Regulation of cholesterol storage in adipose tissue

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Abstract Adipose tissue is a major site of cholesterol storage. In an attempt to define mechanisms controlling this process, a variety of nutritional and metabolic alterations were employed and their effects on adipose tissue cholesterol levels were determined by direct chemical analysis. When rats were raised on Purina chow, a linear increase in the cholesterol/DNA ratio in relation to animal weight (from 120 g [5–6 wk] to 700 g [2 yr]) occurred. The rate of cholesterol accumulation was related to the dietary cholesterol load. Cholesterol accumulation by adipose tissue also occurred in rats raised on a cholesterol-free diet and reached levels exceeding those observed in animals fed on a diet containing 0.05 or 0.1% (w/w) cholesterol. In rats maintained on semisynthetic diets containing 0 to 5% (w/w) cholesterol, the serum cholesterol concentration was inversely related to the dietary concentration, suggesting that feedback inhibition of cholesterol formation may be an important determinant of serum cholesterol levels in this species. Early dietary alterations affected adipose tissue levels later in life. Net cholesterol mobilization from adipose tissue also occurred after acute starvation. Comparison of obese mice with nonobese littermate controls showed that the size of the adipose cholesterol pool was proportional to the degree of adipocity because the amount of cholesterol stored per unit glyceride mass was identical. Adipose tissue cholesterol was not affected by animal sex. Thus, adipose tissue cholesterol levels were dependent on animal age, dietary cholesterol load, early nutritional deprivations, and the size of the adipose organ itself.

Supplementary key words cholesterol mobilization · diet · age · cholesterol pools · slowly exchangeable cholesterol

Adipose tissue contains one of the largest pools of exchangeable cholesterol in man as well as in lower animals (1, 2). Although it has long been assumed that cholesterol in tissues exists as a structural component of membranes, adipose tissue is an exception to this general rule. Adipose tissue accumulates cholesterol in amounts far exceeding those required for cellular membrane elements. Thus, the cholesterol/phospholipid molar ratio in fat tissue exceeds that of any known mammalian cell or membrane, including red blood cells or myelin (3). Analysis of the cellular and the subcellular distribution of cholesterol in fat tissue (2) showed that most of the cholesterol in fat tissue was localized in the central oil droplet of the fat-storing adipocyte. Although the precise mechanism responsible for the accumulation of the cholesterol in fat tissue is not known, its dynamic nature was established by analysis of tissue specific activity and serum specific activity at various times after intravenous administration of [4-^{14}C]cholesterol to rats. It was shown that adipose tissue cholesterol exists in dynamic equilibrium with serum cholesterol and that the cholesterol in adipose tissue has the kinetic characteristics of a slowly exchangeable pool ($t_{1/2}$ = 27 days) consistent with its role as a storage form of cholesterol (2).

In the present study we examined a number of nutritional and metabolic factors that may affect cholesterol storage in rat adipose tissue. The results show that the size of the adipose tissue cholesterol pool was affected by animal age, acute fasting and refeeding, early nutritional experiences, the dietary cholesterol load, and, in one form of genetic obesity (ob/ob mice), the size of the adipose organ itself.

MATERIALS AND METHODS

Animals, diets, and preparation of tissue fractions

Male Wistar rats and male genetically obese hyperglycemic mice (C57BL/65-ob Bar Harbor) used in this study were separately caged and were fed Purina chow and tap water ad lib. The total lipid content of the chow was examined on two occasions and found to be 4.3 and 5.4 g/100 g dry weight. Analysis of the nonsaponifiable fraction showed a total ferric chloride–sulfuric acid-reactive sterol content of 60–77 mg/100 g dry weight. Of this material, 82.2%, as measured chemically, cochromatographed with [4-^{14}C]cholesterol on plates using ethyl ether–pet ether–glacial acetic acid 80:20:2 as the solvent

