Human adipocyte cholesterol. Concentration, localization, synthesis, and turnover.

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Research Article

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Human Adipocyte Cholesterol

CONCENTRATION, LOCALIZATION, SYNTHESIS, AND TURNOVER

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ABSTRACT By analysis of 124 specimens in 16 different patients, isolated human adipocyte cholesterol concentration is highly correlated with fat cell size but not with plasma cholesterol concentration. Less than 6% of total cholesterol is esterified; after subcellular fractionation, 88% of the cholesterol is recovered in the triglyceride-rich supernatant oil. This latter finding supports the observation that fat cell cholesterol is determined by triglyceride content, and hence by fat cell size.

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Obesity is associated with an increased cholesterol synthetic rate equal to 20 mg/day for each kilogram of body fat. To test (by an independent method) if this synthesis might be occurring in adipose tissue, human fat cells were obtained under a wide variety of dietary conditions and incubated in vitro with radioactive glucose or acetate. Incorporation of these precursors into sterol could account for no more than 1 mg cholesterol synthesis/kg fat per day. These in vitro data taken togethed with the in vivo mammillary compartmental analysis data are compatible with the possibility that the excess cholesterol synthesis of obesity occurs in pool 1, most likely from hepatic or intestinal sites.

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INTRODUCTION

Obese humans have increased cholesterol turnover rates as measured by isotope kinetics (1, 2) or by sterol balance methods (2, 3). This excess cholesterol synthesis may be twice normal (2) but can be reversed by weight reduction (3). Compartmental analysis of plasma specific activity curves suggests corresponding enlargement of a slowly turning over body cholesterol pool in obesity (2). Adipose tissue analyses in rats (4) and in a small number of obese humans (5) provide data for cholesterol content in the fat organ that can account for the increased body cholesterol storage.

Farkas, Angel, and Avigan (4) have recently reported detailed studies of rat adipose tissue with special emphasis upon accumulation, distribution, and local synthesis of cholesterol. It is the purpose of this study to extend these findings to man with regard to the following parameters of adipocyte cholesterol: subcellular localization and degree of esterification; effects of cell number, cell size, and plasma cholesterol on adipocyte cholesterol concentration; in vitro cholesterol biosynthetic rates from radioactive glucose and acetate; and compartmental analysis of in vivo adipocyte cholesterol turnover after intravenous infusion of radioactive cholesterol. The last procedure requires the sum of a three-exponential equation to adequately describe cholesterol turnover in man (6, 7). By fitting such an equation to both plasma and adipocyte specific activity curves simultaneously, it is possible to compare different three-pool models (mammillary and catenary, Fig. 1) and to determine if synthesis occurs in the slowly turning over pool 3 of adipocyte cholesterol.

METHODS

All patients were hospitalized on the metabolic ward of The Rockefeller University Hospital. Diets consisted solely of liquid formula feedings with the following composition: 15% of total calories as milk protein (RI-5, Ross Labs., Div. of Abbott Laboratories, Columbus, Ohio); 15–85% of calories as dextrose; 0–70% of calories as polyunsaturated vegetable oil. All formulas were cholesterol-free and the amount of plant sterols was determined on each batch by gas-liquid chromatography (GLC).

Plasma cholesterol and triglycerides were measured by autoanalyzer methods (8, 9).

Compartmental analysis of radioactive cholesterol turnover. A clinical description of the six patients receiving radioactive isotopes is given in Table I. Cholesterol isotope studies were performed after a single 100-μCi intravenous administration of [4-^{14}C]- or [1,2-^{3}H]cholesterol as a 1% ethanolic-saline suspension. Before use, the isotopes were purified on silica gel H thin-layer chromatography (TLC) plates, ethyl ether: heptane, 55:45. All radioactivity was measured in a Packard Tri-Carb scintillation counter (model 3380, Packard Instrument Co., Inc., Downers Grove, Ill.) using an automated channels ratio technique for conversion of gross counts to disintegrations per minute.

