Cellularity of adipose depots in the genetically obese Zucker rat

P. R. JOHNSON, L. M. ZUCKER, J. A. F. CRUCE, and JULES HIRSCH

The Rockefeller University, New York 10021, and the Harriet G. Bird Memorial Laboratory, Stow, Massachusetts 01775

ABSTRACT Cell size and number of three adipose depots, epididymal, retroperitoneal, and subcutaneous, were determined during growth of the obese Zucker rat ("fatty") and nonobese Zucker control. Cellularity of these depots in the adult "fatty" was compared with that in nonobese controls and in nonobese Zucker rats made obese by ventromedial hypothalamic lesions. Epididymal and retroperitoneal depots in the nonobese rat grew by cell enlargement and increase in cell number until the 14th wk, when number became fixed; further increase in depot size occurred by cell enlargement. The subcutaneous depot added cells until the 26th wk. In the Zucker "fatty," cell number increased until the 26th wk in all depots, accompanied by extreme cell enlargement. The enlarged adipose depots of the adult Zucker "fatty," when compared with the nonobese control, are the result of both hypertrophy and hyperplasia. Depot enlargement in the lesioned animal is the result of hypertrophy. "Fatties" have more cells in adipose depots than do lesioned rats. Genetic obesity in the Zucker rat is clearly different from the obesity produced by hypothalamic lesioning.

SUPPLEMENTARY KEY WORDS adipocyte · cell size · cell number · hypothalamic obesity · subcutaneous fat depot

THE CONCEPT that mature adipocytes are fixed in number in the adult organism is of interest in relation to both a general understanding of growth and the clinical problem of the development of obesity in humans. Particularly crucial is the finding that cell number and cell size of a given adipose depot influence its metabolic activity (1). Hirsch and Han (2) have reported that adipose cell number becomes fixed by the 15th wk of life in the Sprague-Dawley rat; neither starvation nor obesity produced by hypothalamic injury was followed by any permanent change in cell number in the adult animal. Preweaning undernutrition, on the other hand, did influence cell number in the rat (3). Hirsch, Knittle, and Salans (4) found the increased size of the adipose depots in very obese human adults to be related primarily to an increased cell number, although that number remained constant after weight reduction. These findings point to the conclusion that adipose cellularity is determined during early growth and development and that it is subject to environmental and metabolic influences which occur during that time.

Further studies of adipose cellularity during the development of obesity are warranted. The Zucker rat, designated "fatty," provides an excellent model for such investigations. The Zucker "fatty" carries a mutation for obesity that is inherited as a Mendelian recessive (5). The mutant is marked by extreme obesity of early onset, recognizable by 3 wk of age.

The present report describes the changes in adipose tissue cellularity that occur during growth and development of the Zucker "fatty" and its lean littermate. In addition, the cellularity of three adipose depots, epididymal, retroperitoneal, and subcutaneous, in the adult "fatty" is compared with the cellularity of the same sites in two types of control animals, namely, the Zucker lean littermate and the lean littermate made obese by hypothalamic lesion. The adult lesioned animal is considered as a control in the sense that it is a normally lean animal in which adipocyte precursor cells have been filled with lipid as a result of the pressure of hyperphagia.

METHODS

Animals

The rats used in this study were all of the Zucker 13M stock, which originated as a cross between Sherman and NIH black, and has been continued at the Harriet

G. Bird Memorial Laboratory, Stow, Mass., for many generations of random breeding. This colony carries the mutant recessive gene "fatty" (*fa*), which when present in the homozygous form (*fa/fa*) produces extreme obesity of juvenile onset (6). Heterozygotes and normal homozygotes (*fa/Fa* and *Fa/Fa*) are lean and phenotypically indistinguishable. The nonobese controls used in the present study were littermates of the "fatties," and were of undetermined genotype (2/3 probability of being *fa/Fa*, 1/3 *Fa/Fa*).

The animals were raised in litters of eight on the regular stock colony regimen. The regimen included ad lib. feeding of a commercial pelleted rat diet of conventional type (fat content 5%; Hemlock Hollow Rat Diet, Agway, Waverly, N.Y.), spinach fed once a week to the older rats, and a lactation diet fed to nursing mothers with pups, and to the weanlings up to 7 wk of age, in addition to the pelleted diet. The lactation diet had the following composition (%): wheat 45, skim milk solids 35, lactalbumin 10, yeast 2.2, cottonseed oil 5, salts (including trace elements) 2.8, sulfamerazine 0.025, and vitamins A, E, and B complex. The rats, which were shipped to the New York laboratory, were maintained thereafter on Purina Lab Chow fed ad lib. All animals were caged individually in a temperature-controlled room with a 12-hr light–dark cycle.

