Fat mobilization in vitro and in vivo in the genetically obese Zucker rat "fatty"

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Abstract Fat mobilization was studied in vitro with epididymal fat pad tissue and also with cell suspensions from epididymal, retroperitoneal, and subcutaneous fat from the obese mutant "fatty" (*fafa*) and control rats of four different ages. Fat mobilization per cell in response to epinephrine was well above normal in young "fatties"; in older "fatties" the output per cell fell to near normal, but the much greater number of fat cells per rat indicated that the fat mobilizing capacity of the older "fatty" is well above normal. The "fatty" showed normal reactions to epinephrine in vivo: hyperglycemia, glycogenolysis, lipolysis with elevated free fatty acids and glycerol, and increased entry of free fatty acids into muscle and liver. Response was at least as great in "fatty" as in control animals. Metabolic indices—levels of circulating free fatty acids, glycerol, and in some cases glucose and lipid—determined at various ages in fed "fatties" and controls, and at intervals during prolonged fasting (70 days), were consistent with a picture of excessive adipose tissue lipolysis, excessive reesterification in the adipose tissue, fat mobilization in excess of need, and return of the excess to the adipose tissue via lipoproteins.

Supplementary key words free fatty acids · free glycerol · fatty acid recycling · isolated fat cells · fat cell size · fat cell number · subcutaneous fat

RATS homozygous for the recessive mutant gene *fa* (fatty [1]), develop an extreme obesity of juvenile onset. Fat mobilization per aliquot of tissue in vitro is greatly depressed in "fatties" (2, 3), yet fat is effectively mobilized in vivo, supplying fuel for prolonged survival without food (4). The enormous expansion of adipose tissues in this type of obesity makes it difficult to interpret in vitro metabolic studies without knowing how many fat cells are present per aliquot of tissue. With the completion of a study on cellular growth of three adipose tissues in the "fatty" (5), such interpretation is now possible. Accordingly, observations of in vitro effects of epinephrine on fat mobilization are presented, and they are discussed in the light of the cellularity data and in relation to in vivo experiments with both epinephrine and fasting.

Abbreviations: FFA, free fatty acids; TG, triglyceride.

METHODS

The rats in the present study were all of the Zucker 13M strain (6), which carries the fatty mutation (1). "Fatties" are genotypically *fafa*. Nonobese controls were generally littermates and genotypically *Fa/?*. The dietary regimen was based on a commercial pelleted rat food and has been fully described (5). Unless otherwise stated, all rats were fed ad lib. Effects of excitement and stress were minimized by keeping the rats in their usual environment and cages up to the moment that they were killed.

Analytical procedures

FFA were determined by the Dole microtitration procedure (7), with the modification of a dilute acid wash of the heptane extract (8). Free glycerol was determined according to Wieland (9), using glycerokinase from Boehringer-Mannheim, obtained from Fisher Scientific Co., Medford, Mass., and ATP and NAD from Sigma Chemical Co., St. Louis, Mo. Alterations in the method included a fivefold increase in the concentration of the glycine buffer and the use of freshly mixed hydrazine in buffer. Glucose was determined according to the Nelson-Somogyi method (10). Glycogen was determined by the usual procedure of alkaline hydrolysis of the tissue followed by glucose determination on the isolated glycogen. Serum lac-

tescence was determined as previously described (4), by measuring the light-scattering power of 50 μl of serum freshly diluted with 1 ml of physiological saline.

In vitro studies

Two studies were made on groups of male rats, "fatties" and paired controls, over the age range 4–40 wk. In the first study, epididymal fat tissue was incubated 4 hr at 37°C in Krebs-Ringer bicarbonate buffer (11), with 0.1% glucose and 3% bovine serum albumin (fatty acid-poor, Pentex Inc., Kankakee, Ill.). Paired studies were carried out in the absence of epinephrine and with epinephrine added at either 0.5 or 10 μg/ml. A few observations with 50 μg/ml of epinephrine established that 10 μg/ml gives a maximal effect. FFA were measured in the adipose tissue, initially and after incubation, and in the incubation fluid. Rats in this experiment were fasted overnight. In the second study all rats were full-fed. Observations were made on cell suspensions from epididymal, retroperitoneal, and subcutaneous (dorsal scapular) fat, as well as on intact epididymal fat. Both FFA and free glycerol were measured in the incubation fluid. Suspensions of fat cells were prepared with collagenase (crude collagenase, Worthington Biochemical Corp., Freehold, N.J.) by Rodbell's technique (12). Collagenase incubations were for 35 min for epididymal fat, 60 min for retroperitoneal fat, and 75 min for subcutaneous fat. Suspensions were filtered through two layers of surgical gauze (12 ply, 20 × 12 mesh), washed twice, and incubated for 2 hr with epinephrine at 0.5 μg/ml. Intact epididymal fat was carried through a blank incubation for 1 hr, then transferred to fresh epinephrine-containing medium and incubated another 2 hr. For lipid determination, the material to be analyzed was extracted with chloroform–methanol 2:1, the chloroform phase was separated with water, and its lipid content was determined gravimetrically. Output per fat cell was calculated for cell suspensions by the use of cell size estimated by measuring diameters of 100 cells. Output per fat cell for intact epididymal fat tissue was calculated using cell size measurements obtained in a matching set of rats (5) by the osmium tetroxide method of Hirsch and Gallian (13).