Both the plasma and adipocyte specific activity time curves were fitted simultaneously with sum of three-exponential equations. The rate constants of the two equations are the same but the coefficients or intercepts are different for each curve. Thus:

$$Y_1 = \sum_{i=1}^{3} A_{1i}e^{-\alpha_i t}$$

$$Y_3 = \sum_{i=1}^{3} A_{3i}e^{-\alpha_i t},$$

where Y_1 and Y_3 are the specific activity curves for pools 1 and 3, A_{1i} is the ith coefficient for the equation for pool 1, A_{3i} is the ith coefficient for the pool 3 equation, and α_i is the ith rate constant (same for equal i for both equations). Note that $\sum_{i=1}^{3} A_{3i} = 0$ so that there are eight independent parameters among the two equations. The fitting is accomplished by finding values for the eight independent parameters which will simultaneously minimize the sum of squared errors about both fitted lines. It should be noted that the plasma and adiocyte were not fitted separately but rather all points, whether plasma or adipocyte, were fitted by the two equations at the same time. The equation for pool 1 is partly determined by the plasma data and partly by the adipocyte data and likewise with the equation for pool 3. Thus the curves cover the time interval studied for all data points regardless of whether plasma or tissue or both was sampled. If the magnitude of the errors about each curve is approximately the same, then each set of data receives the same weight. However, Fig. 5 shows that the variation in the adipocyte specific activity curve is larger than the variation in the plasma specific activity curve. Therefore, one should weight the plasma curve more heavily than the adipocyte curve to rely more fully upon the more accurate data. This weighting can be established a priori if one knows the relative accuracy of the two sets of data (which is seldom known) or one can estimate the proper weights by the method of maximum likelihood (10). The maximum likelihood procedure for simultaneously estimating the eight parameters of the two equations from the two specific activity time curves was incorporated into a computer program (11) which uses a modification of the Marquardt convergence technique (12). The adequacy of the fit was assessed by a runs test on both curves. Both the plasma and adipocyte data were closely fitted by this simultaneous estimation procedure except for patient R. C. (Fig. 5) in whom the later adipocyte data points were not well fitted. The curve for plasma passes through the plasma data on this patient because there are more plasma points and they have less variability and, hence, a higher weight.

TABLE I
Clinical Data

Patient	Sex	Age	Weight	Height	Desirable body weight*	Diet	Diagnosis‡
		yr	*kg*	*cm*	%		
R. B.	F	60	52.5	158	100	45% safflower	CHD; FHC (type IIa); tendinous xanthomatosis
R. H.	M	40	90.0	175	136	70% cottonseed	obesity; HC and HTG (type III)
A. G.	M	50	71.5	169	118	45% safflower	obesity; CHD; PVD; HC and HTG (type IV) with tuberous xanthomatosis
R. C.	F	47	53.5	157	105	70% cottonseed	FHC (IIa) with tendinous xanthomatosis
F. G.	F	62	67.0	157	134	70% cottonseed	FHC + HTG (IIb); CHD
J. G.	M	55	63.0	176	97	70% cottonseed	sporadic HC (IIa); CHD; xanthelasma

* From Metropolitan Life Insurance Company Statistical Bulletin **40**: Nov.–Dec. 1959. Based on estimated frame size and desirable weight for each sex grouped as ages 25 and over.

‡ CHD, coronary heart disease; PVD, peripheral vascular disease; FHC, familial hypercholesterolemia; HC, hypercholesterolemia; HTG, hypertriglyceridemia; types IIa, IIb, III, IV based on WHO classification; 1970, *Bull. W. H. O.* **43**: 891.

TABLE II

Adipocyte Cholesterol: Analytic Precision

Cells	Cholesterol Mean ±SD (*n*)	Coefficient of variation
mg lipid	*mg/g lipid*	
7.5	0.87±0.13 (10)	14.9
15.0	0.82±0.05 (10)	6.1
30.0	0.78±0.05 (10)	6.4
45.0	0.77±0.07 (10)	9.1

Adipocyte cholesterol determination. Subcutaneous adipose tissue was obtained by needle aspiration from the buttocks or anterior abdominal wall by the method of Hirsch and Gallian (13). A portion was mixed in OsO_4 and cell size was determined by a Coulter Counter (Coulter Electronics Inc., Hialeah, Fla.). The tissue shreds were kept at 37°C in a Dewar flask containing gassed Krebs-Ringer bicarbonate buffer. Within the next 15 min the tissue was washed with warm saline over a 253-μm nylon filter. The cells were dispersed after a 1-h incubation with crude collangenase (Worthington Biochemical Corp., Freehold, N. J.), 1 mg/3 ml buffer. They were then washed three times with buffer and dispersed for the appropriate metabolic or analytic experiments.

