Growth and lipolysis of rat adipose tissue: effect of age, body weight, and food intake

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ABSTRACT The purpose of the present work was to study age- and weight-controlled rats to determine which is the primary factor in reducing the lipolytic response of free fat cells and which has the greater effect on the ratio of fat cells to nonfat cells in adipose tissue. The method for estimating fat cell and nonfat cell numbers is based on the analysis of adipose tissue and fat cell DNA and lipid. In adequately fed rats, epididymal adipocyte hyperplasia is complete between 9 and 14 wk of age. Chronic underfeeding delays, but does not eliminate, normal fat cell hyperplasia and is accompanied by a net loss in the nonfat cell population. During 9–14 wk of age, rat epididymal adipose tissue enlarges mainly through adipocyte hypertrophy. Total fat cells from the epididymal adipose tissue of control rats represent only 20–23% of the total cell population. Chronic underfeeding increases the percentage of fat cells in the fat pad from 23 to 28%. Noradrenaline-stimulated lipolysis is proportional to fat cell numbers but is inhibited when fat cell lipid increases to over 80% of fat pad wet weight. Rat age is apparently not primarily responsible for the decreased noradrenaline-stimulated lipolysis in fat cells of 350-g rats in vitro.

SUPPLEMENTARY KEY WORDS isolated fat cells · adipocyte hypertrophy and hyperplasia · noradrenaline-stimulated lipolysis · fat cell DNA to triglyceride ratio · chronic underfeeding

RECENT STUDIES by Salans, Knittle, and Hirsch (1) have demonstrated the importance of correlating the metabolic activity of adipose tissue with both the size and number of its adipocytes. They found that glucose metabolism was closely related to the number of fat cells within the tissue, but insulin responsiveness was dependent upon adipose cell size. It follows from these results that metabolic comparisons should be expressed per adipose cell rather than per unit of tissue lipid or mass. This consideration is of particular importance when the lipid content or the number of fat cells within adipose tissue is altered by differences in the age and body weight (1, 2), food intake (3), or environment of the experimental groups (4).

The paired epididymal fat pads of the rat are well suited for quantitative studies on the size and number of adipocytes. The pads have specific and discrete boundaries throughout all ranges of enlargement and can be quantitatively removed at different ages and degrees of fat accumulation. Compositional analysis indicates that these organs differ little in total weight (5) and their fat content is confined to the adipocytes (6). It has also been determined that there are no significant differences between the right and left epididymal pads with regard to their total lipid content, adipose cell size, and adipose cell number (3). Therefore, total lipid and DNA analysis of one depot, together with measurements of DNA and lipid on isolated fat cells from the other, should provide valid estimates of adipocyte and nonfat cell numbers as well as fat per cell (2). By using this technique, we have noted a decreased lipolytic response to noradrenaline in fat cells isolated from older, heavier rats compared with younger, lighter controls (4, 7). These results, however, may have been related to either the age of the rats or the fat cell lipid content.

The purpose of the present work was to study age- and weight-controlled animals to determine which is the primary factor in reducing the lipolytic response of free fat cells and which has the greater effect on the ratio of fat cells to nonfat cells in adipose tissue.

MATERIALS AND METHODS

Animals and Feeding

Random-bred albino rats were obtained from the Charles

Abbreviations: GFA, glyceride fatty acid; TG, triglyceride.

River CD Strain (Charles River Breeding Laboratories, Inc., Wilmington, Mass.) at equivalent ages and approximate weights (79 ± 5 g, mean ± SD). Each rat was placed into one of four groups, caged individually, and maintained at 25°C with water supplied ad lib. By restricting the food intake (Purina Lab Chow), it was possible to obtain rats of equivalent ages, but with different mean fat cell sizes. All rats were fed each day at 4:00 PM and were subjected to 12 hr of darkness (6:00 PM–6:00 AM). Group A (n = 18) received 20 g of ground lab chow per day in individual feeding cups designed to reduce spillage (Arthur H. Thomas Co., Philadelphia, Pa.); these rats were killed after approximately 5 wk. Group B (n = 18) received a restricted food supply (10 g of ground lab chow per day); these animals were killed at the same age as group A. Group C rats (n = 15) were maintained similarly as group A, and after approximately 10 wk they were killed along with the rats in group D (n = 15) that received a restricted food supply (10 g per day).