In vivo studies with epinephrine

Four matched groups of 15-wk-old female rats, 12 per group, received epinephrine injections (20 μg/100 g, given subcutaneously) and were killed 2 hr later (±5 min). These conditions were chosen to produce a barely maximal rise in plasma FFA in normal rats, and they resulted in a considerable, although much less than maximal, blood sugar rise. At autopsy heparinized blood samples were collected; they were immediately chilled and centrifuged cold. Aliquots of plasma were treated, not later than 20 min after removal of the live rat from its cage, with Dole's extraction mix for determining FFA, or with perchloric acid for determining free glycerol. Immediately after blood collection, samples of leg muscle and liver were removed for glycogen determination, and other samples of leg muscle and parametrial adipose tissue were frozen for later determination of tissue FFA and free glycerol. Collection of all material was completed within 8 min. To estimate true body size and degree of obesity, we measured the length of the tibia and weighed the retroperitoneal and parametrial fat, although more recent observations indicate that these fat pads considerably underestimate the relative obesity because of the enormously greater expansion of the subcutaneous fat in the "fatty" (5).

Effect of fasting

Two experiments were performed to explore short- and long-range effects. In the first experiment, 40-wk-old "fatties" and paired controls, eight female and eight male pairs, were killed after 0, 1, 2, and 6 days of fasting. Plasma FFA, free glycerol, glucose, and lactescence were measured. In the second experiment, 15-wk-old female "fatties", four or five rats per group, were killed after 5, 10, 15, 20, 38, and 70 days of fasting; nonobese controls were killed after 4 and 8 days. Plasma FFA, free glycerol, and lactescence were determined.

Plasma FFA and glycerol in young rats

Both quantities were measured in 12 pairs ("fatty" and control) of 4-wk-old male rats. Free glycerol was determined in somewhat larger groups of 2-wk-old rats; samples of tail blood were analyzed, and from the later growth of these rats the correct assignment to "fatty" and control groups could be made.

RESULTS

In vitro studies

Results of the first study using intact epididymal fat are presented in Table 1 (FFA in the incubation fluid) and Table 2 (tissue levels of FFA). The original measurements were FFA per 0.1 g of tissue, and these figures with their SEMs are shown first. Next to these figures are calculated values of FFA/cell, for those studies in which there were appropriate cell measurements. As is commonly found for obese subjects, FFA output per aliquot of tissue was uniformly significantly depressed, and this was also true for net change in FFA, which can be calculated from the combined data of Tables 1 and 2. Responses to low and high epinephrine stimulus were parallel. Recalculation to FFA/cell produced a complete reversal of the sense of the comparison between "fatties"

TABLE 1. Epididymal fat pad incubated in vitro: FFA in incubation fluid[a]

	Age	No Epinephrine			0.5 μg/ml Epinephrine			10 μg/ml Epinephrine		
		n	FFA/0.1 g	FFA/cell	n	FFA/0.1 g	FFA/cell	n	FFA/0.1 g	FFA/cell
"Fatty"	4 wk	12	0.04 ± 0.003		12	0.24 ± 0.026		12	0.55 ± 0.041	
Normal		12	0.04 ± 0.009		12	0.41 ± 0.021		12	0.80 ± 0.034	
"Fatty"	13 wk	11	0.04 ± 0.008	0.7[b]	11	0.16 ± 0.015	2.7[b]	7	0.25 ± 0.020	4.1[b]
Normal		31	0.04 ± 0.003	0.15	21[c]	0.40 ± 0.028	1.3	10	0.61 ± 0.041	2.0
"Fatty"	25 wk	5	0.04 ± 0.005	0.5[b]	5	0.10 ± 0.011	1.2[b]			
Normal		21	0.05 ± 0.003	0.25	16[c]	0.27 ± 0.028	1.25	11	0.50 ± 0.048	2.35
Normal	40 wk	11	0.03 ± 0.006		11	0.13 ± 0.013				

[a] FFA output per hour, either μmoles/0.1 g ± SEM, or μmoles × 10^6/cell, as indicated.
[b] The output per cell of "fatty" rats 13 wk of age exceeded the normal output; for 0, 0.5, and 10 μg/ml epinephrine the P values were < 0.001, 0.001, and 0.003, respectively. At 25 wk there was no difference in output per cell.
[c] Approximately half of these rats were known heterozygotes (*Fafa*), the rest were normal homozygotes; there was no difference in response.

TABLE 2. Epididymal fat pad incubated in vitro: tissue levels of FFA[a]

					After 4-hr Incubation								
		Initial			No Epinephrine			0.5 μg/ml Epinephrine			10 μg/ml Epinephrine		
	Age	n	FFA/0.1 g	FFA/cell	n	FFA/0.1 g	FFA/cell	n	FFA/0.1 g	FFA/cell	n	FFA/0.1 g	FFA/cell
"Fatty"	13 wk	11	0.24 ± 0.015	4.0[b]	11	0.12 ± 0.010	2.0	11	0.21 ± 0.036	3.5	7	0.37 ± 0.083	6.1
Normal		11	0.57 ± 0.050	1.9[b]	10	0.14 ± 0.022	0.5				11	1.88 ± 0.060	6.2
Normal	25 wk	12	0.57 ± 0.060	2.7[b]	10	0.13 ± 0.015	0.6	4	0.40 ± 0.070	1.9	8	1.17 ± 0.15	5.5

[a] FFA in tissue, μmoles/0.1 g ± SEM, or μmoles × 10^6/cell, as indicated.
[b] These rats were fasted overnight. Levels per cell in fed rats are closer to those found after incubating without epinephrine.

and normal rats, with output from "fatties" now either significantly in excess of normal (at 13 wk of age), or not different from normal (at 25 wk). Similarly, when tissue levels are expressed on a per cell basis, the "fatty" concentration is characteristically above normal at submaximal epinephrine stimulus. It is of some interest however that with maximal epinephrine stimulus, FFA/cell seems to be identical in "fatty" and control.