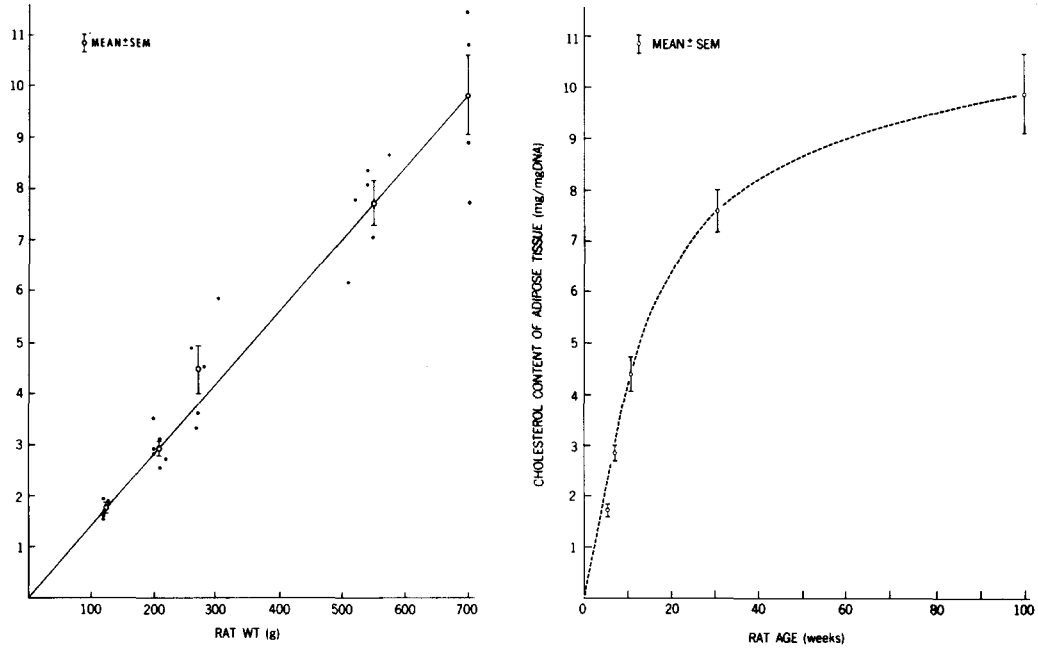

Fig. 1. Effect of rat age and weight on cholesterol content in adipose tissue. Epididymal fat pads of five groups of rats ranging in age from 6 wk (120 g) to 2 yr (700 g) were homogenized and extracted, and cholesterol and DNA were measured as described in Materials and Methods.

system. As judged by gas–liquid chromatography, 36% of the feed sterols was cholesterol, 15% campesterol, 49% sitosterol, and a trace amount of high molecular weight sterol. Thus the cholesterol content of Purina chow was 20 mg/100 g dry weight. In experiments on the effects of dietary cholesterol on growth (Fig. 3) and adipose cholesterol content (Figs. 4 and 5), a semisynthetic diet obtained from Nutritional Biochemicals, Cleveland, Ohio, was employed. The composition of this diet was "vitamin-free" casein, 20.4%; Alphacel cellulose, 16.0%; sucrose, 56.7%; salt mixture U.S.P. XIV, 3.9%; corn oil, 3.0%; and vitamin supplements. Cholesterol was added to the diet in final concentrations of 0.05%, 0.1%, 0.5%, and 5% (w/w) by first dissolving the cholesterol in corn oil, which was then mixed with the other dietary constituents.

The animals were stunned by a sharp blow to the head and killed by cervical dislocation. Epididymal fat pads were excised and weighed, taking care to include all tissue distal to the major vessel. Isolated fat cells were prepared by collagenase digestion of epididymal fat as described by Rodbell (4), with minor modifications (5). The bulk lipid fraction (lipid storage pool) was prepared by homogenizing epididymal fat tissue in 0.9% NaCl 1:4 (w/v) in a Potter-Elvehjem homogenizer and centrifuging the mixture at 300 *g* for 30 sec in a clinical benchtop centrifuge (International). The opaque infranatant fluid together with the stromal and vascular sediment was aspirated, and the oil layer including a white emulsion at the interface was heated at about 60°C for 3 min then centrifuged for 5 min at 300 *g*. The clear oil layer was assumed to be stored lipid and will be referred to as the "bulk lipid fraction."

Surgical procedures

In certain paired studies requiring animal survival, one epididymal fat pad was removed under light ether anesthesia through a 1-cm oblique skin incision superficial to the inguinal canal. The abdominal muscle fibers were separated by blunt dissection, and the peritoneal cavity was opened up to reveal an epididymal fat pad. The pad was delivered through the surgical opening, clamped just distal to the major vessels, and tied with size 0 silk before excision. The abdominal wall and peritoneum were sewn together with 0 silk and the skin was separately sutured using 0 silk.

Chemical analysis of lipids

Intact tissue fragments were homogenized in 10–15 ml of chloroform–methanol 2:1 (v/v) using a Potter-Elvehjem homogenizer; isolated cells were extracted with 10 ml of chloroform–methanol 2:1 (v/v) by mixing with a Vortex agitator. The extract was filtered through prewashed Whatman no. 1 filter paper to remove denatured protein, and the filter paper was rinsed twice with chloroform–methanol 2:1 (v/v).

