Rates of sterol synthesis in the liver and extrahepatic tissues of the SHR/N-corpulent rat, an animal with hyperlipidemia and insulin-independent diabetes

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Abstract The SHR/N-corpulent rat is a new genetically obese strain that exhibits both insulin-independent diabetes and hyperlipidemia. The present studies were undertaken to characterize various parameters of cholesterol metabolism in this model. At 11 weeks of age, the obese animals had markedly elevated plasma cholesterol, triglyceride, glucose, and insulin concentrations and elevated hepatic triglyceride concentrations compared to their lean littermates. The additional cholesterol in plasma was carried in the fractions of density < 1.006, 1.020–1.055, 1.055–1.095, and 1.095–1.21 g/ml. In the obese rats the level of free cholesterol in the liver was decreased significantly while that of cholesteryl ester showed little change. Hepatic sterol synthesis was markedly suppressed in the obese animals. However, the rate of sterol synthesis in the small intestine and other extrahepatic tissues generally remained unchanged. Although hepatic synthesis was suppressed, whole animal sterol synthesis in the obese rats was similar to that in the lean controls. This resulted because, in the obese animals, not only was the reduced rate of hepatic synthesis partly balanced by a greater than 70% increase in liver mass, but the mass of the small intestine and adipose tissue was also increased more than 30% and 4-fold, respectively, thereby making these tissues quantitatively more important sites of sterol synthesis. When obese rats were pair-fed to the intake of their lean littermates for 10 weeks, there was only a modest reduction in body weight and plasma cholesterol concentration, and the rate of hepatic sterol synthesis remained very low. The suppression of synthesis in the liver also persisted when the obese rats were fed surfomer, a drug that specifically blocks cholesterol absorption. In contrast, feeding cholestyramine restored the rate of hepatic sterol synthesis to that found in lean animals. Bile acid pool size in the obese males and females was 2.5-fold greater than in their lean controls. The suppression of hepatic sterol synthesis in this model may be due to a change in the enterohepatic circulation of bile acids arising from an expanded pool or, alternatively, it may represent a compensatory response to overproduction of sterol and its precursors in the intestinal and adipose compartments. —**Turley, S. D., and C. T. Hansen.** Rates of sterol synthesis in the liver and extrahepatic tissues of the SHR/N-corpulent rat, an animal with hyperlipidemia and insulin-independent diabetes. *J. Lipid Res.* 1986. **27:** 486–496.

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Hyperlipidemia and conditions such as coronary heart disease and insulin-independent diabetes are frequently associated with obesity in humans (1–5). Furthermore, sterol balance and isotope kinetic studies have established that, on an ideal body weight basis, obese subjects produce up to twice as much cholesterol per day as lean individuals (6–9). Although it is not certain whether this overproduction contributes to the hypercholesterolemia that often occurs with excess body weight, it is known to be associated with the secretion of excess cholesterol in bile, a condition that predisposes obese individuals to the development of cholesterol gallstone disease (10–15).

It remains to be established whether the over-production of cholesterol in obesity occurs in the liver and/or in the extrahepatic compartment. Several studies have been carried out in which the rate of sterol synthesis has been measured in vitro in the liver and various extrahepatic tissues taken from obese and normal-weight individuals (16–20). Although the mass of the adipose organ is greatly expanded in the obese subject, it would appear not to be the principal site of overproduction because the rate of sterol synthesis in adipose tissue is only a fraction of that found in the liver and intestine (17). It has generally been found that there is no change in the rate of cholesterol synthesis in the liver of obese subjects (17, 19, 20). If this is the case, then, because of an expanded liver mass, total hepatic sterol production in overweight subjects must exceed that in lean individuals (17). However, this would have a significant impact on cholesterol production in the body as a whole only if the liver in humans is normally a major site of cholesterol production as it is in the rat (21, 22). Unfortunately this question has not been resolved, but the balance of evidence tends to favor the possibility

Abbreviations: SHR, spontaneously hypertensive rat; *cp*, corpulent; DPS, digitonin-precipitable sterols; SA, specific activity.

that in humans, as in the rabbit, guinea pig and hamster, the liver is not the most important site of cholesterol production in the body (23, 24). Therefore, the excess cholesterol produced in obesity may not be generated entirely in the liver.

Although several strains of genetically obese rats, mice, and hamsters have been developed over the past two decades, relatively little use has been made of these models to further study the question of how obesity alters the production of cholesterol in the body as a whole (25–29). In the obese female Zucker rat and the obese mouse (strains *ob/ob* and *db/db*), hepatic sterol synthesis is suppressed, whereas in the obese male Zucker rat the rate of cholesterol synthesis both in the liver and in the animal as a whole is apparently unchanged (27, 28, 30–32).

The SHR/N-corpulent rat is a new genetically obese strain that develops hyperlipidemia and insulin-independent diabetes, a condition not found in either the Zucker or Koletsky strains (33). The objective of the present studies was to characterize various parameters of sterol metabolism in this model, in particular to study the impact of obesity on hepatic cholesterol production. The studies show that while the model exhibits many of the metabolic defects found in obese humans including hypercholesterolemia, hypertriglyceridemia, hyperinsulinemia and hyperglycemia, an enlarged steatic liver, and an expanded bile acid pool, overall it does not manifest the profound increase in whole body sterol synthesis found in obese humans because of marked suppression of cholesterol synthesis in the liver.