Results of the second study are expressed directly as output of FFA and free glycerol per cell. There were four "fatties" and four controls at each age, 5.8, 8, 14, and 28 wk. Each rat contributed four different tissue sources, intact epididymal fat, and cell suspensions made from epididymal, retroperitoneal, and subcutaneous fat. Variability was great enough to make it necessary to combine data into larger groups in order to define real trends. Fig. 1 displays the effect of age, and each plotted value represents all four rats and all four tissues, i.e., 16 different determinations at each age. There is a strong tendency for the output of "fatty" cells to exceed the normal, most clearly expressed in the younger groups, and absent from the oldest. The absence of any difference at 28 wk is in agreement with the results of the first study, where output was well above normal at 13 wk but not different from the normal at 25 wk. In Fig. 2 the same data have been regrouped in order to display the effect of different tissue sources. Again there is a strong tendency for "fatty" cells to show excess output, particularly of FFA. Differences are smallest for retroperitoneal fat, and perhaps greater for subcutaneous than for epididymal fat. There is essentially no difference between output of intact epididymal tissue and epididymal cell suspensions; average values for the paired difference (tissue minus suspension) are 0.08 ± 0.31 for FFA and 0.28 ± 0.24 for glycerol. Two different statistical calculations have been made on the differences between "fatty" and normal, one for intact epididymal tissue alone, the other for all tissue preparations combined. In the first case, the difference ("fatty" minus normal) was 2.72 ± 0.85 for FFA, and 1.02 ± 0.35 for glycerol; both of these differences meet the 5% level of significance, and if they are combined, the probability of a chance origin for the difference becomes less than 1%. Significance is also greatly improved by omitting the 28-wk data. Using the data for all four tissue sources, differences are 2.27 ± 0.48 for FFA, and 0.46 ± 0.24 for free glycerol; the FFA difference is statistically significant.

It may seem surprising that output from intact epididymal tissue is in such good agreement with output from cell suspensions, in view of Rodbell's report that "lipolysis in isolated fat cells in response to ACTH or epinephrine is 20 to 30 times greater than that observed in intact adipose tissue" (14). This statement applies to conditions of maximal hormonal stimulation, when the

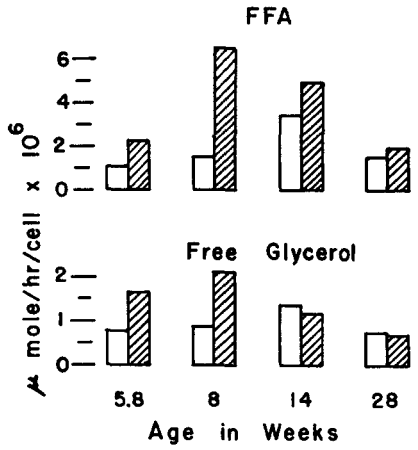

FIG. 1. Effect of age on in vitro fat mobilization in "fatties" (shaded columns) and normals (clear columns), expressed as output per cell of FFA (above) and free glycerol (below). Incubation was for 2 hr in Krebs-Ringer bicarbonate buffer with added glucose, serum albumin, and epinephrine (0.5 μg/ml). There were four "fatties" and four normal rats at each age. There were four different tissue preparations from each rat: intact epididymal fat, and suspensions of epididymal, retroperitoneal, and subcutaneous fat. To illustrate the effect of age, the results for the different tissue preparations have been averaged. Thus, each value in the figure is the mean of 16 different determinations (four rats × four tissue preparations per rat).

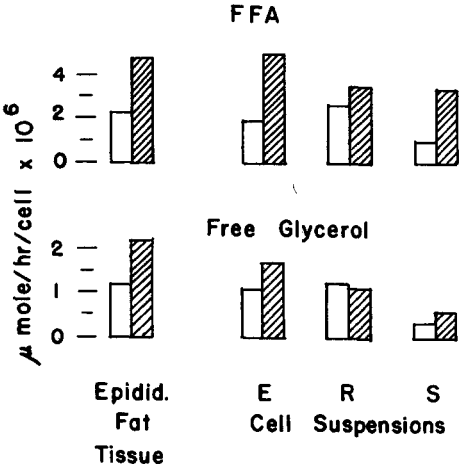

FIG. 2. Effect of tissue source on in vitro fat mobilization in "fatties" (shaded columns) and normals (clear columns). The four different tissue preparations are intact epididymal fat, and cell suspensions from epididymal (*E*), retroperitoneal (*R*), and subcutaneous (*S*) fat. The values in this figure represent a regrouping of the data of Fig. 1. Each value is the mean of 16 different determinations, combining results from 16 different rats (four rats at each of the four ages) for the particular tissue preparation.