The extracts were split into two phases by the addition of 0.2 vol 0.74% KCl, and the lower phase was washed three times with 8 ml of pure solvents upper phase containing 0.74% KCl (6). Aliquots of the resulting lipid extracts in chloroform were evaporated and weighed in tared

TABLE 1. Effect of sex on adipose tissue cholesterol levels

No.	Sex	Age	Weight	Cholesterol Content		
				Bulk Oil Phase[a]	Tissue Fragments	
		wk	*g*	*mg/g lipid*	*mg/g lipid*	*mg/mg DNA*
1	M	13	420	0.58	1.03	5.38
2	M	13	450	0.45	0.92	6.55
3	M	13	435	0.41	0.89	6.04
	Mean ± SEM			0.48 ± 0.05	0.95 ± 0.04	5.99 ± 0.46
4	F	13	270	0.49	1.13	6.48
5	F	13	305	0.48	0.88	6.94
6	F	13	275	0.56	0.86	6.71
	Mean ± SEM			0.51 ± 0.03	0.96 ± 0.09	6.71 ± 0.13

[a] 300–500-mg portions of epididymal and parametrial fat were homogenized in 4 ml of 0.9% NaCl and centrifuged at 700 *g* for 10 min at 4°C. The floating fat cake (bulk oil phase) was clarified by heating (60°C) and recentrifuged for 10 min at 20°C. Aliquots of the clarified oil were analyzed for cholesterol.

vessels to measure total lipid weight. To measure total cholesterol, portions of the lipid extracts were saponified, and the nonsaponifiable fraction was analyzed by the colorimetric procedure of Chiamori and Henry (7), employing a ferric chloride–acetic acid–sulfuric acid reagent as previously described (2). Serum cholesterol was measured by Chiamori and Henry's procedure (7). Previous gas–liquid chromatography and recovery experiments using argentation thin-layer chromatography have shown that more than 90% of the ferric chloride–acetic acid–sulfuric acid reaction material in the nonsaponifiable fraction was cholesterol (2, 8, 9).

DNA measurements

DNA was measured in the residue after extraction of adipose tissue or isolated cells with acetone and ether; the procedure of Burton was used (10). The solvents, inorganic materials, and unlabeled substrates were reagent grade. Solutions were made up with double-distilled water (glass).

RESULTS

Effect of age and animal weight on adipose tissue cholesterol levels

Adipose tissue cholesterol levels increased progressively and linearly in relation to animal weight in rats maintained on Purina chow for up to 2 yr (**Fig. 1,** *left*). When the data were expressed in terms of animal age, a rapid initial increase was observed between ages 6 and 11 wk, followed by a slower rate of increase beginning at approximately age 15–20 wk (Fig. 1, *right*). A similar increase in the adipocyte cholesterol levels was observed on analysis of cholesterol content of isolated adipocytes (**Fig. 2**). To account for the sharp increase in fat cell cholesterol in relation to age, the data were expressed in terms of total cell lipid (triglyceride). As shown in the legend to Fig. 2, the cholesterol/triglyceride ratio was also greater in the larger cells of old rats compared with those derived from 5-wk-old animals.

It is important to note that the cholesterol/DNA level in isolated fat cells (Fig. 2) was consistently greater than the corresponding levels in intact tissue fragments (Fig. 1). This difference has been noted before (2) and occurs because fat cells contain most of the tissue cholesterol but only one-half the tissue DNA, as about half the cells in this tissue are mast cells, fibroblasts, endothelial cells, and white blood cells.

Effect of sex on adipose cholesterol levels

Two groups of age-matched male and female rats (90–100 g, 4–5 wk) were maintained for a further 9 wk on Purina chow. At the time of tissue removal, the male rats weighed 50% more than the female ones. As shown in **Table 1,** the cholesterol content of epididymal fat expressed per unit lipid or DNA did not differ significantly from parametrial fat tissue. In addition, the cholesterol concentrations of the bulk oil fractions were the same, indicating an absence of difference due to sex.

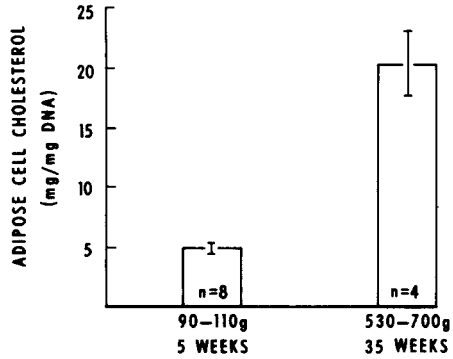

Fig. 2. Effect of age on cholesterol content in isolated adipose cells. Isolated epididymal fat cells from two groups of rats, 90–110 g (5–6 wk) and 530–700 g (35–36 wk), are compared. A fourfold increase in the cholesterol/DNA ratio occurred. The cholesterol/triglyceride ratio was also significantly higher in the older animals (0.71 ± 0.01 vs. 0.86 ± 0.08, mean ± SEM; n = 8 and 4, respectively; $P < 0.02$).