MATERIALS AND METHODS

Experimental animals and diets

The SHR/N-*cp* strain was developed by Dr. C. T. Hansen at the National Institutes of Health (33). The initial step in developing the strain was mating a Koletsky rat (26), which was heterozygous for the *cp* gene, to a spontaneously hypertensive rat (SHR). The SHR/N inbred strain was derived at the NIH from the Okamoto strain. Twelve backcrosses were carried out to eliminate the noncorpulent genes of the Koletsky strain. The SHR/N-*cp* strain differs from the SHR/N strain by the presence of the *cp* gene. Since corpulent rats do not reproduce, heterozygotes are used for breeding purposes. Mating yields three genotypes but only two phenotypes, i.e., homozygous (*cp/cp*) corpulent and heterozygous (*cp/+*) and homozygous (*+/+*)[1] lean rats. Based on normal genetic distribution, lean rats consist of two-thirds *cp/+* rats and one-third *+/+* rats.

The litters were weaned at 4–5 weeks at which time the corpulent offspring were visibly different from their lean littermates. All breeding and experimental stock were housed in plastic boxes containing wood shavings and were maintained in a room with alternating 12-hr periods of light (1500–0300 hr) and dark (0300–1500 hr). All animals were fed a plain pelleted diet (GR2+, Clark King & Co., Melbourne, Victoria) and had free access to water. The diet contained 53% carbohydrate, 20% protein, and 3.9% lipid. The fat content was 3.3% of which one-third was saturated and two-thirds was unsaturated. The cholesterol content of the diet was 0.23 mg/g (0.023%). The plant sterol content was not determined. In one study, lean and obese male rats were fed the plain diet containing either surfomer[2] (Monsanto Chemical Company, St. Louis, MO) or cholestyramine (Mead Johnson Research Center, Evansville, IN) each at a level of 2% (wt/wt) for 3 weeks.

In all studies except one involving pair-feeding, the diets were fed ad libitum and the animals were not deprived of food before the experiments. In one experiment obese males and females were pair-fed to the intake of their lean littermates for 10 weeks starting at 11 weeks of age. The amount of diet consumed by lean animals each day was measured and the same quantity was then given to each obese animal over the next 24 hr. The pellets were placed in a special compartment contained within the wire-mesh top that was fitted to each cage. Most studies were carried out when the animals were either 11 or 21 weeks of age. However, in the experiment with surfomer and cholestyramine, the animals were 14 weeks old at the time of study. All experiments were carried out at about the mid-dark phase of the lighting cycle.

Determination of sterol synthesis in vivo

Lean and obese animals were lightly anesthetized with diethyl ether, a 50-mCi bolus of [^{3}H]water (Amersham Australia Pty. Ltd., Sydney, New South Wales) was administered directly into a femoral vein, and they were left for 1 hr during which no food or water was given. The administration of the [^{3}H]water and all subsequent procedures were carried out under well-ventilated fume-hoods. The animals were then anesthetized again and 5 ml of blood was taken from the abdominal aorta and the specific activity of plasma water was measured (21). The whole animal was saponified with alcoholic KOH and aliquots were taken for the determination of [^{3}H]DPS (digitonin-precipitable sterol) content (21, 22). The rate of sterol synthesis was expressed as the μmol of [^{3}H]water incorporated into DPS per hr per whole animal.

[1]Normal allele is designated *+*.

[2]Surfomer (AOMA) is a nonabsorbable copolymer of α-olefin and maleic acid. It has been shown to block cholesterol absorption in humans and experimental animals (34, 35).

Determination of sterol synthesis in vitro

In several studies the rate of sterol synthesis in the liver and various extrahepatic tissues was measured in vitro. Lean and obese animals were anesthetized with diethyl ether and bled from the abdominal aorta. The liver, proximal and distal segments of the small intestine, white adipose tissue (from the retroperitoneal space), adrenal glands, kidney, and spleen were quickly removed and placed in cold 0.15 M NaCl solution. The segments of small intestine were rinsed and weighed, as was the liver. Tissue slices were prepared and incubated in 5 ml of Krebs bicarbonate buffer containing 10 mCi of [^{3}H]water (50 mCi in the case of adipose tissue) and 10 mM glucose. For the adipose tissue, several incubation blanks were set up in which 0.25 ml of 10 N sulfuric acid was added to the flask before the tissue slices. The flasks were gassed with 95% O_2 and 5% CO_2 and incubated for 90 min at 37°C in a metabolic shaker set at 120 oscillations per min (36). Under these conditions, tissue slices incorporate radiolabeled precursors into sterol as a linear function of time for at least 90 min (36, 37). The tissue sterols were extracted quantitatively and precipitated as the digitonides. The DPS were dried under vacuum and the digitonides were split with pyridine. The free sterols were extracted with diethyl ether, dried under vacuum, and assayed for ^{3}H content (21). The rate of sterol synthesis was expressed as the nmol of [^{3}H]water incorporated into DPS per g of tissue per hr.