isolated cells produced up to 60 μmoles of FFA/100 mg of lipid per hr. Our only comparison of intact tissue and isolated cells is at low epinephrine concentration, with a maximal output of only 1.4 μmoles/100 mg per hr. Obviously, slow diffusion out of intact tissue is much more rate limiting under Rodbell's conditions, with consequent accumulation of FFA in the cell and shutdown of lipolysis. At our much lower level of stimulation, slow FFA diffusion is probably of little consequence. If there is any residual effect of slow diffusion, it should be greater for "fatties" than for controls, because of the larger fat cells of "fatties." This would have the effect of underestimating the excess lipolytic capacity of the "fatty."

Consideration must be given to the effect of cell breakage during incubation of cell suspensions on the reliability of the conclusions drawn from our data. Fat cells from "fatties" are much more fragile than control cells, and this difference is apparently related to cell size. The extent of cell breakage can be judged from a direct comparison which has been made[1] in the same rats between estimates of average cell size according to Hirsch and Gallian (13), where cell breakage is avoided by fixation with osmium tetroxide, and sizing by the measurement of cell diameters in collagenase suspensions. There was excellent correlation between the two sets of measurements applying equally to "fatties" and controls, young and old, but there was numerical agreement for the smallest average cell sizes only, with progressive divergence as cell size increased. Eventually, for the largest cells, the size determined after collagenase action was less than half of the true in vivo size. Using this numerical comparison as a criterion, one can conclude that cell breakage is not significant for epididymal fat cell suspensions from normal rats under 10 wk of age, but it becomes moderately significant in normal adults; it is already considerable in 5.8-wk-old "fatties" and rapidly becomes more and more massive in older "fatties." Subcutaneous cells are generally smaller than epididymal and therefore less subject to breakage; retroperitoneal cells are generally larger and therefore more easily broken. However, in older "fatties" all three cell types approach the same large size (5). We have corrected for cell breakage during preparation of the cell suspension with collagenase by using actual in vitro cell sizes to calculate the output per cell. However, cell breakage continues during the incubation with epinephrine, although at a slower rate than in the presence of collagenase (and accompanying enzyme impurities). Three effects of cell breakage on the results can be envisaged. (*a*) The destroyed cell puts out no more FFA and glycerol for the rest of the incubation period, contributing to a low result. (*b*) At the time of destruction, the cell's content of FFA and glycerol is added to the medium, contributing to a high result. This effect is much greater for FFA than for free glycerol since, because of easy diffusibility, there is very little

[1] Zucker, L. M., R. Andersen, and J. Hirsch. Unpublished observations.

glycerol in the fat cell. (*c*) Retroperitoneal cells being the largest, these should be the most affected, subcutaneous cells the least. For FFA values, errors (*a*) and (*b*) should approximately cancel each other. A constant rate of cell breakage over the 2-hr incubation period is a reasonable assumption to make, and this would be comparable to a model in which all the cells that are going to break up do so after exactly 1 hr. Then for the first hour all the cells would contribute their FFA output, for the second hour intact cells would continue to do so, and the broken cells would contribute their content, which is of the same order of size as output per hour (compare Tables 1 and 2). For the free glycerol, purely negative errors are expected, from error (*a*). There are three comparisons in Figs. 1 and 2 which do not show a higher output by the "fatty," and all three involve free glycerol output: these are retroperitoneal fat cells (Fig. 2) and ages 14 and 28 wk (Fig. 1). These are the three groupings of the data which are most subject to effects of greater cell breakage in the "fatty." Free glycerol output of epididymal cell suspensions from "fatties" is appreciably below the output of intact tissue, and this is the only one of the four comparisons of tissue and cell suspensions to show a difference. Again, the probable explanation is the uniformly depressing effect of cell breakage upon apparent free glycerol output. In summary, different error sources for tissue and cell suspensions and for FFA and glycerol output introduce bias into the results in different ways, but they generally tend to minimize, not falsely magnify, the observed phenomenon of an increase in fat mobilization by the fat cell of the "fatty."

In vivo studies with epinephrine

Some effects of epinephrine injected into 15-wk-old "fatties" and controls are shown in Table 3. Plasma glucose rose similarly in both groups. Plasma FFA and free glycerol, already high in untreated "fatties," rose considerably more in epinephrine-treated "fatties" than in controls. The ratio of FFA to free glycerol is of interest in relation to the value of 3 resulting from simple breakdown of TG. In the untreated nonobese the ratio was 3. In the untreated "fatty" it was 1.4, an indication of recycling of FA to TG in the adipose tissue. In the treated obese and nonobese alike, the ratio was very close to 3. Lactescence was low and remained low in the blood of the nonobese; in the "fatty" it was very high and rose further after epinephrine, although not significantly.