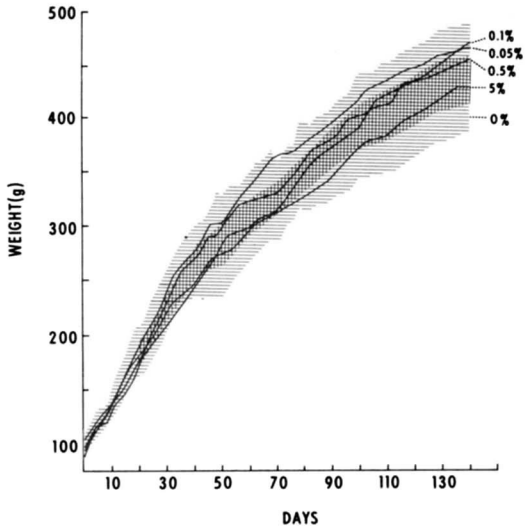

Fig. 3. Effect of dietary cholesterol level on rat growth curves. Five groups of rats weighing 90–110 g were kept on a semisynthetic diet supplemented with cholesterol (0–5%). The rats maintained on a cholesterol-free diet served as the reference group. The horizontal shading describes the range and the cross-hatching defines the means ± SD (n = 6) of the weights of the reference group. Each line describes the mean weight of a group of rats fed cholesterol at the levels shown. No significant differences between groups occurred.

Effect of dietary cholesterol load on adipocytes and serum cholesterol levels

To study the effect of dietary cholesterol level on adipose cholesterol storage, groups of rats were maintained on semisynthetic diets containing various levels of cholesterol as described in Materials and Methods. The growth curves (**Fig. 3**) show that animal growth patterns were identical and were not adversely affected by diets containing 0–5% cholesterol.

The adipocyte cholesterol level was greatest in rats maintained for 2 months on the 5% cholesterol diet, and the level decreased as the dietary concentration fell (**Fig. 4,** *left*). Of equal significance was the finding that adipocyte cholesterol also accumulated in rats maintained on a cholesterol-free diet and to a level significantly greater than that observed in rats on 0.05% and 0.1% cholesterol. Serum cholesterol levels were also affected by dietary cholesterol load (Fig. 4, *right*). The highest serum cholesterol concentration occurred in rats on zero cholesterol intake and the lowest in rats on the highest dietary cholesterol level. These observations obtained from rats fed the diets for 2 months differed significantly from those obtained after 5 months. After 5 months, serum and perirenal adipose tissue were analyzed for cholesterol (**Fig. 5**). It was not possible to prepare isolated fat cells from perirenal adipose tissue because of cell breakage (this is a common problem in obese or large [500 g] rats); therefore, total adipose tissue cholesterol and DNA were measured. Since well over 80% of fat tissue cholesterol in the older animals is in fat cells, the results are comparable to the data observed in isolated fat cells as shown in Fig. 4 (*left*). In Fig. 5, *left*, the results are expressed as percent deviation, using the zero cholesterol group as the reference level. The increase in adipose tissue cholesterol in rats maintained on 0.05% and 0.1% cholesterol was small (10%) compared with the zero cholesterol group and was not statistically significant. However, in rats maintained on 0.5% and 5.0% cholesterol, tissue levels were significantly higher than all the other groups. Serum cholesterol concentrations after 5 months on the diet (Fig. 5, *right*) were uniformly elevated and were not different between groups, in contrast to results obtained after 2 months (Fig. 4, *right*) when an inverse relationship between dietary cholesterol level and serum cholesterol concentration was observed.

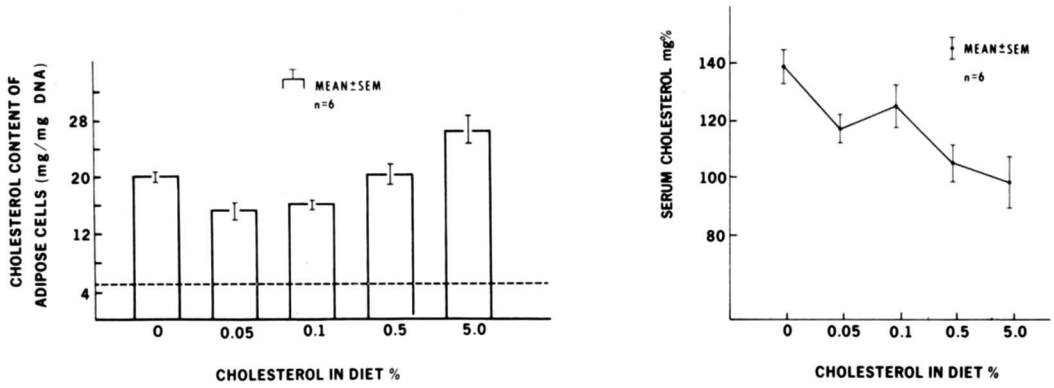

Fig. 4. *Left,* effect of dietary cholesterol level on adipose cell cholesterol concentration. Five groups of rats (90–110 g) were fed semisynthetic diets containing the amounts of cholesterol as shown. After 2 months the rats were anesthetized, one epididymal fat pad was removed from each rat, and isolated fat cells were prepared. The rats were kept on the diet for a further 3 months and examined (see Fig. 5). A control group of rats was processed at the beginning of the experiment to determine adipocyte cholesterol level; this group is represented by the broken line. The portions of the bars above the broken line represent the net increments of cholesterol during the 2-month period of feeding. Significance of differences between means (*t* test): 0 vs. 0.05%, $P < 0.02$; 0 vs. 0.1%, $P < 0.01$; 0 vs. 5.0%, $P < 0.02$; 0.05 and 0.1% vs. 5.0%, $P < 0.01$. *Right,* effect of dietary cholesterol level on serum cholesterol concentration. Blood was withdrawn from the rats by heart puncture just prior to removal of epididymal fat pads. The highest serum cholesterol level was in the animals on the cholesterol-free diet.