Cholesterol was quantitated in the following manner: 1 or 2 ml of a known volume of suspended adipocytes was extracted overnight into 40 ml $CHCl_3$:MeOH, 2:1. β-Sitosterol was added as an internal recovery standard. Two phases were obtained after addition of 20 ml distilled H_2O. The lower $CHCl_3$ phase was transferred to a tared glass weighing bottle and evaporated to dryness under N_2. After determination of total lipid gravimetrically, the contents were dissolved in 18 ml absolute ethanol and transferred to 125-ml glass-stoppered bottles. 2 ml of 10 *N* NaOH was added and the mixture was refluxed at 78°C for 2 h. The nonsaponifiable fraction was extracted three times with 40-ml volumes each of petroleum ether (bp 66–69°C). After trimethyl silylation sterols were determined in this fraction by GLC using a 5α-cholestane as an internal reference standard (Steraloids, Inc., Pawling, N. Y.). A 6-foot glass column packed with silanized Gas-Chrom P (100–120 mesh) coated with 1–2% film of DC 560 (Applied Science Labs, Inc., State College, Pa.) was run at 240°C in an instrument equipped with a hydrogen flame ionization detector.

Cholesterol synthesis in vitro. Shreds of human subcutaneous adipose tissue were incubated with collagenase, 1 mg/3 buffer, for 1 h at 37°C, and adipocytes were isolated (14) and incubated for 2 h in the following proportions:

1 ml—cells (50–100 mg/ml suspension)

1 ml—Krebs-Ringer bicarbonate buffer with 0.65 mM Ca^{++} concentration, gassed with 95% O_2/5% CO_2, pH 7.40; 4 g/100 ml dialyzed albumin (Abbott fraction V bovine with low insulin-like activity [Abbott Laboratories, North Chicago, Ill.]); [U-^{14}C]glucose 1 μCi/ml; glucose 1 mM; insulin (Porcine, Eli Lilly and Company, Indianapolis, Ind.) 1 mU/ml or [U-^{3}H]acetate 10 μCi/ml; sodium acetate 1 mM.

The incubation was terminated by adding 15 ml isopropanol: heptane, 1:1. The heptane extract was used to quantitate lipid gravimetrically and was then saponified for 2 h in 1 *N* ethanolic KOH at 78°C. A β-sitosterol internal recovery standard was added to the heptane before saponification. The nonsaponifiable fraction was removed in petroleum ether (bp 66–69°C), washed repeatedly with water (until no radioactivity was detectable in aqueous layer) and counted in a liquid scintillation counter. Sitosterol mass was analyzed by GLC and losses of radioactive sterols were corrected on the basis of sitosterol recovery.

In two separate experiments, pooled heptane extracts were saponified in an identical fashion, and the nonsaponifiable fraction was then separated in a silica gel H plate with heptane: ether, 55:45. The plate was developed with rhodamine G staining and all bands were eluted and counted for radioactivity.

RESULTS

Adipose tissue cholesterol: precision, regional variation. To test the precision of the method for adipocyte cholesterol analysis in a fat cell, a suspension was prepared from pooled adipose tissue (approx. 250-g male white rats), and 10 aliquots of 7.5, 15.0, 30.0, and 45 mg lipid each were analyzed for cholesterol content. The mean, standard deviation of the mean and coefficient of variation are listed in Table II. The precision was increased substantially when 15 mg or more cells were available.

To examine cholesterol concentration in adipose tissues taken from various body fat depots, we obtained autopsy specimens from two different patients (Table III). Patient 1 (S. S.) was a 56-yr-old male with hypercholesterolemia and severe atherosclerotic heart disease. Cholesterol content of whole adipose tissue taken from omental, subcutaneous, substernal, and perirenal regions averaged 1.6 mg/g lipid and varied little from site to site. A second patient, L. S., was a 54-yr-old female with a 7-yr history of primary biliary cirrhosis. Her adipose tissues contained over three times the