Determination of adipose tissue mass

The mass of adipose tissue in lean and obese rats was calculated using values for the lipid content both of the whole animal and of the various individual tissues that together represent the bulk of the animal. The procedure for determining whole animal lipid content was similar to that already described (38). After removing the contents of the gastrointestinal tract and the hair, the animal was cut into pieces and mixed with two weights of anhydrous sodium sulfate. This mixture was passed through a tissue mincer and was then homogenized using a blendor. The resulting uniform mixture was weighed and quadruplicate 20-g aliquots were taken for the measurement of lipid content as described below. In a separate experiment, all the nonadipose organs (except pancreas, thymus, and ovaries) were removed and weighed. The mass of skeletal muscle in the lean animals was calculated using a ratio of 45.5 g of muscle/100 g body weight (39). This value was then multiplied by 0.74 to obtain an estimate of the skeletal muscle mass in the obese littermates (40, 41). The mass of the skin in each case was calculated using a value of 18.0 g/100 g (39). Total blood volume was estimated using a ratio of 6.0 ml/100 g for the lean animals, 5.1 ml/100 g for the obese males, and 4.1 ml/100 g for the obese females (42, 43). Aliquots of all the nonadipose organs, as well as subcutaneous and mesenteric adipose tissue and whole blood, were extracted in chloroform–methanol 2:1 and their lipid content was determined as described. From the values for the weights and lipid contents of all the individual organs, it was possible to obtain an estimate of the total amount of lipid that was contained in the nonadipose compartment of the animal. The difference between this value and the value for whole animal lipid content was taken to represent the quantity of lipid in the adipose tissue compartment. In the lean animals, the lipid content of subcutaneous adipose tissue (59%) was less than that of mesenteric tissue (83%) so an average lipid content of 71% was assumed. In contrast, the lipid content of adipose tissue from different sites in the obese animals was uniform at about 85% of the wet tissue weight. From the data for the amount of lipid present in the adipose compartment and the average lipid content of adipose tissue in the lean and obese animals, an approximate value for the mass of adipose tissue per 100 g body weight was determined for each group.

Determination of the distribution of cholesterol in plasma lipoproteins

Lean and obese rats were bled from the abdominal aorta into syringes containing a concentrated EDTA solution. Plasma from two animals was combined and equal volumes were adjusted to densities of 1.006, 1.020, 1.055, 1.095, and 1.21 g/ml and centrifuged simultaneously at 165,000 *g* for 40 hr. The cholesterol concentration in each density fraction was then measured (44).

Analytic procedures

The level of cholesterol in the plasma, liver, and diet was measured as described (44–46). Bile acid pool size was determined using the hydroxysteroid dehydrogenase assay and [24-^{14}C]taurocholic acid (New England Nuclear, Boston, MA) as an internal standard (47, 48). Plasma glucose and triglyceride levels were measured enzymatically using kits obtained from Boehringer Mannheim, West Germany. Hepatic triglyceride levels were also assayed enzymatically after aliquots of liver had been extracted in chloroform–methanol 2:1 (v/v). Plasma insulin levels were measured by radioimmunoassay using kits obtained from Amersham Australia Pty. Ltd. In the procedure for determining tissue lipid content, aliquots of minced tissue were extracted twice in chloroform–methanol 2:1 (v/v) and the combined extracts were washed with one volume of water for each four volumes of extract (49). The upper phase was discarded and the lower phase was filtered into a preweighed container, the solvent was evaporated, and the lipid content was determined gravimetrically.

Calculations

The equations used to calculate the specific activity of plasma water and the rates of whole animal sterol synthesis have been described in detail (21). In all experiments the data are presented as the mean ± 1 SEM. Differences in mean values were tested for significance using the unpaired two-tailed Student's *t*-test.

RESULTS

An initial study was carried out to measure the food intake of groups of lean and obese rats from 11 to 21 weeks of age. The average intake (g per animal per day) of the obese females (23.5 ± 0.3, n = 5) was about 60% higher than that of their lean littermates (14.8 ± 0.2, n = 7, $P < 0.05$). A similar difference was found in the intake of obese (32.7 ± 0.3, n = 6) and lean (20.8 ± 0.2, n = 8, $P < 0.05$) males.

The nonfasting plasma levels of cholesterol, triglyceride, glucose, and insulin in lean and obese animals at 11 weeks of age are shown in **Table 1**. The obese females and males each manifested about 2- to 5-fold higher plasma cholesterol and triglyceride concentrations than their lean littermates. While plasma glucose levels were also elevated by a similar extent in the obese males, the degree of hyperglycemia was much less in the obese females. However both groups of obese animals manifested pronounced hyperinsulinemia. Fasting for 12 to 14 hr did not abolish the hyperlipidemia but it did significantly lower plasma glucose concentrations in these animals. However, when given an oral glucose load, the corpulent animals rapidly became hyperglycemic confirming that they were diabetic (33).

The distribution of cholesterol in the various density fractions of plasma is shown in **Table 2**. In both the obese females and males, the excess cholesterol in plasma was carried in the fractions of density <1.006, 1.020–1.055, 1.055–1.095, and, to a lesser extent, in the 1.095–1.21 g/ml fraction. Although the data are not shown, almost 90% of the plasma triglyceride in the obese animals appeared in the d <1.006 g/ml fraction.

In the obese animals the mass of the liver was more than 70% greater than in the lean controls and the hepatic triglyceride level was elevated about 4-fold (Table 1). In contrast, the level of free cholesterol in the liver of the obese animals was significantly reduced while there was little change in the hepatic cholesteryl ester concentration.

The data in **Table 3** show that the higher body weights of the obese animals were due mainly to a 4- to 8-fold increase in body lipid content. In both absolute and relative terms, the obese females manifested a greater increase in body lipid mass than did their male counterparts. The data for the lipid content both of the whole animal and of essentially all the individual nonadipose organs were used to determine the proportion of body mass in lean and obese animals that consisted of adipose tissue. This estimate was obtained by first calculating the approximate quantity of body lipid that was contained in all the nonadipose tissues. Since the lipid content of individual tissues did not show a sex difference within the lean or obese groups, the values for males and females were averaged. The average lipid content of each tissue, expressed as a percentage of wet tissue weight, in four males and four females was as follows (values are mean ± SEM for the lean group followed by the obese group; an asterisk indicates that the values are significantly different at the $P < 0.05$ level): liver (4.9 ± 0.1; 10.2 ± 0.9*), small intestine (2.6 ± 0.1; 2.6 ± 0.1), large intestine (2.4 ± 0.4; 2.6 ± 0.1), stomach (3.5 ± 0.3; 4.7 ± 0.3*), spleen 2.3 ± 0.1; 2.2 ± 0.1), kidneys (3.7 ± 0.2; 3.9 ± 0.1), lungs (3.6 ± 0.7; 4.1 ± 0.7), heart (2.9 ± 0.1; 2.6 ± 0.4), thigh muscle (1.8 ± 0.1; 3.9 ± 0.2*), skin (5.9 ± 0.6; 7.2 ± 0.5), brain (7.7 ± 0.1; 7.8 ± 0.1), testes (2.6 ± 0.1;