Tissue Isolation

The rats were rapidly decapitated and both epididymal fat pads were immediately excised, blotted, and weighed. The entire left epididymal fat pad (pad L) of each rat was taken for total neutral lipid and DNA determinations, and the right epididymal fat pad (pad R) was used to prepare isolated fat cells. Within each group (A–D) there were 5–6 subgroups made by pooling the isolated fat cells from three rats (total of 15–18 rats per group). Pooling was necessary in order to have sufficient numbers of cells for both adipocyte DNA, triglyceride, and lipolysis determinations.

Adipose Tissue Lipid and DNA Analysis

One entire epididymal fat pad per rat (pad L) was placed into a 50-ml graduated plastic centrifuge tube containing 5 ml of methanol. The tube was closed with a screw-cap and stored 2–3 days at 5°C prior to analysis. The fat pad was later removed and hand-homogenized in 10 ml of 2% perchloric acid (8). In the meantime, the methanol remaining in the plastic tube was evaporated under nitrogen at 70°C. The homogenized fat pad was incubated for 20 min at 90°C while stirring with the homogenizer pestle every 3–5 min. After hydrolysis, the homogenizers were brought to 30–40°C in a water bath and 10 ml of hexane was added. The contents were mixed with the homogenizer pestle and transferred to the original 50-ml plastic centrifuge tube. The homogenizer was washed by the addition of 10 ml of hexane and 5 ml of 2% perchloric acid, and the wash was added to the same tube. After mechanically shaking for 5 min, the phases were separated by centrifugation. The hexane was carefully transferred to a preweighed plastic tube containing a boiling chip. The homogenizer was washed with an additional 10 ml of hexane and the process was repeated. After evaporation at 70°C under nitrogen, the slightly discolored lipids were dried to constant weight in a dessicator under negative pressure. Aliquots of the perchloric acid solution were taken and the DNA was determined spectrophotometrically after reaction with indole according to Ceriotti (9, 10). Since adequate hexane extraction of the homogenate required shaking and centrifugation, the lipids were not extracted in the homogenizer prior to hydrolysis.

Preparation of Isolated Fat Cells

Isolated fat cells were prepared by a modification of the procedure of Rodbell (6, 7).

Isolated Fat Cell DNA and Lipid Analysis

The isolated fat cells from three rats in each subgroup were combined and diluted to 60 ml with Krebs-Ringer phosphate buffer (pH 7.4) containing 4% albumin. An approximation of the lipid content of the 60-ml cell suspension was obtained by noting the weight of tissue treated with collagenase and assuming that lipid weight equals 80% of the tissue wet weight and that 80% of tissue lipid is recovered as fat cell lipid (11). Two aliquots (10 and 20 ml) were removed from the pooled fat cell suspension while stirring, and each was placed in a separate 50-ml plastic centrifuge tube. After the fat cells had floated to the surface, the suspension medium was aspirated away and DNA was added as an internal standard to the 10-ml aliquot of fat cells. The cells, unlike the tissue, did not require mechanical homogenization for adequate DNA recovery, and they were brought to a volume of 10 ml with 2% perchloric acid (for this reason, lipid extraction preceded DNA hydrolysis). Hexane (10 ml) was added and the tubes were screw-capped, mechanically shaken, and centrifuged. The hexane phase was transferred into a preweighed tube and the process was repeated. The combined hexane phases were evaporated at 70°C under nitrogen and dried to constant weight in an evacuated dessicator. There was no apparent discoloration of the lipid fraction under these conditions. The 2% perchloric acid phase was heated for 20 min at 90°C, with stirring every 3–5 min on a Vortex mixer. Suitable aliquots were taken for DNA determination.

Incubation of Fat Cells

The incubation was started by adding 1.0 ml of the fat cell suspension (preequilibrated at 37°C), by means of a large-bore plastic pipette, to polyethylene vials containing 2.9 ml of buffered 4% albumin and 0.1 ml of norepinephrine (preequilibrated at 37°C). In control tubes the appropriate carrier solution was substituted for either fat

cells or norepinephrine. Incubation was carried out at 37°C in a gyratory water bath (New Brunswick Scientific Co., Inc., New Brunswick, N. J.) at 200 rpm. At the end of the incubation period, the reaction was stopped by the addition of 5 ml of Dole's acid extraction medium (12). Appropriate zero time controls as well as experimental tubes containing everything but adipocytes were run concurrently.