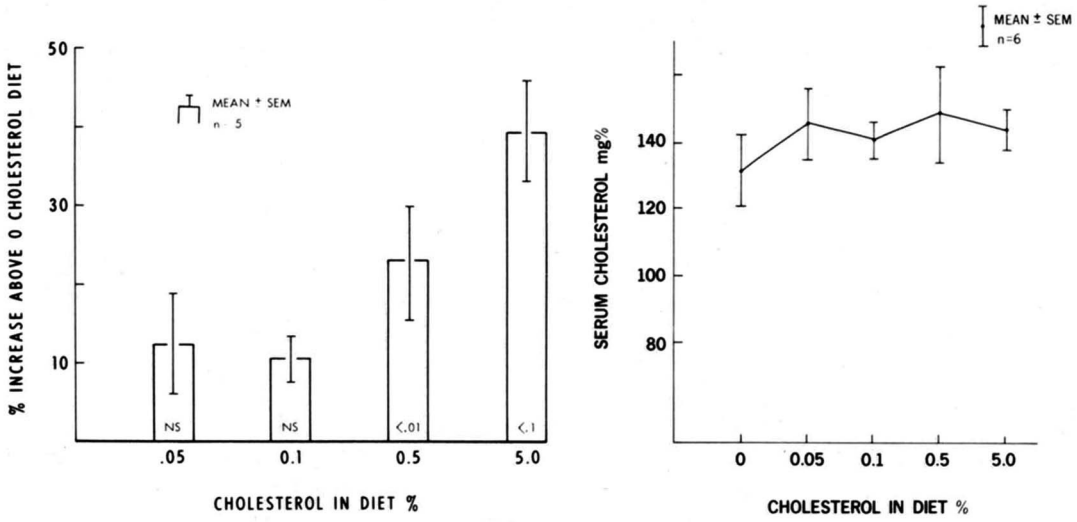

Fig. 5. *Left,* effect of dietary cholesterol level on adipose tissue after 5 months of feeding. Perirenal adipose tissue was taken from the rats surviving the experiment described in Fig. 4 and analyzed for cholesterol and DNA. Free cells were not isolated because of extreme fragility of adipocytes. Results are expressed as percentage increase of accumulated cholesterol using the zero cholesterol group as reference value. *Right,* effect of dietary cholesterol level on serum cholesterol level. After 5 months the serum cholesterol levels were uniformly elevated.

Effect of acute starvation on adipose tissue cholesterol levels.

Acute starvation resulted in a net mobilization of cholesterol from adipose cells. When the cholesterol content of isolated fat cells was measured on contralateral pads before and after 6 days of fasting (**Fig. 6**), a fall in the cholesterol/DNA ratio occurred in five out of six instances. The triglyceride content decreased in all six instances and at a rate appreciably greater than the fall in cholesterol level. This latter observation is more evident in **Fig. 7,** where the effect of fasting on cholesterol content is expressed in terms of triglyceride content. Here the cholesterol/triglyceride ratio increased in fat tissue, isolated cells, and the bulk lipid fraction in spite of net loss of cholesterol per cell. This indicates incomplete correspondence between cholesterol and triglyceride mobilization processes and suggests the preferential mobilization of glycerides over cholesterol.

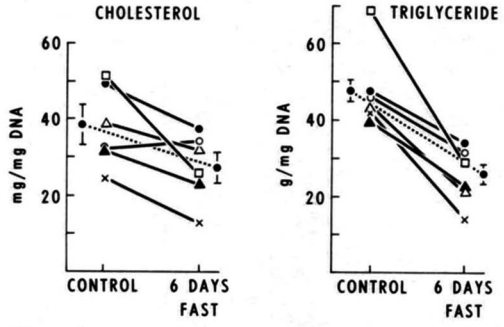

Fig. 6. Effect of acute starvation on cholesterol content in adipose cells. Six rats weighing 500–620 g (36 wk) were fasted 6 days. Body weight loss was 14.6 ± 1.0% (mean ± SD, n = 6). One epididymal fat pad was removed and, after 6 days of fasting, the contralateral pad was excised. Cholesterol/DNA and lipid/DNA ratios in isolated adipocytes were measured (see Materials and Methods). Each symbol represents one rat before and after fasting, so that each animal served as its own control. Broken line is the mean ± SEM.