TABLE 1. Plasma cholesterol, triglyceride, glucose, and insulin levels and hepatic cholesterol and triglyceride levels in lean and obese rats at 11 weeks of age[a]

Experimental Group	Body Weight	Liver Weight	Concentration in Plasma				Hepatic Cholesterol Concentration		Hepatic Triglyceride Concentration
			Cholesterol	Triglyceride	Glucose	Insulin	Free	Esterified	
	g	*g*	*mg/dl*			*μU/ml*	*mg/g*		*mg/g*
Female									
Lean (8)	174 ± 5	7.4 ± 0.2	63.9 ± 1.7	94.5 ± 15.3	145.5 ± 8.0	87.9 ± 14.0	2.47 ± 0.04	0.19 ± 0.01	10.7 ± 0.4
Obese (8)	285 ± 8[b]	13.4 ± 0.5[b]	114.8 ± 8.7[b]	355.1 ± 38.4[b]	199.1 ± 11.0[b]	1714 ± 192[b]	1.89 ± 0.04[b]	0.22 ± 0.03	40.9 ± 9.2[b]
Male									
Lean (8)	280 ± 15	11.6 ± 0.5	54.2 ± 2.2	73.2 ± 4.9	167.9 ± 5.4	414 ± 48.4	2.22 ± 0.04	0.33 ± 0.04	14.4 ± 2.3
Obese (8)	374 ± 16[b]	21.9 ± 0.9[b]	125.3 ± 4.3[b]	368.4 ± 67.2[b]	429.6 ± 24.7[b]	2255 ± 256[b]	1.66 ± 0.02[b]	0.47 ± 0.05	51.9 ± 10.2[b]

[a] Lean and obese rats that had been fed a plain chow diet ad lib were anesthetized, and bled from the abdominal aorta. The liver was removed, weighed, and aliquots of tissue were placed in chloroform–methanol 2:1. The plasma levels of cholesterol, triglyceride, glucose, and insulin and the hepatic levels of cholesterol and triglyceride were determined as described in Materials and Methods. The data represent the mean ± SEM of values obtained in the number of animals shown in parentheses.

[b] Significantly different from the value for the respective lean group at the $P < 0.05$ level.

TABLE 2. Cholesterol levels in different density fractions of plasma in lean and obese rats at 11 weeks of age[a]

Experimental Group	Cholesterol Concentration in Density Fraction					
	< 1.006 g/ml	1.006–1.020 g/ml	1.020–1.055 g/ml	1.055–1.095 g/ml	1.095–1.21 g/ml	> 1.21 g/ml
	mg/dl					
Female						
Lean (8)	5.9 ± 1.3	1.1 ± 0.6	6.9 ± 0.8	20.7 ± 1.7	26.6 ± 1.9	2.9 ± 1.1
Obese (8)	12.9 ± 2.7	0.7 ± 0.2	17.2 ± 4.3	47.4 ± 8.1[b]	34.5 ± 3.7	2.2 ± 0.7
Male						
Lean (8)	5.2 ± 0.6	0.6 ± 0.3	6.5 ± 0.6	15.1 ± 1.6	23.7 ± 1.5	3.3 ± 0.6
Obese (8)	17.9 ± 3.1[b]	0.7 ± 0.5	24.5 ± 2.8[b]	40.5 ± 4.0[b]	37.6 ± 3.1[b]	4.3 ± 0.6

[a] In the experiment described in Table 1, aliquots of plasma from two animals were combined, adjusted to different densities (g/ml), and fractionated. The cholesterol level in each fraction was determined as described in Materials and Methods. The data represent the mean ± SEM of values obtained in the number of animals shown in parentheses.

[b] Significantly different from the value for the respective lean group at the $P < 0.05$ level.

2.5 ± 0.1) and blood (0.53 ± 0.07; 0.74 ± 0.07). The higher lipid content of thigh muscle in the obese animals was probably due mainly to adipose contamination. From these data and the values for organ weights, it was calculated that there was about 2–3 g of lipid per 100 g body weight present in the nonadipose compartment. In the case of the lean animals, this represented a significant proportion of whole body lipid content (9–10 g of lipid per 100 g body weight). However, in the case of the obese animals, it was a much smaller fraction of the total lipid mass in the animal (34–42 g of lipid per 100 g body weight). The difference in the values for the lipid content of the whole animal and the nonadipose compartment was taken to represent lipid present in the adipose organ. Since the proportion of adipose tissue weight represented by lipid had also been measured (lean 71%, obese 85%), the data for the lipid content of the entire adipose compartment could be converted to a wet tissue weight. As shown in Table 3, on a whole animal basis, there was 4- to 8-fold more adipose tissue in the obese animals than in their lean littermates.