The total fatty acid content in each flask was determined as previously described (4). Glycerol in the aqueous phase was measured using a micromodification of the enzymatic method of Vaughan (13), in which DPNH formation was assayed fluorometrically.

L-Arterenol bitartrate, monohydrate (noradrenaline, USA grade; Sigma Chemical Co., St. Louis, Mo.) was dissolved in 0.01 mM ascorbic acid immediately before use. The concentration chosen (1.5 μM) provides maximal stimulation of lipolysis under these conditions (7).

Salmon testes DNA was obtained from Worthington Biochemical Corp., Freehold, N.J., and was used as a reference standard (10).

Calculations

The above procedures yield the following data: wet weight of epididymal fat pads L and R, total DNA to wet weight ratio of pad L, total lipid to wet weight ratio of pad L, DNA to lipid ratio of pooled fat cells from fat pads (pad R) of three rats, and net stimulated glycerol release (30 min minus zero time) and total fatty acids in each incubation flask.

The total number of cells of both epididymal fat pads per rat was calculated as follows: total cells = [μg DNA in pad L + (μg DNA/g wet wt) in pad L × (g wet wt of pad R)] × (cells/μg DNA).

The total number of adipocytes in both fat pads was estimated as follows: total adipocytes = [g lipid in pad L + (g lipid/g wet wt in pad L) × (g wet wt of pad R)] × (μg DNA/g lipid in pooled adipocytes) × (cells/μg DNA).

Values for GFA release per μg of DNA per 30 min were obtained as follows: nmoles GFA/μg DNA/30 min = [3 × (nmoles glycerol per 30 min/incubation flask)/(μmoles lipid/incubation flask)] × (μmoles lipid/μg DNA in pooled adipocytes).

The above calculations are based upon the following assumptions and values:

1. The percentage of epididymal fat pad wet weight as lipid is the same for both pads.
2. The total DNA to lipid ratio is the same for both epididymal fat pads.
3. The DNA to lipid ratio obtained from the pooled fat cells is an average value, representative of the normal distribution of fat cell sizes in the pads.
4. The calculation of cells per μg of DNA was based on the commonly accepted value of 7×10^{-6} μg of DNA per cell (14).
5. The average cell neutral lipid has a molecular weight of 809.

RESULTS

Rat Growth

The results of several feeding experiments (15–18) have indicated that approximately 20 g of laboratory chow per day will support normal growth in male rats. Groups A and C received only 20 g of chow per day so that growth would be as normal as possible, but the intragroup variability in total fat pad lipid would be reduced. The average body weight of rats in group C (Fig. 1) was somewhat less than the average for this strain (data obtained from Charles River Breeding Labs), but was nearly identical to that of rats fed ad lib. (17–19). On the other hand, the rats in groups B and D had restricted growth curves similar to others intentionally underfed (19) and nearly identical to those trained to eat their entire daily ration in a 2-hr meal (17).

Tissue Growth

The effects of rat age and underfeeding on the total wet weight, lipid as percent wet weight, and total DNA content of the epididymal adipose tissues are tabulated in