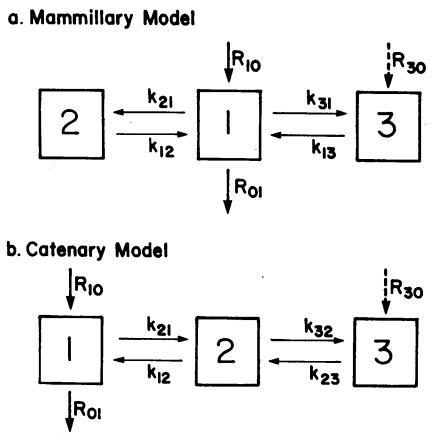

FIGURE 1 Three-pool models of cholesterol turnover in man. The pools are denoted by arabic numbers 1, 2, and 3. Rate constants are denoted by k where k_{12} for example is the rate of cholesterol transfer in units of day-1 from pool 2 into pool 1. The total removal of cholesterol mass (grams per day) is denoted by R; R_{01} for example refers to rate of mass transfer out of the body from pool 1.

cholesterol seen in patient 1 and all others so examined including hypercholesterolemic patients without liver disease but with comparable plasma cholesterol levels of over 400 mg/100 ml (Fig. 2). The site to site variation was greater than the coefficient of variation for repetitive analyses but this may also be explained by the use of intact tissue rather than isolated fat cells.

Adipose tissue cholesterol: percent ester. The percentage of total adipose tissue cholesterol that was esterified was determined on specimens from six normolipidemic, obese patients and three rats. The lipid extract was separated into free and esterified cholesterol by TLC (silica gel H, hexane: ethyl ether, 55:45), saponified separately and then quantitated by GLC. Radioactive cholesterol and cholesterol palmitate were co-chromatographed as internal recovery standards. The results in Table IV show that only 5.6% of cholesterol is esterified in human subcutaneous adipose tissue and only 3.8% in rat adipose tissues taken from various regions.

Adipocyte cholesterol: correlations with plasma lipids, adipocyte size and number. Adipocyte cholesterol from 16 patients was compared with their average plasma cholesterol concentrations determined in most instances over a period of several months. The adipocytes were also analyzed on different occasions and represent 124 specimens in total (Fig. 2). No correlation could be proven between adipocyte cholesterol and type of hyperlipidemia or to the mean plasma cholesterol concentration obtained during the 2–3 mo of our studies.

There was a highly significant correlation, however, with cell size (Fig. 3). As the amount of triglyceride per cell increases so does the amount of cholesterol. Cholesterol storage in the fat organ is apparently determined by the amount of body fat and not necessarily the number of cells containing that fat. Two adipocytes containing 0.5 μg lipid each would store almost the same amount of cholesterol as one large adipocyte containing 1.0 μg lipid even though the two small cells contribute more membrane than the single large one.

TABLE III

Adipose Tissue Cholesterol: Regional Differences

	mg cholesterol/g lipid
Patient 1 (S. S.), Hypercholesterolemia and atherosclerosis	
Omental	1.5
Subcutaneous—abdominal	1.5
Substernal	2.1
Perirenal	1.4
Mean	1.6±SD 0.3
Patient 2 (L. S.), Primary biliary cirrhosis	
Omental	4.7
Subcutaneous	
Thoracic	6.6
Abdominal	5.1
Mean	5.5±SD 1.0

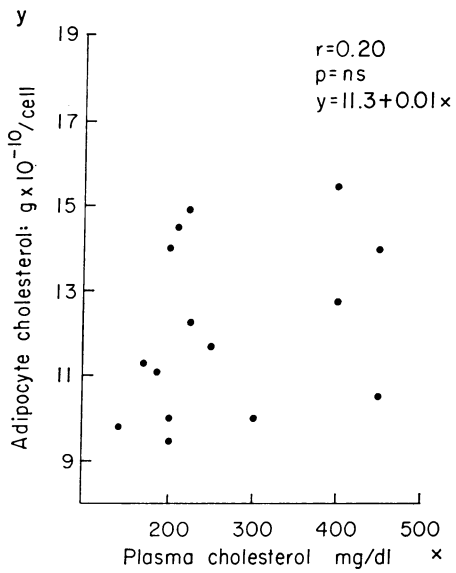

FIGURE 2 Correlation of adipocyte cholesterol with plasma cholesterol. Adipocyte cholesterol is plotted on the vertical axis as $g \times 10^{-10}$/cell.