Lactescence is closely related to triglyceride level of the serum (15), and is principally influenced by levels of very low and low density lipoproteins. Since substrate for lipoprotein lipase action appears to be at a high concentration in the blood of "fatties," consideration must be given to this as contributing to high blood values of FFA and free glycerol. Conditions for the correct determination of FFA have proved to be rather critical. Not only do FFA and free glycerol rise steadily during standing of blood, but they also rise if plasma is held in Dole's extraction mix (which would be an analytically convenient procedure). Errors were minimized by processing the blood as rapidly as possible and allowing just 10 min (the interval specified by Dole) for the extraction of FFA. The increase which could take place in the blood samples before initiation of the FFA extraction can be estimated from the following observations. Plasmas at room temperature for 3 hr showed a constant rate of increase in FFA, successively 7.5, 8.7, and 8.2 μmoles/100 ml/hr. For plasmas kept in the cold room, the average rates fell to about 4 μmoles/100 ml/hr. Plasmas obtained 2 hr after epinephrine showed more lipolysis, at the rate of 7 μmoles/100 ml/hr in the cold

TABLE 3. Effects of epinephrine in vivo

	Body Wt	Tib.[a]	Weight of:			Plasma			Lactescence[a]
			Muscle	Fat	Liver	Glucose	FFA	Free Glycerol	
	g	*cm*[3]	*mg*[b]	*g*[b]	*g*[b]	*mg*[c]	*μmoles*[c]	*μmoles*[c]	
"Fatties"									
Epinephrine	440	45.8	26	0.88	0.33	395 ± 15	111.0 ± 5.5	46.4 ± 2.0	0.86 ± 0.10
No epinephrine	422	45.4	26	0.80	0.32	134 ± 4	29.7 ± 3.2	21.7 ± 1.3	0.67 ± 0.14
Normals									
Epinephrine	230	50.4	26	0.15	0.14	362 ± 9	53.3 ± 3.5	16.2 ± 0.57	0.077 ± 0.015
No epinephrine	236	50.6	26	0.14	0.15	123 ± 4	17.5 ± 0.9	5.9 ± 0.51	0.087 ± 0.010

Entries are means ± SEM. There were 12 rats in each of the four groups, matched females, aged 15 wk, fed ad lib. The epinephrine dose was 20 μg/100 g of body wt, injected subcutaneously 2 hr before killing. Tib.[3] is the cube of tibia length, used as a measure of skeletal size. Muscle is calf muscle mass. Fat is weight of retroperitoneal plus parametrial fat pads. This criterion underestimates the relative obesity of the "fatty," since subcutaneous fat is much more expanded in the "fatty" than the abdominal fat pads are.

[a] Lactescence is in arbitrary units (% of solids in an equivalent suspension of Pyrex glass powder of defined sedimentation properties used as a nephelometric standard).

[b] Per cm^3 of tibia.

[c] Per 100 ml of plasma.

TABLE 4. Effects of epinephrine in vivo

	Fatty Acids			Glycogen	
	Liver TFA[a]	Muscle FFA	Fat FFA	Liver	Muscle
	%	μmoles/100 g		%	%
"Fatties"					
Epinephrine	7.0 ± 0.60	0.49 ± 0.03	0.38 ± 0.03	5.2 ± 0.29	0.12 ± 0.012
No epinephrine	5.1 ± 0.45	0.41 ± 0.02	0.21 ± 0.02	6.2 ± 0.30	0.46 ± 0.014
Normals					
Epinephrine	3.9 ± 0.11	0.36 ± 0.015	0.32 ± 0.03	3.7 ± 0.27	0.15 ± 0.015
No epinephrine	3.1 ± 0.06	0.32 ± 0.02	0.14 ± 0.01	5.2 ± 0.25	0.43 ± 0.016

[a] TFA (total fatty acids), determined titrimetrically, has been converted to g/100 g of liver assuming a molecular weight of 284; this is to allow ready comparison with liver fat values as usually reported.

room. Under all three conditions, lipolysis proceeded at the same average rate in "fatties" and controls. Also, there was no individual correlation between degree of lactescence and rate of lipolysis. Apparently, under the conditions studied this rate is zero order with respect to substrate and there is no excessive lipolytic activity in "fatty" blood. It can be concluded that recorded values of FFA and free glycerol may be a little high, but they faithfully reflect in vivo differences, and these differences are not attributable to excessive lipolysis in the bloodstream.

Other effects of epinephrine in vivo are shown in Table 4. Liver fat was higher in "fatties" than in controls and rose in both after epinephrine. The liver fat level in untreated "fatties," although above normal, was by no means in a pathological range. Muscle FFA was significantly higher in untreated "fatties" than in untreated controls and rose after epinephrine in both. A scatter diagram of the relation between individual muscle FFA and plasma FFA in 41 rats showed the same linear trend for all four groups; the correlation coefficient had the value 0.56 ± 0.10, and this is highly statistically significant. It is to be expected that plasma FFA level has a controlling effect on muscle FFA (16); it is of interest that muscles from obese rats respond as muscles from normal rats. Mean FFA levels in adipose tissue showed about the same pattern as muscle FFA in the four groups; however, there was little correlation between individual fat pad FFA and plasma FFA. Muscle glycogen was about the same in resting "fatties" and controls, and the low levels after epinephrine also did not differ significantly. Liver glycogen was a bit higher in untreated "fatties" than in controls, but the difference is not significant; in another experiment[2] we have found numerically identical liver glycogen levels in resting "fatties" and controls.