The next experiment examined whether the marked difference in body composition between lean and obese animals resulted in any change in the rate of sterol synthesis in the animal as a whole. As shown in **Table 4**, the rates of incorporation of [^{3}H]water into DPS in vivo in the lean and obese animals were generally not different. This was the case whether the data were expressed on a whole animal basis or in terms of lipid-free body weight. In a separate experiment, the rate of sterol synthesis in the liver, small intestine, and adipose tissue was measured in vitro so that the relative importance of each organ as a site for sterol production in obese animals could be assessed. The rates of incorporation of [^{3}H]water into DPS by slices of liver, small intestine, and mesenteric adipose tissue prepared from lean and obese rats at 11 weeks of age are shown in **Table 5**. In the obese females and males, hepatic sterol synthesis was suppressed 48% and 75%, respectively, but overall there was no change in synthesis in either the proximal or distal segments of the small intestine. Mesenteric adipose tissue, whether taken from lean or obese animals, manifested an extremely low rate of sterol synthesis.

The extent to which the suppression of hepatic sterol synthesis in the obese animals was balanced by the increase in liver mass differed between males and females. From the data in Table 5 it can be calculated that, on an in vitro basis, total hepatic sterol synthesis (nmol per liver

TABLE 3. Whole animal lipid content and estimated adipose tissue mass in lean and obese rats at 11 weeks of age[a]

Experimental Group	Body Weight	Whole Animal Lipid Content	Percent of Body Weight as Lipid	Estimated Adipose Tissue Mass	
	g	*g*	*%*	*g/100 g body wt*	*g/whole animal*
Female					
Lean (5)	177 ± 6	17.43 ± 1.13	9.8 ± 0.4	10.6	18.8
Obese (5)	329 ± 12[b]	137.11 ± 7.35[b]	41.6 ± 1.4[b]	45.7	150.4
Male					
Lean (5)	260 ± 6	26.09 ± 2.49	10.0 ± 0.8	10.8	28.1
Obese (5)	336 ± 18[b]	112.49 ± 7.83[b]	33.5 ± 1.5[b]	35.5	119.3

[a] Whole animal lipid content in lean and obese rats was measured as described in Materials and Methods. These data, together with the values for the lipid content of the tissues (given in the text) were used to estimate the mass of adipose tissue in the whole animal. The data represent the mean ± SEM of values obtained in the number of animals shown in parentheses.

[b] Significantly different from the value for the respective lean group at the $P < 0.05$ level.

TABLE 4. Rates of whole animal sterol synthesis determined in vivo in lean and obese rats at 11 and 21 weeks of age[a]

Experimental Group		Body Weight	Whole Animal Sterol Synthesis
		g	*μmol/whole animal per hr*
Female			
Lean	11 wk (7)	187 ± 3	20.31 ± 0.90
Obese	11 wk (7)	301 ± 7[b]	29.86 ± 2.51[b]
Lean	21 wk (5)	226 ± 6	20.38 ± 2.47
Obese	21 wk (4)	484 ± 14[b]	19.23 ± 1.85
Male			
Lean	11 wk (8)	289 ± 7	28.87 ± 1.92
Obese	11 wk (7)	346 ± 9[b]	27.01 ± 1.25
Lean	21 wk (4)	372 ± 5	25.32 ± 2.89
Obese	21 wk (5)	525 ± 29[b]	18.87 ± 2.77

[a] Lean and obese rats that had been fed a plain chow diet ad lib were given an intravenous injection of 50 mCi of [^{3}H]water and killed 1 hr later. The SA of plasma water and the content of [^{3}H]DPS in the whole animal were determined as described in Materials and Methods. The rate of sterol synthesis is expressed as the μmol of [^{3}H]water incorporated into DPS per whole animal per hr. The data represent the mean ± SEM of values obtained in the number of animals shown in parentheses.

[b] Significantly different from the value for the respective lean group at the $P < 0.05$ level.

per hr) in the obese females (3732 ± 331) was about the same as in their lean controls (3736 ± 362). However, in the males where hepatic sterol synthesis was more suppressed, the rate of synthesis in the whole liver was much less in the obese animals (1141 ± 343, $P < 0.05$) than in their lean littermates (2727 ± 451). In the obese animals, the mass of the small intestine was on average 37% greater than in the lean animals. Thus, while the rate of intestinal sterol synthesis was similar in both groups, overall the small intestine produced significantly more sterol in the obese animals than in the lean controls. This also applies to the adipose tissue, the mass of which was 4- to 8-fold greater in the obese rats.

Bile acid pool size was also measured in animals at 11 weeks of age. As shown in **Table 6**, the size of the bile acid pool, whether expressed on a whole animal or lipid-free body weight basis, was almost 2.5-times greater in the obese males and females than in their lean littermates.

The final series of experiments was designed to assess to what extent the suppression of hepatic sterol synthesis and the elevated plasma cholesterol levels in the obese animals may have resulted from the uptake of excess cholesterol from the intestine. It seemed that this may have occurred not only because the obese rats consumed 60% more food and hence more dietary cholesterol per day, but also because bile acids, which help facilitate cholesterol absorption (24), were present in much greater concentration in the intestinal lumen of the corpulent rats. In one study, male and female obese rats were pair-fed to the intake of their lean littermates for 10 weeks starting when the animals were 11 weeks of age. As shown in **Fig. 1**, the pair-fed obese animals, particularly the females, gained less weight than those that were fed ad lib. However, the weight of the pair-fed animals always remained significantly greater than that of the lean controls. The data in **Table 7** show that pair-feeding lowered, but did not abolish, the elevation in plasma cholesterol levels in the obese rats, and also that the suppression of sterol synthesis in the liver persisted despite the reduction in dietary cholesterol intake.