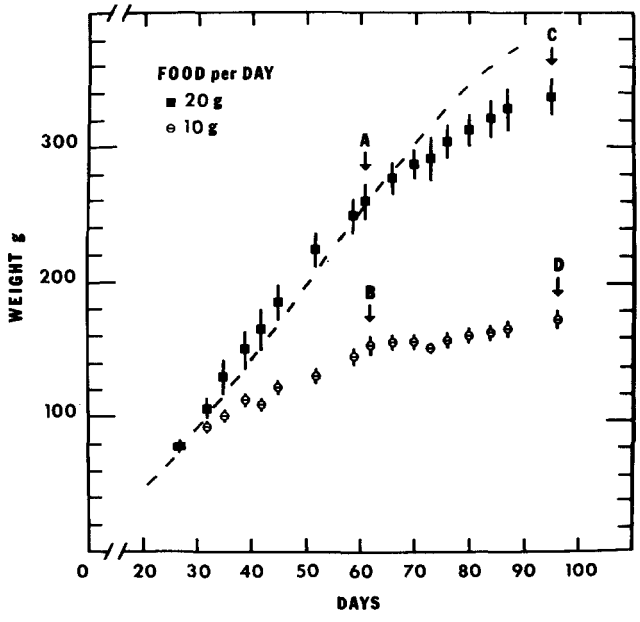

FIG. 1. Growth rates of rats with restricted food supplies. Letters indicate the time of killing of each group (*A–C*). Other details are in text.

Table 1. The increase in age of group C (compared with group A) resulted in a 30.1% increase in body weight. This change is reflected by significant increases in fat pad wet weight and percent lipid, but not in total pad DNA. Conversely, underfeeding resulted in significantly lower body and fat pad weight gains in groups B and D. It is noted that group B, but not group D, is significantly different from group A in the percentage of fat pad wet weight as lipid. On the other hand, both groups B and D developed significantly less total fat pad DNA than group A. It is an interesting question whether this failure to increase total pad DNA by underfeeding is analogous to the loss of depot lipid and DNA during weight reduction (20). If fat depots contained mostly fat cells as opposed to nonfat cells (reticuloendothelial cells, etc.), the data would suggest that between 9 and 14 weeks of age, lipid is deposited mainly in preexisting cells and that underfeeding results in fewer fat cells containing less lipid. This suggestion, however, is not defensible without qualification, since Rodbell's data (21) indicate that a large fraction of adipose tissue DNA is not found within adipocytes themselves but within the stromalvascular cells. Thus, whether the development of less total fat pad DNA with underfeeding represents less fat cells, nonfat cells, or both, becomes an important consideration.

The results of the fat cell analyses are shown in Table 2. Since there were no obvious signs of adipocyte breakage (oil droplets) and since some unavoidable losses occur during the washing procedures, the final high yield of fat cell lipid as percent of pad lipid indicates that the adipocyte DNA:triglyceride ratios were based on highly representative samples (78–83%) of the total fat cell populations. The lack of any statistically significant differences between these yields indicates that, within this range, the amount of lipid per cell does not affect adipocyte recovery. Salmon testes DNA was added to the fat cells as an internal standard and was completely recovered in all groups except group A (86.9% recovery).

TABLE 1 EFFECT OF AGE AND DIETARY RESTRICTION ON GROWTH OF EPIDIDYMAL ADIPOSE TISSUE OF RATS

Group	n_1*	Age	Food per Day	Body Wt	Epididymal Fat Pads			Total Fat Pad DNA
					Total Wt	n_2*	% Lipid	
		days	*g*	*g*	*g*			*μg*
A	18	61	20	259† ± 13	2.299 ± 0.356	17	78.2 ± 2.1	681 ± 138
B	18	62	10	154 ± 8	0.825 ± 0.178	18	63.1 ± 7.0	559 ± 95
P (B vs. A)‡				<0.0005	<0.0005		<0.0005	<0.0005
C	15	95	20	337 ± 13	3.292 ± 0.742	14	85.7 ± 2.3	646 ± 206
P (C vs. A)				<0.0005	<0.0005		<0.0005	
D	15	96	10	173 ± 7	1.023 ± 0.330	14	77.7 ± 4.9	450 ± 66
P (D vs. A)				<0.0005	<0.0005			<0.0005
P (B vs. C)				<0.0005	<0.0005		<0.0005	
P (B vs. D)				<0.0005	<0.0005		<0.0005	<0.0005
P (C vs. D)				<0.0005	<0.0005		<0.0005	<0.0005

* n_1, number of animals measured in each group; n_2 reflects accidental sample losses at later stages in the assay procedures.
† Mean ± SD.
‡ Significance testing was carried out using Student's "*t*" test. *P* values >0.05 are omitted from the table.