Effect of early nutritional deprivation on cholesterol stores

In this study (**Fig. 8,** *open circles*) rats were intermittently fasted and refed for 10–11 wk in order to prevent weight gain. They were then allowed free access to food for a further period of 10 wk. Epididymal fat pads were taken at zero time, at 10 wk, and at 20 wk for analysis, and results were compared with tissue from the control animals (Fig. 8, *closed circles*), which were allowed free access to food throughout. As shown in **Table 2,** the cholesterol content per epididymal fat pad increased progres-

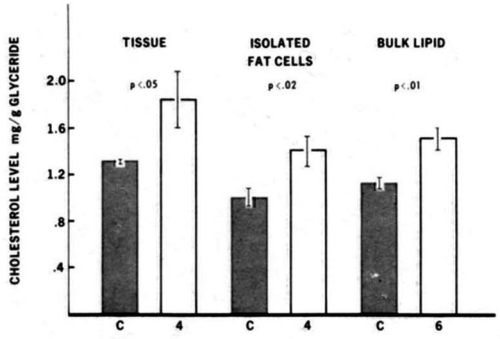

Fig. 7. Effect of acute starvation on cholesterol content in different adipose tissue preparations. In the pair of columns on the left, one epididymal fat pad from each of four rats (480–550 g) was taken before and after fasting for 4 days. In the center two columns, isolated fat cells were prepared from epididymal fat taken from each of seven rats (430–550 g) before and after fasting for 4 days. On the right, the epididymal fat from each of nine rats (450–600 g) was homogenized and centrifuged to obtain the bulk lipid fraction (see Materials and Methods) from specimens taken before and after fasting for 6 days. C, control; 4 or 6, number of days fasting. Rat wt, 480–550 g. The vertical bracket = mean ± SEM.

TABLE 2. Effect of chronic intermittent starvation on cholesterol content in adipose tissue

	Cholesterol Content in Epididymal Fat			
	F–R	Control	F–R	Control
	mg/fat pad		*mg/g lipid*	
Zero time	0.29 ± 0.02	0.29 ± 0.02	0.46 ± 0.05	0.46 ± 0.05
11–14 wk	0.45 ± 0.11	1.72 ± 0.44	0.70 ± 0.04	0.66 ± 0.05
20–24 wk	1.83 ± 0.46	3.29 ± 0.26	0.50 ± 0.03	0.93 ± 0.06

Tissues from rats used in the experiment described in Fig. 8 were analyzed for cholesterol and total lipid, and the results were expressed in terms of cholesterol per epididymal fat pad or per unit weight of stored lipid. F–R represents fasted–refed group. Values are means ± SEM; n = 7–8.

sively with age, as did the cholesterol/triglyceride ratio, which is consistent with our previous observation (2). In the animals that were intermittently fasted and refed, the rate of cholesterol accumulation per pad was significantly reduced (in the test animals the cholesterol content increased 0.16 mg/pad, while the control increased 1.43 mg/pad during the same interval). After the test group had been fed ad lib. for 10 wk the cholesterol content of epididymal fat increased 1.38 mg, which is not significantly different from that of controls, which increased by 1.57 mg/pad. Nevertheless, at the end of the experiment the cholesterol content of fat tissue, whether expressed per pad or per unit lipid weight, was significantly lower in the animals subjected to the intermittent deprivation.

Effect of genetic obesity on adipose tissue cholesterol stores

Male ob/ob mice and littermate controls were used in this study. The body weight of obese mice was 72.5 ± 2.1 g, and that of littermates was 38.5 ± 6.4 g (mean ± SD). Total visible body fat was dissected and weighed and compared with the weights of liver and carcass. It is apparent in **Fig. 9** that total body adipose tissue in ob/ob mice was 5 times larger than the control value (20.3 ± 2.2 g vs. 4.10 ± 0.95 g, mean ± SD), and liver weight was 2.5 times higher in ob/ob mice than in the controls (5.1 ± 0.82 g vs. 1.9 ± 0.27 g), while carcass weight was less than 50% greater in ob/ob mice than in controls.

Analysis of cholesterol content of carcass, liver, and adipose tissue (**Table 3**) showed that in the obese mice 12.6% of total body cholesterol was in fat tissue compared with 2.55% in control animals. Also, the amount of cholesterol in fat tissue from ob/ob mice is proportional to the glyceride mass, as the cholesterol/lipid ratios were identical (0.88 in ob/ob mice vs. 0.82 in controls).