Effect of fasting

The 6-day fasting experiment is presented in Fig. 3. The "fatties" started with higher FFA than the normal rats, but the two groups were approaching the same level by the 6th day. The "fatties" showed a much larger excess of free glycerol than of FFA, and maintained a large difference during fasting. Plasma glucose was a bit higher in these 40-wk-old rats than in younger ones, and the "fatty" levels were persistently slightly above control levels, although there was no difference initially. Plasma lactescence fell immediately in controls to a stable fasting level. In "fatties" lactescence started very high (characteristically much higher in females than in males), fell significantly only on the 2nd day, and was still above the normal fasting level on the 6th day.

Fig. 4 shows the results of more prolonged fasting. After 8 days the nonobese were apparently coming to the end of their fat stores and could no longer maintain their FFA level. "Fatties" maintained a stable elevated

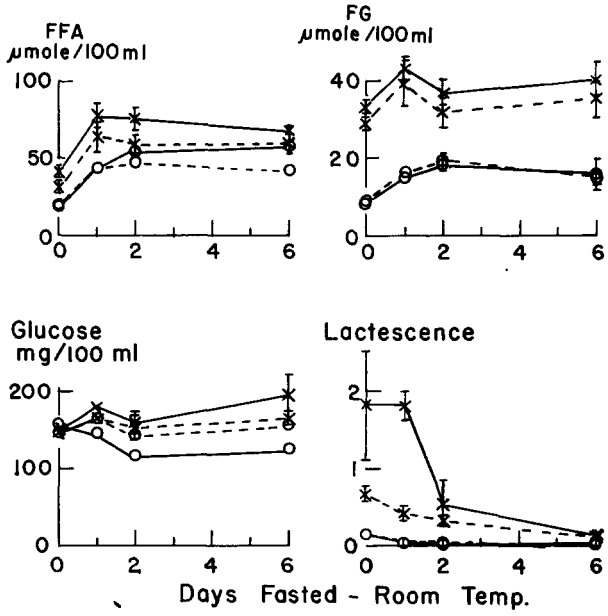

FIG. 3. Effect of fasting for 6 days. Each plotted point represents eight rats, aged 40 wk. "Fatties" are indicated by crosses, normal rats by circles. Data for females are connected by a solid line, for males by a broken line. SEM is indicated by the vertical line through each point, shown wherever this is longer than the symbol. FG is free glycerol. Lactescence is in arbitrary units (nephelometric), but is related to TG by the equation $TG = 0.58\ (Lact)^{0.9}$.

[2] Zucker, L. M. Unpublished observations.

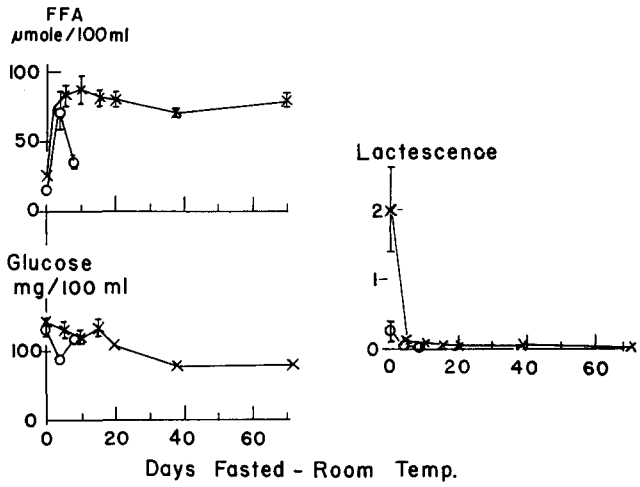

FIG. 4. Effect of fasting for 70 days. Each plotted point represents four or five females, initially aged 15 wk. Symbols are the same as in Fig. 3.

FFA level for 70 days. Glucose in the "fatties" fell to well below 100 mg/100 ml. Lactescence reached the normal fasting level by the 40th day. In a parallel series (17) with occasional body lipid determinations in "fatties," we found 36% body lipid after 42 days; on the 79th day, two out of eight rats died with no visible fat store, and the six survivors averaged 19.5% lipid (a normal value).

Metabolic indices of fat mobilization in fed rats of varying ages

At 2 wk of age, the free glycerol level was normal ("fatty" minus normal = -1.3 ± 1.6 μmoles/100 ml). At 4 wk of age, glycerol was significantly high; values for "fatties" and controls were, respectively, 17.9 ± 0.6 and 7.9 ± 0.5. The FFA level in the "fatty" at this age was still quite normal. FFA is above normal in the "fatty" at 5–6 wk of age (18). Data for other (older) ages are summarized in Table 5. Both FFA and free glycerol levels appear to be quite stable in the normal control, and the data show no obvious sex difference. In the "fatty," on the other hand, the excess in FFA and glycerol already noted (glycerol by the 4th wk, FFA by the 6th wk) continues to increase until about 25 wk of age, and females are more abnormal than males.