TABLE 2 EFFECT OF AGE AND DIETARY RESTRICTION ON FAT CELL–NONFAT CELL POPULATION OF EPIDIDYMAL ADIPOSE TISSUE

Group	n_2*	Total† Cells (×10⁶)	n_3*	Total Adipocyte DNA	Total Adipocytes (×10⁶)	Adipocytes % of Total	% Pad Lipid Isolated as Fat Cell Lipid	% Recovery of DNA Internal Std.	μg Adipocyte DNA/g TG
				μg					
A‡	17	97.5 ± 19.8§	6	138 ± 22	19.8 ± 3.2	20.3 ± 2.0	82.5 ± 5.8	86.9 ± 10.2	77.2 ± 6.8
B	18	80.0 ± 13.7	4	106 ± 8	15.1 ± 1.2	19.1 ± 1.5	83.1 ± 8.6	98.9 ± 7.1	209 ± 20
P (B vs. A)‖		<0.005		<0.05	<0.05			<0.05	<0.0005
C	14	90.3 ± 29.6	5	140 ± 9	20.0 ± 1.3	23.2 ± 7.1	83.4 ± 4.0	100.0 ± 8.1	51 ± 5
P (C vs. A)								<0.05	<0.0025
D	14	64.4 ± 9.5	5	128 ± 21	18.3 ± 3.0	28.1 ± 3.9	78.0 ± 6.5	100.7 ± 4.5	154 ± 33
P (D vs. A)		<0.0005				<0.01		<0.05	<0.0025
P (B vs. C)				<0.01	<0.05				<0.001
P (B vs. D)		<0.0025				<0.0125			
P (C vs. D)		<0.005							<0.001

* n_2, number of individual animals measured; n_3, number of pooled subgroups of 2–3 animals used for isolated fat cell DNA, triglyceride, and lipolysis determination.
† Total cells = total DNA (Table 1) × 1.43 × 10⁵ cells/μg DNA (14).
‡ Values in group A depending upon isolated fat cell DNA were corrected to 100% recovery of DNA added as internal standard.
§ Mean ± SD.
‖ Significance testing carried out using Student's "*t*" test. *P* values >0.05 are omitted from the table.

In Table 2, the data for group A were corrected, where indicated, to 100% DNA recovery. The fat cell DNA:triglyceride ratios reflect how very different, and yet reproducible, are the characteristic yields of DNA and triglyceride for the 5–6 subgroupings of animals within each group (A–D). These different ratios clearly represent how the average lipid content per cell (Table 3) is altered by age and food intake of the rats (2).

When the size of the total adipocyte population is calculated, the data indicate that between 61 and 95 days of age there is no significant increase in the fat cell population of rats fed 20 g of chow per day. On the other hand, underfeeding group B has retarded the normal increase in fat cell numbers seen in group A. Since the fat cell population of group D was not significantly reduced by underfeeding (D vs. A or C), this indicates that underfeeding may delay fat cell hyperplasia, thereby extending this phase of development.

The determinations of total cell numbers of the individual fat pads show no significant changes between groups A and C. Thus, the number of adipocytes ranged from 20.2 to 23.2% of the total number of fat pad cells in groups A and C, respectively. These measurements are less than the 33% estimated by Rodbell (21) but similar to that of Hirsch and Han (3). Underfeeding, on the other hand, reduced the normal increase in total fat pad cells in groups B and D. This reduction in group D occurred mainly in the nonfat cell population and resulted in an increased proportion of fat cells in the fat pad (28.1%). Since the total number of cells in group D is less than in group B but the number of adipocytes (B vs. D) is not significantly different, the results indicate a net loss in the nonfat cell population.

The data relating noradrenaline-stimulated GFA release to fat cell size and number are found in Table 3. In groups A, B, and D, the highly significant differences in lipolysis based on adipocyte lipid content are obviously proportional to the number of cells represented by a unit of triglyceride in each group. Thus, when lipolysis is calculated as a function of the number of cells reacting (lipolysis per μg of DNA), these differences disappear. There is, however, one exception, group C. In this case, the rate of lipolysis (per μg of DNA) is significantly less than in any other group. Since there is no decrease in lipolysis in groups B and D, the effect in group C is apparently not one of age, per se. This decrease in lipolysis does concur, however, with the average increase in lipid content per cell in group C over group A. Thus, a critical lipid content per cell may have been reached, whereby the adipocytes failed to respond maximally to the hormonal stimulus of catecholamine. This hypothesis is supported by the observation that lipolysis in group D, where the increase in lipid content with age failed to develop, is not different from that in group A.