Adipocyte cholesterol: subcellular fractionation. After complete cell disruption (Fig. 4), 88% of adipocyte cholesterol was recovered in the supernatant oil with the remainder evenly divided between the membranous pellet and an opaque lipid fraction partitioning between the oil and water phases.

Adipocyte cholesterol turnover. The relationship of plasma and adipocyte cholesterol turnover is illustrated in Fig. 5. A single 100-μCi dose of [4-^{14}C]cholesterol was given intravenously to six hyperlipidemic patients. Plasma and adipocyte cholesterol specific activities were measured for periods of 10–20 wk thereafter. The resultant curves were very similar. In each case, adipocyte specific activity reached its maximum within 4–6 wk at a value very close to the plasma specific activity but decayed much more slowly thereafter.

Three-pool mammillary models, without and with synthesis into pool 3, were simultaneously fitted to both the plasma and adipocyte specific activity time curves. Assuming a mammillary model, synthesis into pool 3 was zero in all of the six subjects studied. The values of the six significant model parameters (R_{01}), the production rate; M_1 the mass of rapidly miscible pool; and k_{12}, k_{21}, k_{13}, k_{31} (the exchange rates) plus the mass of pool 3 ($M_3 = M_1k_{31}/k_{13}$ if synthesis into pool 3 is zero) are given in Table V. Because of the short to medium

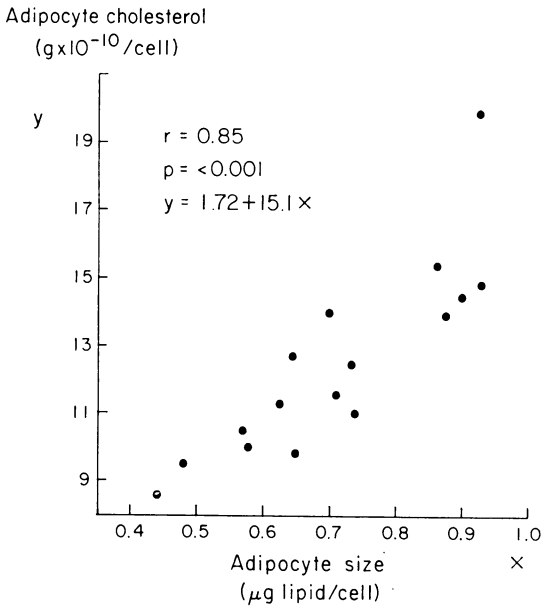

FIGURE 3 Correlation of adipocyte cholesterol with adipocyte size.

length of the studies (64–149 days) some of the estimated parameter values had wide confidence limits, particularly k_{12} and k_{21} for subject R. C.

Three-pool catenary models, without and with synthesis into pool 3, were fitted to the six sets of data. The results show that estimated synthesis into pool 3 (R_{30}) had a finite, non-zero value in three of the six subjects studied although it was more than 5% of the overall production rate (R_{10}) only in subject F. G. No one model showed a significant reduction in the residual error about the fitted curve and we are therefore unable to choose between the three on a mathematical basis.

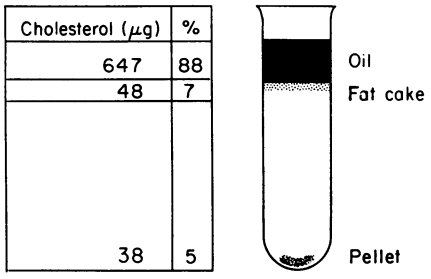

FIGURE 4 Cholesterol distribution in human adipocytes. Isolated fat cells pooled from several different patients were completely disrupted by (*a*) repeated freezing and thawing, (*b*) repeated rapid perfusions through a 26-guage needle, or (*c*) by sonication. The broken cell suspensions were then centrifuged for 1 h at 100,000 *g*. The three visible fractions were isolated by tube slicing, saponified and analyzed separately for cholesterol mass by GLC. Results were comparable by all three methods of cell disruption.