DISCUSSION

In vitro and vivo studies are all consistent with the conclusion that the "fatty" mobilizes fat at an above normal rate. It was pointed out several years ago (3), before cellularity data were available, that the depression of in vitro fat mobilization per aliquot of adipose tissue is smaller than the factor by which the fat tissue is expanded in the "fatty," so that calculated whole body fat mobilizing capacity is excessive. Analysis of the fat mobilization in vitro in terms of cellularity of the tissue now establishes that there is also increased fat mobilization per cell in the "fatty"; this increased cellular activity provides the basis for increased fat mobilization per whole rat in younger "fatties," where cell number is the same as in the nonobese (5). At later ages, fat mobilizing capacity is supported at a high level by the increasing number of cells in the whole adipose organ (5). Herberg, Gries, and Hesse-Wortmann (19) reported a similar situation in obese mice. In young obese animals (both *obob* and NZO), there was elevated lipolytic activity per cell in vitro and, in fact, close parallelism between cell size and lipolytic activity. This parallelism continued during the life of the NZO mouse, which also showed no fat cell proliferation. In *obob* mice, however, after cell proliferation began lipolytic activity per cell dropped.

Parallelism between fat cell size and lipolytic activity has also been discussed by Goldrick and McLoughlin (20), who studied free glycerol production in cells from two different adipose tissues in human adults. Subcutaneous cells were considerably larger than omental cells. Lipolytic activity generally paralleled cell volume, under either basal conditions or with theophylline

TABLE 5. Plasma FFA and free glycerol in untreated "fatties" and controls

	Sex	15 wk[a]		15 wk (purified diet)[b]		25 wk	40 wk[c]	
		FFA	Free Glycerol	FFA	Free Glycerol	FFA	FFA	Free Glycerol
		μmoles/100 ml						
"Fatties"	F	29.7 ± 3.2	21.7 ± 1.3	28.5 ± 4.1	33.6 ± 2.0	48.1 ± 7.9	40.4 ± 4.1	33.1 ± 1.5
	M					28.5 ± 2.4	30.4 ± 4.7	28.4 ± 1.0
Normals	F	17.5 ± 0.9	5.9 ± 0.5	15.5 ± 0.9	7.5 ± 1.6	16.0 ± 2.3	18.1 ± 2.0	8.1 ± 0.9
	M					16.2 ± 1.6	19.7 ± 1.5	8.5 ± 0.7

Entries are means ± SEM.
[a] Data from epinephrine in vivo experiment (Table 3).
[b] The purified diet resembles the stock diet in gross composition except for a low fiber content.
[c] Data from the first fasting experiment (Fig. 3).

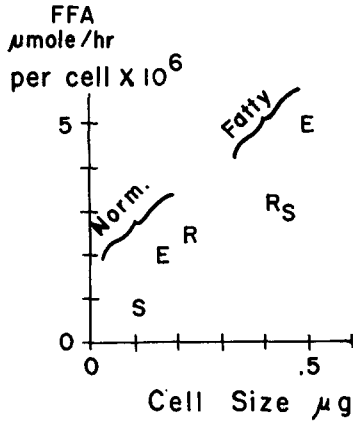

FIG. 5. Relation between cell size and FFA output per cell for three tissues. The location of each plotted value is indicated by the letter appropriate to the tissue, *E* for epididymal, *R* for retroperitoneal, and *S* for subcutaneous.

stimulation, although surprisingly, in view of our results and those of Herberg et al. (19), there was no such relation for epinephrine stimulation. As Goldrick and McLoughlin (20) point out, since cells obtained from different tissues of the same subject must have been under the same in vivo environmental conditions, a relation between cell size and lipolytic activity in the same individuals is much more convincing than such a relation based on comparison of large cells from obese subjects with small cells from lean subjects. We can make both kinds of comparison. Fig. 5 shows the relation between cell size and FFA output for the three kinds of fat cells. The parallelism between cell size and lipolytic activity appears to hold both for different cells from the same rats and between "fatty" and lean rats provided these are of the same average age. But as Fig. 1 shows, the oldest rats have the largest cells and the smallest FFA output, so the parallelism between cell size and lipolytic activity falls down completely if one compares rats of different ages.

In vivo, the "fatty" displays all the normal reactions to epinephrine injection: hyperglycemia, glycogenolysis, lipolysis with elevated levels of FFA and free glycerol, and increased entry of FFA into muscle and also into liver. In the liver, the FFA is incorporated into lipids which are secreted into the blood as lipoproteins. The 2-hr experimental period is too short to allow for much increase in circulating lipid, but the indication is present in the "fatty." Many of the responses of the "fatty" appear to be greater than the changes in the control, and none is smaller. Quantitative comparison of response is, however, difficult because the starting points, the levels in the untreated rats, are not always the same for "fatty" and control (e.g., FFA and glycerol), and also because dosage in the two kinds of rats, equalized on the basis of body weight, cannot be fully comparable, since not all epinephrine-affected tissues are equally expanded in the "fatty." Specifically, the following ratios of "fatty"/control have been noted: body weight 1.85, blood volume about 1.5, liver 2, musculature (as represented by leg muscle) 0.9, and body lipid 7. The effective fat ratio is actually higher than 7, a figure calculated from the total lipid content of 192 g for "fatties" and 28 g for controls (from data obtained in similar rats [17]). Some of this lipid is nervous system and membrane-structural lipid, not part of the fat storage system supporting fat mobilization. If we subtract 10 g, the ratio becomes 182/18 = 10, instead of 7. While for these reasons exact comparison of "fatty" and control responses is not possible, the general impression given by the data is of a slightly greater responsiveness of the "fatties," consistent with the results of the in vitro studies.