DISCUSSION

The present study confirms and extends earlier work from this laboratory (2) in which it was shown that adipose tissue is a major site of cholesterol storage, and it goes on to show that the amount of cholesterol in fat tissue is affected by a variety of nutritional and growth-related events. It would appear that the adipocyte cholesterol level was linearly related to animal weight. When expressed in terms of animal age, the rate of increase in adipocyte cho-

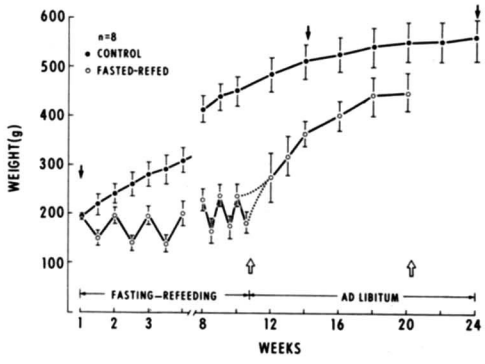

Fig. 8. Effect of intermittent fasting and refeeding on rat growth. In this experiment, 200-g (8 wk old) rats were either fed ad lib. (*closed circles*) or were alternately fasted for 4 days and refed for 4 days for 10 wk (*open circles*). One epididymal fat pad was removed and analyzed as discussed in Table 2. The rats were then fed ad lib. for 2 months and killed, and the remaining fat pad was analyzed as described above. The arrows indicate times of removal of fat pads.

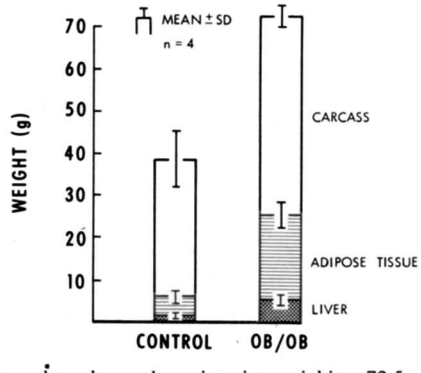

Fig. 9. Four obese hyperglycemic mice weighing 72.5 ± 2.1 g and four littermate controls weighing 38.5 ± 6.4 g were examined. To estimate total adipose tissue, all visible fat was dissected by hand and weighed. Total adipose mass was five times higher in ob/ob mice than in controls.

TABLE 3. Total body cholesterol in adipose tissue of ob/ob mice and littermate controls

		Cholesterol Distribution			
		Carcass	Adipose Organ	% in Adipose Organ	Cholesterol Content in Adipose Tissue
		mg	*mg*		*mg/g lipid*
ob/ob	1	100.0	13.04	11.5	1.00
	2	114.6	11.12	8.8	0.83
	3	80.4	16.56	17.0	0.83
	4	99.8	15.11	13.1	0.87
Mean ± SEM		98.7 ± 7.0	14.0 ± 1.2	12.6 ± 1.7	0.88 ± 0.04
Littermate	1	72.2	1.54	1.5	0.77
	2	67.2	1.8	2.7	0.88
	3	58.3	2.01	3.3	0.70
	4	46.9	1.32	2.7	0.99
Mean ± SEM		61.2 ± 5.6	1.67 ± 0.15	2.55 ± 0.38	0.82 ± 0.06

After complete dissection of adipose tissue, all remaining tissues, excluding brain, were saponified in 120 ml of 33% ethanolic KOH for 4 days and then processed as described in Materials and Methods.

lesterol was greatest during the first 15 wk of life, corresponding to the period when growth of adipose tissue occurs by cell division as well as by enlargement of existing fat cells (11). Net accumulation of cholesterol occurred after 25 wk of age, but at a much reduced rate compared with the earlier period of rapid growth. Thus, at a time when expansion of fat tissue mass was primarily due to an increase in cell size, the rate of cholesterol accumulation was slow. The increase in fat cell cholesterol in relation to rat age, and hence fat cell size, is evident in Figs. 1 and 2 and is compatible with the report of Björntorp and Sjöström (12) showing a linear correlation between fat cell size and cholesterol content in adipocytes from human subcutaneous fat.

The importance of cell triglyceride content as a primary determinant of cholesterol shortage is most evident from the studies on ob/ob mice and littermates. In spite of a fivefold difference in adipose mass (Fig. 9) and 1.2–2-fold differences in fat cell diameter (13), the cholesterol/triglyceride ratios were identical in the two groups. This indicates the close correlation between adipose mass (triglyceride stores) and the size of the cholesterol storage pool and is consistent with the previous predictions based on kinetic analysis of plasma cholesterol specific activity die-away curves in obese man, which show a linear correlation between excess body weight and the size of the slowly exchangeable cholesterol pool (1, 14, 15).

Although fat cell size, and hence triglyceride content, might be an important determinant of cholesterol content, it is clear that other factors play a part because the cholesterol content in relation to glyceride mass also increases with animal age and weight (Fig. 2).

Cholesterol feeding for prolonged periods has profound effects on cholesterol storage in most tissues, with the exception of muscle and brain (16). Although significant variations in susceptibility between species and between tissues do exist in cholesterol-fed rabbits, the size of the tissue cholesterol pool is generally related to the serum cholesterol level (16). It seems that the rat differs from the rabbit in that after 5 months of cholesterol feeding, adipose tissue cholesterol levels seemed to be better related to dietary load than to serum cholesterol levels, which were uniformally elevated (Fig. 5).