DISCUSSION

The present study was initiated to determine whether the age of the animal or the average lipid content of its adipocytes were factors in the lipolytic response of fat cells to noradrenaline. Since our previous results indicated that fat cell hyperplasia was nearly complete in the epididymal adipose tissue of 260-g rats, this average weight was considered a probable starting point for changes in the tissue related primarily to fat cell hypertrophy. Of special interest were the observations that an average increase in rat body weight to 350 g was related to decreased insulin sensitivity (19) and progressive hyperinsulinism (22). Thus, the control rats were killed when they reached this weight. It is clear from these results and those of Salans et al. (1), however, that lipolysis and perhaps other alterations in adipose tissue

TABLE 3 NORADRENALINE-STIMULATED GFA* RELEASE OF ISOLATED ADIPOCYTES: EFFECT OF ADIPOCYTE AGE, NUMBER, AND LIPID CONTENT

Group	n_s†	μmoles GFA Release/ mmoles TG‡/30 min	μg Lipid/Adipocyte	nmoles GFA Release/ μg DNA/30 min	Adipocytes/g TG ($\times 10^6$)
A§	6	41.2‖ ± 9.0	0.0913 ± 0.0079	671 ± 182	11.0 ± 1.0
B	4	120.4 ± 12.9	0.0336 ± 0.0039	710 ± 17	29.9 ± 2.9
P (B vs. A)¶		<0.0025	<0.0005		<0.0005
C	5	16.1 ± 6.4	0.1388 ± 0.0122	395 ± 157	7.3 ± 0.7
P (C vs. A)		<0.005	<0.0025	<0.05	<0.0025
D	5	82.7 ± 13.2	0.0472 ± 0.0101	674 ± 81	22.0 ± 4.8
P (D vs. A)		<0.0025	<0.0025		<0.005
P (B vs. C)		<0.0005	<0.001	<0.05	<0.0005
P (B vs. D)		<0.0125			<0.025
P (C vs. D)		<0.0005	<0.001	<0.05	<0.0025

* Net glycerol × 3 = glyceride fatty acids released.
† n_s, number of pooled subgroups of 2–3 animals used for isolated fat cell DNA, triglyceride, and lipolysis determinations.
‡ Net glycerol × 3 per mmole fat cell triglyceride (TG).
§ Values in group A depending upon isolated fat cell DNA were corrected to 100% recovery of DNA added as internal standard.
‖ Mean ± SD.
¶ Significance testing carried out using Student's "*t*" test. *P* values >0.05 are omitted from the table.

metabolism, previously related to differences in the age and body weights of animals, are primarily proportional to the number of cells present in a unit weight of the tissue. There are *two* major variables, however, which determine the number of adipocytes within a unit weight of adipose tissue. The first of these is the fat cell triglyceride to DNA ratio, which can obviously reflect the enormous increases in lipid content per cell attainable by adipocytes. The second is the fat cell to nonfat cell ratio, which is considered important because the adipocytes represent only 20–25% of the total adipose tissue cell population, and this percentage can be altered experimentally. In the present work there was significant increase in this ratio due largely to net loss in nonfat cells brought about by underfeeding. In a previous study, we have shown that chronic cold exposure can produce a net increase in the fat cell population (4). Thus, when metabolic comparisons must represent equivalent numbers of adipocytes, factors such as underfeeding, chronic cold exposure, or even normal growth make quantitative fat cell analysis imperative. For example, the following values calculated from the tables demonstrate how different methods of comparison alter the number of fat cells represented in groups A and D.