TABLE IV
Adipose Tissue Cholesterol: Percent Ester

	%
Human: Subcutaneous	
Patient	
1	3.0
2	6.2
3	7.3
4	2.7
5	7.1
6	7.1
Mean	5.6±SD 2.1
Rat: 300 g ♂	
Rat	
1(a) Epididymal	2.3
(b) Retroperitoneal	3.4
(c) Subcutaneous	1.4
2(a) Epididymal	5.6
(b) Retroperitoneal	5.6
(c) Subcutaneous	3.0
3(a) Epididymal	3.1
(b) Retroperitoneal	6.7
(c) Subcutaneous	3.5
Mean	3.8±SD 1.7

Adipocyte cholesterol biosynthesis. Table VI lists the in vitro cholesterol synthesis rates in isolated human adipocytes under a variety of conditions. Each of the first two patients were studied under metabolic ward conditions on four different occasions. The first two adipose aspirations were performed while the patients were eating a liquid formula diet consisting of either 90% dextrose or 90% corn oil for at least 10 days; the remaining 10% of the total calories was protein. Their body weights were 221 and 191 kg, respectively. After approximately 6 wk of weight reduction the same studies were repeated at body weights of 175 and 158 kg, respectively. Neither dietary alterations, weight reduction or addition of insulin in vitro (1,000 uU/ml) increased glucose incorporation into cholesterol to more than 2 nmol g cells/2 h. This value is equivalent to less than 1 mg cholesterol/kg body fat per day. In our earlier report (2) and in Miettinen's (3) study, obese patients synthesized 20 mg/kg body fat per day in excess of normally weighted controls.

In two additional patients (Table VI) fed a liquid formula consisting of 40% corn oil (cholesterol-free), 45% dextrose, and 15% protein, intact shreds of subcutaneous adipose tissue were incubated with radioatcive acetate. Duplicate experiments were performed 2 wk apart after weight stabilization. The results again

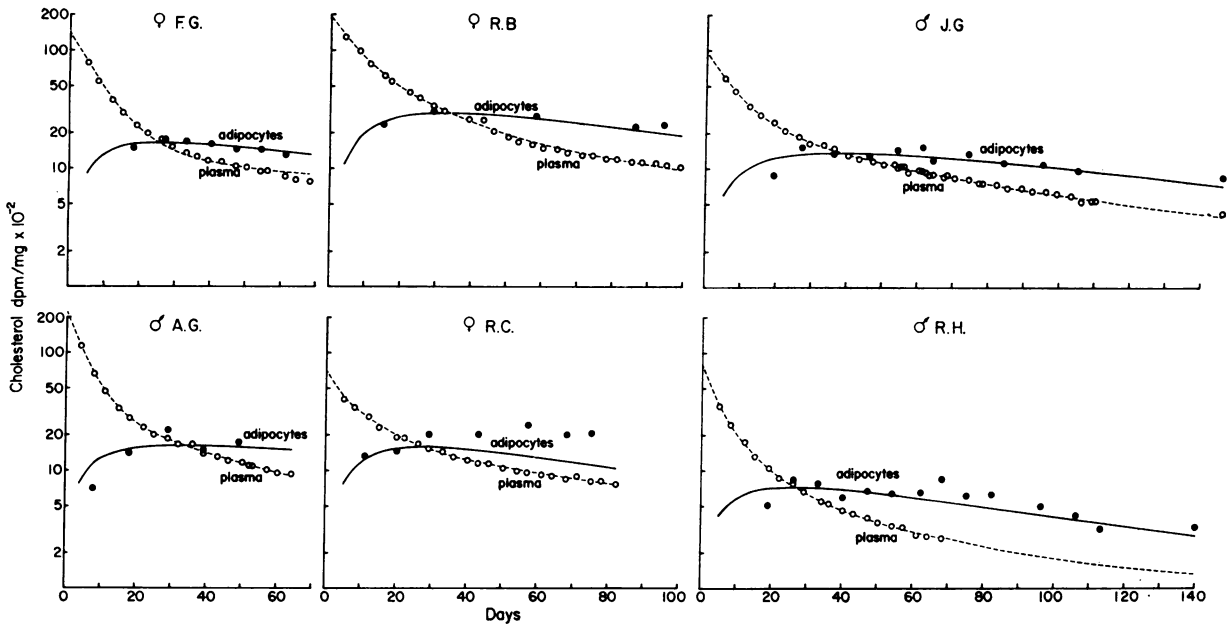

FIGURE 5 Plasma and adipocyte cholesterol specific activities in six patients. The broken line represents the computer's best fit to the plasma data. The solid line represents cholesterol specific activities in pool 3 as determined by computer program (see Methods).

