observed in obese rats are some of the contributing factors in the etiology of obesity. Lakshman et al.[19] reported greater hepatic activities of FAS, ACX, and HMGR and a lesser activity of 7αH in the BHE rats when compared with the normal Wistar rat. Alterations in the activities of other regulatory enzymes and hormones under different conditions have been reported by other investigators. Ellwood et al.[11] determined liver and epididymal fat glucose-6-phosphate dehydrogenase (G6PD) and malic enzyme and found greater activity in the LA/N-cp rats as compared with the lean ones. Increased lipogenic enzyme activity could account for the increased fat deposition observed in the corpulent rats. In addition, there was also an increased gluconeogenic activity in the corpulent rat. Increases in hepatic and kidney fructose 1,6-diphosphatase and phosphoenolpyruvate carboxylase were observed in the corpulent rats when compared with controls. Kava et al.[39] found increased adipose tissue lipoprotein lipase activity in the Wistar fatty rat. Higher levels of insulin, corticosterone, and growth hormones and lower levels of glucagon in the plasma were reported by Martin et al.[27] in the Zucker obese rat when compared with the controls. Similarly, Bhathena et al.[40] observed plasma glucagon levels, insulin and glucagon binding to target cells to be lower in the LA/N-cp rat when compared with the lean rat. They concluded that these changes are important contributors to hyperlipidemia and obesity.

From this study it can be seen that the LA/N-cp obese rats exhibited at least twice the hepatic activities of the key enzymes of fatty acid and cholesterol synthesis as compared with their lean controls. A lower activity of 7αH in the obese group suggests a lowered rate of cholesterol degradation to bile acid synthesis under obese conditions. These results suggest that enhanced syntheses of body fat and cholesterol in obese animals, accompanied by decreased degradation, might be responsible for the increased accumulation of cholesterol and fat in the LA/N-cp obese strain which may well be controlled by hormonal factors, especially insulin.

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