In a second study, lean and obese male rats were fed a diet containing either surfomer (a drug that blocks the absorption of dietary and biliary cholesterol (34, 35)) or cholestyramine, each at a level of 2% (wt/wt). The diets were fed ad lib for 3 weeks starting when the animals were 11 weeks old. As shown in **Table 8**, surfomer significantly increased hepatic synthesis in the obese animals but the rate remained much lower than in the lean controls. In contrast, in the obese animals fed cholestyramine, sterol synthesis in the liver was increased more than 7-fold to a

TABLE 5. Rates of sterol synthesis determined in vitro in the liver, small intestine, and adipose tissue of lean and obese rats at 11 weeks of age[a]

Experimental Group	Body Weight	Tissue Weight				Tissue Sterol Synthesis			
		Liver	Proximal Small Intestine	Distal Small Intestine	Adipose[b]	Liver	Proximal Small Intestine	Distal Small Intestine	Mesenteric Adipose Tissue
	g	*g*				*nmol/g per hr*			
Female									
Lean (6)	174 ± 5	6.7 ± 0.3	2.93 ± 0.04	2.64 ± 0.09	18.4 ± 0.5	557.2 ± 46.1	47.6 ± 3.4	195.9 ± 18.0	2.2 ± 0.4
Obese (5)	292 ± 9[c]	12.9 ± 0.5[c]	3.75 ± 0.17[c]	3.67 ± 0.20[c]	133.2 ± 4.2[c]	291.5 ± 30.5[c]	41.4 ± 5.8	241.9 ± 9.2[c]	1.8 ± 0.1
Male									
Lean (7)	289 ± 5	11.9 ± 0.3	4.30 ± 0.15	3.78 ± 0.10	31.2 ± 0.6	225.5 ± 32.7	73.5 ± 3.5	209.4 ± 21.4	1.8 ± 0.1
Obese (8)	357 ± 6[c]	20.5 ± 0.6[c]	5.85 ± 0.15[c]	5.57 ± 0.45[c]	126.7 ± 2.0[c]	55.4 ± 16.3[c]	74.2 ± 2.1	235.2 ± 17.7	1.4 ± 0.2

[a] Lean and obese rats that had been fed a plain chow diet ad lib were anesthetized and bled from the abdominal aorta. The liver, small intestine, and pieces of mesenteric adipose tissue were quickly removed and placed in cold 0.9% NaCl solution. The liver and proximal and distal halves of the small intestine were weighed. Aliquots of tissue slices were incubated in Krebs buffer containing [^{3}H]water, and the rate of incorporation of the [^{3}H]water into DPS was determined as described in Materials and Methods. The rate of sterol synthesis is expressed as the nmol of [^{3}H]water incorporated into DPS per g of tissue per hr. The data represent the mean ± SEM of values obtained in the number of animals shown in parentheses.

[b] Values were estimated using the body weight of the animal and the data given in Table 3 for the mass of adipose tissue per 100 g body weight.

[c] Significantly different from the value for the respective lean group at the $P < 0.05$ level.

TABLE 6. Bile acid pool size in lean and obese rats at 11 weeks of age[a]

Experimental Group	Body Weight		Bile Acid Pool Size	
	Actual	Lipid-free[b]		
	g	*g*	*μmol/whole animal*	*μmol/100 g lipid-free body wt*
Female				
Lean (4)	184 ± 7	166 ± 6	64.4 ± 4.3	38.7 ± 1.9
Obese (4)	308 ± 11[c]	180 ± 6	155.2 ± 22.3[c]	86.2 ± 11.7[c]
Male				
Lean (4)	274 ± 7	247 ± 6	100.4 ± 6.4	40.5 ± 1.7
Obese (4)	356 ± 11[c]	236 ± 7	250.3 ± 35.4[c]	107.2 ± 17.2[c]

[a]The whole small intestine and its contents were removed and placed in a beaker containing ethanol. Bile acid pool size was measured as described in Materials and Methods. The data represent the mean ± SEM of values obtained in the number of animals shown in parentheses.
[b]Lipid-free body weights were calculated from the actual weight and the body lipid content data given in Table 3.
[c]Significantly different from the value for the respective lean group at the $P < 0.05$ level.

rate that was similar to that found in the lean group fed the control diet. Neither drug reduced plasma cholesterol levels in the obese animals.

DISCUSSION

These studies demonstrate that the SHR/N-*cp* rat exhibits several major metabolic changes including hyperlipidemia, insulin-independent diabetes, an enlarged steatic liver, an expanded bile acid pool, and a markedly depressed rate of hepatic sterol synthesis. Two points concerning the characteristics of this model warrant emphasis. First, the metabolic changes are present in all the corpulent offspring but are generally more pronounced in the obese males than in their female counterparts. Second, restricting the caloric intake of the obese animals has relatively little effect on their body weight gain. This indicates that this strain of corpulent rat, like the Zucker rat, utilizes dietary energy more efficiently (50). While the degree of corpulence could probably be dramatically reduced by more severe caloric restriction, data relating to hepatic sterol metabolism in such animals would be difficult to interpret because of the compounding effect of fasting.

In recent years, [^{3}H]water has been used extensively to measure sterol synthesis in vivo in several species under a variety of metabolic conditions (23, 51, 52). The application of this technique in the present studies has shown that this strain of genetically obese rat, like the Zucker rat (32), does not exhibit the marked increase in whole body sterol synthesis that is characteristic of obesity in man. This species difference exists irrespective of how whole body sterol synthesis rates are expressed. Thus, the daily rate of sterol synthesis in obese subjects, when expressed per kg of actual body weight, is not different from that in lean individuals (9). In contrast, when the data for sterol synthesis in the corpulent rats are expressed this way, the obese animals actually synthesize less sterol per kg body weight than lean animals. However, such comparisons are much less meaningful than those made on the basis of body weights that have been corrected for differences in body lipid content.