The serum cholesterol levels in rats fed a cholesterol-free semisynthetic diet were double that seen in rats raised on Purina chow. This occurs within 2 wk of feeding the diet[1] and is probably the net result of a number of dietary factors, including high carbohydrate content, absence of dietary fiber, and removal of dietary feedback suppression of hepatic cholesterol synthesis. Because endogenous cholesterol production under these circumstances would be close to V_{max}, it is tempting to speculate that the hypercholesterolemia noted in the present research (Figs. 4, *right,* and 5, *right*), like that reported in baboons fed cholesterol-free semisynthetic diets high in carbohydrates (17), is a function of undampened hypercholesterolgenesis and is not secondary to a reduction in lipoprotein utilization or redistribution of cholesterol from tissues to serum. The validity of this suggestion awaits direct experimental confirmation.

It is of interest that, after 2 months of this diet, serum cholesterol levels were lower in rats receiving cholesterol than in rats on the zero cholesterol diet. Indeed, the lowest serum cholesterol level was observed in rats receiving 5% cholesterol (Fig. 4, *right*). In addition, the adipose tissue cholesterol content was inversely related to the serum cholesterol concentration in those animals receiving cholesterol. In trying to explain this unexpected finding, it should be recalled that cholesterol synthesis in the rat is extremely sensitive to feedback inhibition by dietary cho-

[1] Angel, A., and J. Farkas. Unpublished data.

lesterol (18). Because 90% of endogenous cholesterol synthesis in the rat is hepatic (19) and at least 30–70% of plasma is derived from endogenous sources, presumably liver (20), it is reasonable to argue that the lower serum cholesterol levels in the animals ingesting cholesterol could reflect increasing degrees of feedback inhibition, which could account for the inverse relationship between dietary cholesterol load and serum cholesterol levels.

The accumulation of cholesterol in adipose tissue in the absence of a dietary source occurred in rats fed the cholesterol-free diet (Fig. 4, *left*). Although the source of this cholesterol is endogenous, it is not possible to identify its origin, i.e., whether it represents cholesterol formed in situ or derived from circulating lipoprotein particles. Since adipose tissue is capable of synthesizing cholesterol de novo (8), in situ synthesis must be considered; however, until the quantitative importance of this activity compared with liver is established in rats on a cholesterol-free semisynthetic diet, it is not possible to choose which process predominates.

The lower adipose cholesterol level in rats fed 0.05–0.1% cholesterol compared with those fed no cholesterol is an interesting and provocative observation (Fig. 4, *left*). It is best explained by suggesting that approximately this level of dietary cholesterol is capable of depressing endogenous cholesterol synthesis to a degree that exceeds net cholesterol absorption, resulting in a reduction in the adipose storage pool. However, this effect is not apparent in animals ingesting diets containing 0.5–5.0% cholesterol, presumably because net retention of dietary cholesterol equals or exceeds the reduction in endogenous synthesis, leading to progressive accumulation of cholesterol in the adipose pool. It would appear that the effect of the lower cholesterol level (0.05–0.1%) is not everlasting in that after 5 months of feeding the diet, adipose tissue cholesterol levels did not differ from animals fed the cholesterol-free diet (Fig. 5, *left*).

The present study has also shown that the adipose cholesterol pool is labile and nonfixed, because tissue levels were acutely reduced by fasting. It is interesting that, in fasting, net cholesterol outflow was less than net glyceride release, which accounted for the increase in cholesterol/triglyceride ratios in fat tissue from fasted animals (Fig. 7). This disassociation implies that the mechanisms of cholesterol accretion and/or dissipation are not completely coupled to the size of the glyceride storage pool. This point is reinforced by the finding that, after refeeding of intermittently fasted rats, the rate of cholesterol accumulation in fat tissue was lower than net glyceride accumulation measured simultaneously (Table 2).

The mechanism responsible for net cholesterol inflow or outflow in adipose tissue is not known. Although the uptake or exchange of labeled cholesterol is very rapid, outflow is very slow indeed (2). Lecithin–cholesterol acyltransferase activity (21) has been assigned a role of directing the outflow of cholesterol from tissues to the liver by formation of cholesterol esters. Whether this controls the directional flow of cholesterol in and out of fat tissue is not known. Since serum cholesterol levels are reduced when fasting it is possible that net release of cholesterol from adipose tissue could occur along a concentration gradient through endothelial cells to red blood cells and hence to α-lipoproteins by a mechanism of noncompetitive membrane exchange (22). The significance of this latter suggestion remains speculative, as little is known about the chemically active pools at the fat cell surface and their mobility across the interstitial space to gain access to the capillary lumen. Carrier proteins, analogous to sterol carrier proteins (23), or an apoprotein derivative from circulating lipoproteins may exist and may serve to direct and/or shuttle sterols between the fat cell and the circulation. However, until more direct experimental evidence is obtained, further speculation about control of cholesterol flux in adipose tissue would be injudicious.

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