developed by Hirsch and Gallian (26), our results confirm the finding (3) that rat epididymal adipose tissue hyperplasia is essentially complete at 9 wk of age and that further growth is mainly through a process of cellular hypertrophy. Hirsch and Han (3) have shown that chronic underfeeding of 350-g rats will reduce fat cell size without necessarily affecting cell number. Our results, on the other hand, indicate that chronic underfeeding of young rats (80 g) delays, but does not prevent, the attainment of a normal epididymal fat cell population. Chronic underfeeding, however, results in a net loss of total epididymal fat pad DNA. This confirms the earlier observation of Zingg, Angel, and Steinberg (20) and indicates that chronic starvation may alter the proportions of fat cells to nonfat cells. In this case (B vs. C), underfeeding results in a 3-fold difference in the number of adipocytes per 100 mg of adipose tissue. These data support the contention of Cohn and Joseph (27) that if "underfeeding" is synonymous with "meal eating," in the absence of quantitative data on adipocyte numbers metabolic effects cannot be clearly attributed to the periodicity of eating. Under these circumstances, periodicity studies clearly require pair-fed animals maintaining equivalent weights.

	No. of Adipocytes Represented by:				
	1 μg Adipocyte DNA	1 μg Adipose Tissue DNA	100 mg Adipose Tissue (wet wt)	100 mg Fat-cell Lipid	100 mg Fat-free Adipose Tissue (wet wt)
In group A	143,000	28,900	861,000	1,104,000	3,950,000
In group D	143,000	40,000	1,794,000	2,202,000	8,030,000
$\frac{\text{Adipocyte No. in A}}{\text{Adipocyte No. in D}} \times 100$	100%	72.3%	48.0%	50.1%	49.2%

It is clear from these calculations that only adipocyte DNA provided the necessary basis for comparison.

An awareness of these complex factors has been helpful in sorting out some of the interrelationships between growth and metabolism in both brown and white adipose tissue. For example, the increased brown fat mass occurring in a mammalian hibernator (*Citellus tridecemlineatus*) after cold exposure resulted primarily from the deposition of neutral lipid in existing fat cells and from increased tissue water (23). On the other hand, the decreased epididymal fat pad mass found in chronically cold-exposed rats was accompanied by an increase in total fat cell content proportional to the body weight (250–500 g) (4). In controls of equivalent weight, the adipose tissue grew primarily by the deposition of neutral lipid in existing fat cells (4). The present study has attempted to determine the effects of "underfeeding" (24, 25) on the proportions of fat cells to nonfat cells in epididymal adipose tissue.

By employing a technique different from the one

The increased percentage of fat cells in group D is accompanied by an increase in the percentage of fat pad lipid. This reflects the alteration in cell ratios (fat cells vs. nonfat cells). If it results in a proportionally larger share of circulating nutrients going to fat cells, it may explain, in part, the rapid repletion of lipid within adipose tissue after weight loss and the resumption of a more adequate diet.

The results for the number of fat cells in the epididymal fat pads of approximately 350-g rats agree with the results of Nestel, Austin, and Foxman (28) derived from microscopic measurements. Our calculations of cell number are based on the commonly accepted value 7×10^{-6} μg of DNA per cell (14).

The present results support our previous findings (4) of a decreased lipolytic ability in fat cells from older, heavier rats compared with younger, lighter controls. This is not an effect of age, per se, since it is prevented by underfeeding rats of the same age, nor is it likely to be related to the time of food intake relative to sacrifice

since there was no effect between groups A and B. It is apparently related to the increased lipid content per cell or the decreased surface area to volume ratio. This explanation does not preclude the possibility, however, that insulin, or some other factor, plays a primary role in this process. In this regard, it is interesting to note that the loss of insulin sensitivity and the increased conversion of glucose to glyceride-glycerol are associated with an increase in fat pad lipid to over 80% (19). The decreased lipolytic response to noradrenaline reported here also correlates with an increase in epididymal fat pad lipid from 78 to 86% and it is within this weight range that rat hyperinsulinism is reported to occur (22). Weight reduction can reverse insulin resistance (19) and restore glucose tolerance to normal (29). During weight reduction through underfeeding (19), older, heavier rats showed little or no lipogenesis and a rapid conversion of glucose to glyceride-glycerol. This pattern of carbohydrate metabolism is similar to that reported by Cahill, Leboeuf, and Flinn (30) in response to catecholamines. Thus, weight reduction, like underfeeding, may result in a greater sensitivity to the lipolytic effects of catecholamines and induce, via free fatty acid production, an alteration in the pattern of carbohydrate metabolism.

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