On an ideal body weight basis, obese subjects synthesize up to twice as much sterol per day as lean individuals (6–9). Such marked overproduction does not occur in the obese rat because hepatic sterol synthesis is suppressed, a condition not found in the obese human (17, 19, 20). In the 11-week-old corpulent male rats, sterol synthesis in the liver was suppressed by 75% and, despite an increase in

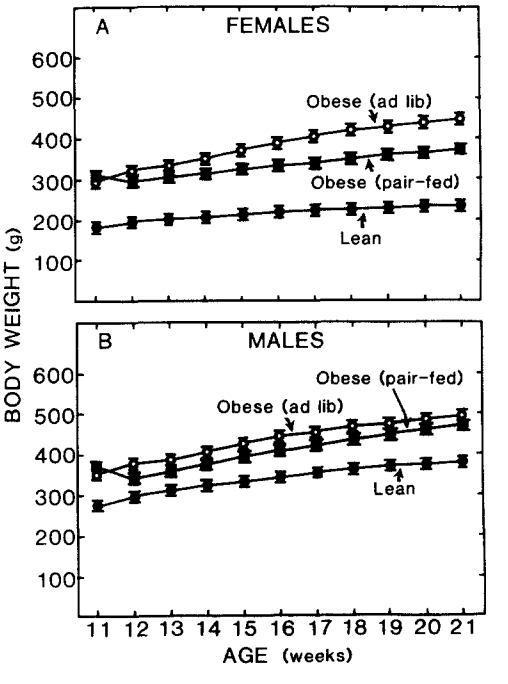

Fig. 1. Effect of restricting food intake on the body weight gain of obese rats. Female (panel A) and male (panel B) obese rats were pair-fed to the intake of their lean littermates for 10 weeks starting when the animals were 11 weeks of age. The data represent the mean ± SEM of values obtained from seven or eight animals in each group.

TABLE 7. Effect of restricting food intake on hepatic sterol synthesis and plasma cholesterol levels in obese rats[a]

Experimental Group	Body Weight	Liver Weight	Tissue Sterol Synthesis					Plasma Cholesterol Level
			Liver	Proximal Small Intestine	Adrenal	Kidney	Spleen	
	g	*g*	*nmol/g per hr*					*mg/dl*
Female								
Lean (8) (ad lib)	235 ± 3	8.1 ± 0.1	266.0 ± 35.4	33.0 ± 2.3	30.3 ± 3.5	6.8 ± 0.7	14.8 ± 2.0	84.1 ± 2.4
Obese (7) (ad lib)	448 ± 16[b]	18.4 ± 0.9[b]	99.7 ± 9.2[b]	46.3 ± 5.6	21.9 ± 2.7	8.8 ± 0.8	12.0 ± 0.6	132.3 ± 10.4[b]
Obese (7) (pair-fed)	372 ± 7[bc]	12.6 ± 0.3[bc]	76.2 ± 12.4[b]	45.5 ± 5.8	31.7 ± 3.2[c]	8.4 ± 0.4	20.1 ± 1.2[bc]	109.8 ± 3.8[b]
Male								
Lean (8) (ad lib)	379 ± 4	12.9 ± 0.1	128.6 ± 25.5	57.2 ± 2.6	21.8 ± 2.1	7.8 ± 0.6	14.3 ± 1.5	54.2 ± 2.0
Obese (8) (ad lib)	498 ± 17[b]	29.2 ± 1.0[b]	14.2 ± 2.1[b]	57.7 ± 9.3	14.8 ± 1.4[b]	8.5 ± 0.7	11.3 ± 0.6	184.1 ± 11.7[b]
Obese (8) (pair-fed)	481 ± 8[b]	19.0 ± 0.4[bc]	18.3 ± 2.3[b]	46.7 ± 8.5	18.6 ± 2.5	8.3 ± 0.5	14.9 ± 1.5	126.0 ± 4.1[bc]

[a] Female and male obese rats were either fed ad lib or were pair-fed to the intake of their lean littermates for 10 weeks starting at 11 weeks of age. The animals were then anesthetized and bled from the abdominal aorta. The liver and various extrahepatic tissues were quickly removed and placed in cold 0.9% NaCl solution. Aliquots of tissue slices were incubated in Krebs buffer containing [^{3}H]water and the rate of incorporation of the [^{3}H]water into DPS was determined as described in Materials and Methods. The rate of sterol synthesis is expressed as the nmol of [^{3}H]water incorporated into DPS per g of tissue per hr. The data represent the mean ± SEM of values obtained in the number of animals shown in parentheses.

[b] Significantly different from the value for the respective lean group at the $P < 0.05$ level.

[c] Significantly different from the value for the respective obese (ad lib) group at the $P < 0.05$ level.

liver mass, total hepatic sterol production in the obese animals was less than half that in the lean controls. Although the small intestine and adipose organ, through hypertrophy, produced more sterol in the obese animals, the rate of whole animal sterol synthesis in the corpulent males did not exceed that in their lean littermates. In the 11-week-old obese female rats, sterol synthesis in the liver was suppressed by 48% but, because of an enlargement of the liver, total hepatic sterol production was similar to that in the lean animals. Hence, since the obese animals also produced additional sterol in the small bowel and adipose tissue, they manifested a marginally higher rate of whole body sterol synthesis. However, this difference is not evident in older corpulent females because sterol synthesis in the liver becomes suppressed to the extent that total hepatic production in the obese animals is less than that in age-matched lean controls (Table 7).