Control data for untreated rats in the epinephrine in vivo study suggest that the resting "fatty" is more actively mobilizing fat than the resting lean rat. Plasma FFA is higher (ratio 1.7), free glycerol is higher (ratio 3.7), adipose tissue FFA is slightly high per tissue aliquot and certainly far above normal per cell, and muscle FFA is high. The higher liver fat and plasma lipoprotein (measured by lactescence) are also consistent with greater fat mobilization, although another contributing factor here is the greater food consumption of "fatties." The findings suggest a greater output of FFA from the adipose tissue, greater muscle consumption of fat for fuel, and return of the remaining excess of mobilized FFA to the adipose depots for storage as fat, necessitating a more active cycle of fat transport between liver and adipose tissue. The high level of free glycerol relative to FFA (ratio FFA/free glycerol = 1.4 instead of 3) suggests recycling of FFA to TG with failure to reutilize the glycerol. If, as has been reported for *obob* mice (21), adipose tissue glycerokinase activity is at a metabolically significant level in the "fatty," the observed excess of free glycerol in the plasma must actually underestimate the extent of the recycling. This recycling could involve not only the cycle within the adipose tissue but also the cycle between liver and fat cells. In either case, higher than normal production of glycerol within the fat cell from carbohydrate precursors is indicated, and this has indeed been found by Bray (22).

One must, of course, consider the possibility that the high plasma free glycerol results not from excessive influx but from slow removal by the liver. However, it is unlikely that there is any depression in glycerol uptake by the liver in view of the failure of free glycerol to accumulate much more extensively in the epinephrine-treated "fatties." Similarly, in the absence of directly measured turnover rates for FFA to support our interpretation, it is possible that the high FFA results from

depressed uptake by peripheral tissues, but this is not very probable. Since free glycerol is also high, it would be necessary to assume defective performance by both liver and muscle. Muscle FFA level is related to plasma FFA level in a quite normal fashion in the "fatty," both basally and after epinephrine. The hyperlipemia of "fatties" which persists during several days of fasting and the rise in liver fat after epinephrine are consistent with efficient removal of FFA from the circulation. There is, after all, a cellular basis for increased lipolytic activity in the "fatty," demonstrable in vitro. The "fatty" successfully stores fat for prolonged fasting (4), with appropriate further rises in FFA and glycerol. The "fatty" thus resembles the obese human subject, described by Bortz (23) as being adapted to increased fat mobilization and primed for starvation while in the fed state.

Increased fat mobilization in vivo by the 15-wk-old "fatty" is indicated by many criteria. The possibility of increased fat mobilization in vivo at other ages is suggested by the in vitro data. Some metabolic indices of fat mobilization in vivo have been measured throughout the life of the "fatty," and these indicate that excessive lipolysis and recycling of fatty acid to triglyceride in the adipose tissue begin by 4 wk of age, and excessive fat mobilization into the blood begins by 5–6 wk and continues for life to an increasing degree. At 2 wk of age, when fattening has already begun (17), no abnormality in these indices could be found. The ratio of FFA/free glycerol at various ages is of interest. In nonobese rats it is about 3 in young rats and in either fasting or epinephrine-treated rats; it is closer to 2 in older rats and also young rats fed a calorically enriched diet, which generally produces a somewhat fatter carcass (24). In "fatties" the ratio is 2 by 4 wk of age, nearer to 1 at all later ages, and even below 1 with a calorically enriched diet; it remains near 2 during the early stages of fasting and it approaches 3 after epinephrine.

The data of Table 5 and of Fig. 3 show a sex difference in fat mobilization and transport in "fatties," females being more abnormal than males. This sex difference was not found in the controls. The higher lactescence in females occurs in spite of an opposing effect that might be expected from the common nephrotic state of male "fatties" (25). Overweight is also greater in females, with the ratio "fatty"/control for adults (25 wk or older) averaging 2.2 in females, 1.75 in males. Available data are not adequate to determine how much of this difference in overweight represents difference in fatness and how much might be due to difference in nonfat body size (i.e., a sex difference in the degree of skeletomuscular stunting which is characteristic of "fatties" [4, 5, 17]). However, there is unquestionably some sex difference in fatness. There are no data on the cellularity of the adipose tissue in females. Conceivably, if there is a species-fixed limit to the number of loadable fat cells and a limit to the possible fat loading per cell, maximally nourished female "fatties" might have a total adipose organ approaching the size of the male's, giving the female with its smaller nonfat body size a greater percentage of fat and a greater fat mobilizing capacity.

In a study of fat mobilization in vitro in the adult "fatty" compared with obese controls produced by hypothalamic lesioning, Bray, Mothon, and Cohen (26) concluded that fat mobilization did not differ for the two kinds of obesity. Cell size was the same, and lipolytic activity per cell was the same. These authors did not measure cell number. We have found, in agreement with them, the same cell size in "fatties" and lesioned rats, but there was a difference in cell number; the lesioned rats had the normal number of fat cells, whereas the cell number was greatly increased in "fatties" (5). This has interesting possible consequences. Granted that fat cells in both types of rat are putting out FFA at the same average rate per cell, there should be more fat mobilization in vivo in the "fatty" because there are more cells. This means more FFA in the plasma, more lipoprotein in the plasma, more FFA in the adipose tissue, and more glycerol synthesis in the adipose tissue to esterify the returning FFA. The last expectation is supported by Bray's findings (22).

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