TABLE V

Three-Pool Model Parameters: Catenary Type without and with Synthesis in Pool 3

Patient	Model and* number of parameters		R_{01}	R_{30}	M_1	M_3	k_{12}	k_{21}	k_{13}	k_{31}	k_{23}	k_{32}
			g/day	g/day	g	g	day^{-1}	day^{-1}	day^{-1}	day^{-1}	day^{-1}	day^{-1}
	M	6	0.59	0	14.4	22.7	0.095	0.023	0.018	0.028		
R. B.	C	6	0.60		14.0	13.7	0.085	0.060			0.042	0.058
	C	7	0.60	0	14.0	13.7	0.085	0.060			0.042	0.058
	M	6	0.85	0	22.8	37.5	0.067	0.038	0.017	0.028		
J. G.	C	6	0.87		22.4	16.7	0.052	0.069			0.034	0.019
	C	7	0.85	0.030	22.4	16.5	0.052	0.069			0.039	0.021
	M	6	2.24	0	30.8	80.4	0.119	0.050	0.017	0.044		
R. H.	C	6	2.31		31.1	42.8	0.070	0.090			0.031	0.034
	C	7	2.31	0	31.1	42.8	0.070	0.090			0.031	0.034
	M	6	0.73	0	12.7	51.8	0.134	0.044	0.018	0.073		
F. G.	C	6	0.74		12.1	34.6	0.079	0.126			0.038	0.067
	C	7	0.71	0.16	12.3	29.2	0.079	0.126			0.054	0.080
	M	6	1.25	0	40.3	61.6	1.373	0.259	0.034	0.052		
R. C.	C	6	1.25		47.9	38.1	0.103	0.051			0.172	0.274
	C	7	1.25	0	47.9	38.1	0.103	0.051			0.172	0.274
	M	6	1.18	0	15.2	25.3	0.052	0.070	0.013	0.021		
A. G.	C	6	1.21		15.1	8.2	0.045	0.090			0.028	0.008
	C	7	1.22	0.070	15.2	2.9	0.044	0.088			0.070	0.004

* M represents a mammillary model and C represents a catenary model.

TABLE VI
Sterol Biosynthesis In Vitro

Patient	Weight	Diet	[^{14}C]Glucose incorporation*
	kg		*nmol/g/2 h*
H. Mc.	221	90% CHO	2.00
			2.10‡
	216	90% Fat	0.71
			0.67‡
	175	90% Fat	0.53
			0.37‡
	176	90% CHO	0.84
			1.20‡
R. W.	191	90% CHO	1.12
			1.10‡
	191	90% Fat	0.58
			0.54‡
	158	90% Fat	0.53
			0.49‡
	158	90% CHO	0.46
			0.29‡
			[^{3}H]Acetate incorporation*
			nmol/g/2h
N. G.	185	40% Fat	Mean 0.49±SD 0.28 (n = 5)§
J. O'G.	155	40% Fat	0.17± 0.05 (n = 6)§

* TLC of this nonsaponifiable lipid fraction yielded greater than 90% of total radioactivity in the free cholesterol band.
‡ Addition of porcine insulin 1 mU/ml.
§ Shreds of adipose tissue; experiments were performed on three different occasions over a 1-mo period.

showed a very low cholesterol synthesis rate equivalent to less than 1.0 mg/kg fat per day.

DISCUSSION

Adipose tissue is heterogenous: it is composed of fat cells interspersed in an abundant capillary bed and is held together by connective tissue. Connective tissue cholesterol concentration may increase with elevated plasma cholesterol levels (i.e., tendinous xanthomata) and also with age (15). We have described a method for determining fat cell cholesterol that eliminates these connective and vascular tissue variables. The GLC analysis provides a specific determination of cholesterol mass plus the mass of β-sitosterol for use as an internal recovery standard. Results show that human adipocytes store cholesterol primarily in their intracellular triglyceride, not as membrane-bound sterol but presumably by a solvation effect. As the cell enlarges with triglycerides so does the amount of cholesterol stored.