While the results of the in vivo studies make it apparent that a significant amount of additional sterol is produced in the extrahepatic compartment of the obese rat, the proportion of this sterol that is generated within the small intestine and adipose organ, or in any other tissue, cannot be determined from the rates of [^{3}H]DPS synthesis described here since they were measured under in vitro

TABLE 8. Effect of feeding surfomer or cholestyramine on hepatic sterol synthesis and plasma cholesterol levels in lean and obese male rats[a]

Experimental Group	Diet	Body Weight	Liver Weight	Hepatic Sterol Synthesis	Plasma Cholesterol Concentration
		g	*g*	*nmol/g per hr*	*mg/dl*
Lean (5)	Control	312 ± 11	11.8 ± 0.4	290.0 ± 24.8	57.5 ± 2.4
Lean (6)	Surfomer	324 ± 3	11.9 ± 0.2	455.8 ± 58.5[b]	59.3 ± 1.7
Lean (4)	Cholestyramine	298 ± 11	11.6 ± 0.6	951.0 ± 60.6[b]	47.0 ± 2.8[b]
Obese (8)	Control	404 ± 6[b]	21.2 ± 0.7[b]	37.6 ± 3.7[b]	134.0 ± 8.7[b]
Obese (5)	Surfomer	411 ± 5[b]	20.0 ± 0.9[b]	70.1 ± 4.7[b,c]	133.6 ± 5.8[b]
Obese (4)	Cholestyramine	387 ± 10[b]	15.7 ± 0.4[b,c]	281.9 ± 33.9[c]	124.7 ± 12.5[b]

[a] Lean and obese male rats were fed either a plain chow diet (control) or the same diet containing either surfomer (2% wt/wt) or cholestyramine (2% wt/wt) ad lib for 3 weeks starting when the animals were 11 weeks of age. The animals were then killed and the rate of hepatic sterol synthesis and plasma cholesterol levels were measured as described in Materials and Methods. The data represent the mean ± SEM of values obtained in the number of animals shown in parentheses.

[b] Significantly different from the value for the lean group fed the control diet at the $P < 0.05$ level.

[c] Significantly different from the value for the obese group fed the control diet at the $P < 0.05$ level.

conditions. This applies particularly to adipose tissue which synthesizes significant quantities of methyl sterols that can be readily transported to other organs for eventual conversion to cholesterol, and squalene which is stored in the adipocyte and turned slowly over to sterol (18). Thus the contribution of the adipose organ to the generation of excess cholesterol in the obese rat has been underestimated in the present studies. It should be emphasized, however, that even when these cholesterol precursors are taken into account, the adipose organ, at least in obese humans, ultimately generates only a small proportion of the excess cholesterol that the body synthesizes (16, 18).

An additional problem in assessing the relative importance of the adipose organ as a site of sterol synthesis in lean and obese rats is that the total mass of the tissue had to be measured indirectly. The values obtained for the mass of adipose tissue per 100 g body wt are probably overestimates because the measurements of total body lipid content included complex lipids in membranes as well as all the lipid that is stored as triglyceride. In the lean animals, the proportion of total lipid consisting of complex lipids would be greater than in the obese animals, and therefore the error in the adipose tissue measurement would be higher. The exact magnitude of this error is difficult to determine. By direct dissection, the adipose tissue mass of lean male rats has been estimated to be about 7% (39) of body weight, whereas a value of 10.8% was obtained in these studies. The difference between these two values could be related to strain differences or to methodology; it is not possible to distinguish which. However, the error in measuring adipose organ mass in the obese animals is much less in view of the overwhelming amount of triglyceride in fat tissue compared to complex lipids.

Since the corpulent rat fails to overproduce cholesterol like obese humans because of suppression of synthesis in the liver, the cause of this condition must now be established. At least four possible explanations can be considered. One is that there is a primary defect in the sterol biosynthetic pathway. However, this seems unlikely because the suppression was evident only in the liver and varied markedly with the age and sex of the animal. In addition, hepatic sterol synthesis in the obese animals could be restored to a rate similar to that in lean animals by cholestyramine feeding, indicating that the liver does not have an inherently limited capacity for sterol production.

A second possibility is that the suppression resulted from the delivery of increased quantities of chylomicron cholesterol to the liver. This seemed likely since the obese animals had a greater dietary cholesterol intake and a much larger bile acid pool, which would promote the absorption of cholesterol from the intestine (24). However, this appears not to be the case since the rate of hepatic sterol synthesis remained very low in the obese animals that were either pair-fed to the intake of their lean littermates or given surfomer to block the uptake of cholesterol from the intestinal lumen. In addition, the marked suppression occurred in the face of very little increase in the hepatic cholesteryl ester level. When the delivery of chylomicron cholesterol to the liver is increased in the Sprague-Dawley rat, the resultant suppression of hepatic synthesis is accompanied by a marked increase in the level of cholesteryl ester (53, 54).

A third explanation is that since excess cholesterol, or its precursors, are synthesized in the small intestine and adipose tissue, the liver compensates for this by reducing its own rate of synthesis. The transport of the excess sterol from the extrahepatic compartment to the liver could be facilitated through lipoproteins, but there is currently no information on how this would occur.

Finally, the suppression of hepatic synthesis may be related to the expansion of the bile acid pool. The increased pool size probably results from the hypertrophy of the liver and small intestine, which would potentially allow an increase in both the production and efficiency of reabsorption of bile acids. While most evidence suggests that bile acids do not directly regulate cholesterol synthesis in the normal rat (53), the finding that cholestyramine derepresses hepatic synthesis in this corpulent strain implies that the low rate of synthesis may be due, at least partly, to a greater flux of bile acids through the liver. Further studies are being carried out to elucidate the mechanisms that regulate hepatic cholesterol production in this model.

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