These findings agree with rat adipose tissue data (4) and also with the interesting observations by LaCroix, Mattingly, Wong, and Alford (16) that cholesterol content of dairy products bears a close and direct relationship with fat content but not protein. Cholesterol solubility in oils derived from both animal and plant sources is approximately 3–4 g/100 ml (17), a value far in excess of that observed in vivo. On the basis of the present data, an obese patient with 25 kg of body fat would be expected to store approximately 50 g of cholesterol in his adipose tissue, an amount equal perhaps to one-third to one-half his total miscible pool of cholesterol.

Adipocyte cholesterol is almost 95% unesterified, as is the sterol in most body tissues with the exception of adrenal cortex, liver, skin, and atheromata. Farkas et al. (4) reported a 75% unesterified cholesterol composition in rat adipocytes which differs from our finding in the same species. Their disproportionately high cholesterol ester content may have been due to the indirect method employed. However, in a small series of human adipocytes they found cholesterol to be 93% in the free form which does agree well with our findings.

These observations do not of course infer that esterified cholesterol cannot enter the cell from plasma and then be hydrolyzed before storage nor do they rule out the possibility that cholesterol is esterified before export into plasma. Whatever the case, cholesterol exchange between plasma and fat tissue is very slow in the steady state. With weight reduction and mobilization of adipocyte triglyceride, cholesterol may be released into the circulation at a much faster rate. The eventual excretion of this adipocyte cholesterol into bile may have important implications related to gallstone formation (18).

The cholesterol specific activity curves illustrated in Fig. 5 are of interest in several regards. The slow exchange between plasma and fat tissue proves the existence of at least two pools of body cholesterol. Recently plasma specific activity curves of longer duration have been best fit by three exponentials, and we have therefore analyzed our data with a three-compartment model (7). Adipose tissue cholesterol kinetics agree quite closely with the hypothetical third pool. Other studies from our laboratory suggest colon, stomach, and muscle as the major tissues comprising the second pool.[1] Connective tissue appears to exchange cholesterol with plasma at a rate still slower than adipose tissue.

Analysis of the two specific activity curves in the six subjects studied did not distinguish between the mammillary or catenary model structure, largely because the adipose tissue was not sampled early enough in the study. If adipocyte cholesterol turns over according to a mammillary model then significant counts of radioactive cholesterol will be seen early while appearance of radioactivity will be delayed if the catenary model is the correct model. On the other hand, the implication of the catenary model is that there is a pool between plasma and adipocyte which may be difficult to justify (particularly if pool 2 is thought to be comprised of colon, stomach, and muscle).

If whole body turnover of cholesterol can be described by a three-pool mammillary model, then the results of

[1] Schreibman, P. H. Unpublished observation.

the modeling show conclusively that synthesis in pool 3 must be less than 0.01% of the total production rate. This conclusion supports the in vitro biosynthesis experiments. If a three-pool catenary model is the best description of cholesterol turnover, then the synthesis into pool 3 ranges from 0 (three of the patients) to 22% of the total production rate (R_{01}) with an average of 5.3%. In the three subjects with some synthesis into compartment three of a catenary model there was not a significant reduction in the residual error about the model. Thus, it was not possible to say that inclusion of the seventh parameter (R_{30}) significantly improved the fit.

To test more directly the possibility that adipose tissue cholesterol synthesis might contribute a substantial portion of the total body sterol turnover, we measured sterol biosynthesis in vitro (Table VI). Using glucose as a substrate in physiologic concentration human adipocytes could account for no more than 5% of total body cholesterolgenesis. Changes in fat cell size (by weight reduction), diet, or addition of insulin to the medium failed to increase the glucose incorporation into cholesterol. Angel and Farkas (19) have previously reported similar low rates of glucose conversion to digitonin-precipitable sterols in rat adipose tissue.

If adipose tissue is not responsible per se for the enhanced cholesterol synthesis of obesity, then the liver is perhaps the next most likely source as suggested by Miettinen (20). A positive correlation between caloric intake and cholesterol synthesis has been made in 65 nonobese and 13 obese patients studied over the past 5 yr at The Rockefeller University.[3] Future experiments examining the influence of calorie intake, hormonal imbalance, and free fatty acid turnover will hopefully provide insight into control mechanisms of hepatic cholesterol biosynthesis in the obese patient.

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[3] Schreibman, P. H., and E. A. Ahrens, Jr. Unpublished observations.

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