In the first experiment, "fatties" and littermate controls were killed at 7, 9, and 37 wk of age. The cell size and cell number of the epididymal pads were determined. In the second experiment, observations on "fatties" and littermate controls were extended to include determinations at 3, 5.5, 8, 14, and 26 wk. The cellularity of three adipose depots, epididymal, retroperitoneal, and dorsal scapular subcutaneous, was determined. In the third experiment, "fatties," littermate controls, and littermates made obese by ventromedial hypothalamic lesions were killed at 26 wk of age. Cellularity was determined for epididymal, retroperitoneal, and dorsal scapular subcutaneous pads. The fourth experiment was a study of the distribution of fat over the entire body of maximally obese "fatties" and controls. Maximal obesity in Zucker "fatties" occurs in year-old females.

Dissection and Processing of Tissue

The left epididymal pad was removed just distal to the major blood vessel in the base of the pad. The left retroperitoneal pad was removed as a triangular section extending from a vertex in the inguinal region up the midline and across at the lower pole of the kidney, extending laterally as far as fat was visible. The subcutaneous pad lying dorsal to the scapular region was removed in the following manner. With the animal lying first on the left and then on the right side, lateral cuts were made through the skin from the top of the haunch to the base of the ear, to reveal the underlying fatty layer. At a point about midway of this cut, where the fatty sheath thins markedly, lateral cuts were made into the fatty pad following the line of the previous skin incision. Next, a cut was made through the skin just below the rib cage across the dorsal surface joining the two lateral incisions. The rectangular flap of skin produced by these procedures was then carefully peeled back, leaving the subcutaneous pad intact. The pad was carefully dissected away from the underlying muscle and fascia and floated intact in a petri dish containing warm saline. The intrascapular brown fat embedded in the white adipose pad was carefully dissected away, as well as any muscle strips that had been removed with the pad. This method results in the removal of a section of the subcutaneous fatty sheath of the rat which has proved to be reproducible. In an earlier report this same fat pad was designated as dorsal subcutaneous fat, cephalad portion (6).

The dissected tissues were washed in warm saline. Two representative samples from each tissue were prepared by cutting bits from the proximal, medial, and distal portions of the pad to obtain a portion of approximately 100 mg wet weight. Each sample was placed on a tared nylon sieve, blotted, and weighed. One piece was placed in chloroform–methanol 2:1 for lipid extraction, and the other piece was fixed in osmium tetroxide for subsequent cell counting. The remaining tissue was blotted and weighed on a tared nylon sieve so that the total wet weight of the tissue could be determined. After a 48-hr extraction, distilled water (0.2 vol) was added to the chloroform–methanol extract to break the phases, and aliquots for lipid determination were taken from the chloroform phase. Lipids were determined gravimetrically.

Determination of Cellularity

The cells separated from the osmium-fixed tissue sample were counted in a Coulter Counter to determine the number of cells in a known wet weight of tissue. The details of the procedure are those reported as method III by Hirsch and Gallian (7). Cell size is expressed as micrograms of lipid per cell. In the first two of the three experiments to be reported, cellularity was determined by a second independent procedure, which has been used in some variation by a number of other investigators (8–11). This procedure involves the measurement of cell diameter in photomicrographs of fat suspensions prepared by collagenase digestion, the calculation of cell volume from the measured diameter, and the calculation of cell number from adipose organ weight and fat content. Since we believe the first procedure to be the method of choice for cell size and number determina-

tions in tissues, the data presented in this report are those obtained by the technique of Hirsch and Gallian (7).[1]

Carcass Analyses

Total carcass fat, water, and nitrogen were determined in some rats in each age group of the second experimental series of animals by a slight variation of a previously described procedure (6). The carcass analysis was carried out by passing the carcass, after washing out the intestines, through a meat grinder with a known weight of sodium sulfate (1–2 times the carcass weight). After several grindings and air-drying in a thin layer for a day, a fairly homogeneous and stable powder was obtained which was recovered with minimal loss of solids; it was weighed and stored in a tightly closed container in the cold. Aliquots of the powder were taken for fat determination (4 g), residual water content (2 g), and nitrogen determination (2 g). The sample for fat determination was extracted with cold chloroform–methanol 2:1. Distilled water (0.2 vol) was added to an aliquot of the extract to break the solution into two phases, and an aliquot of the resulting chloroform phase containing 20–60 mg of fat was measured gravimetrically. Nitrogen was determined by a semimicro-Kjeldahl digestion with mercury as catalyst, followed by steam distillation of the NH_3 freed with sodium thiosulfate. Residual water was determined by oven-drying of a 2-g aliquot of the air-dried material. Total carcass water was calculated by adding the residual water content to the difference between the initial weight of carcass plus salt and the weight of the air-dried preparation.

In the third experiment of this series, slightly different procedures were followed for carcass analyses. The subcutaneous pads of fat which still remained after dissection of the dorsal scapular pad were also carefully dissected away, washed in warm saline, blotted, and weighed. The weight of this fat plus the weight of the dorsal scapular pad is reported as total subcutaneous fat weight, and is used as the measure of fatness in place of percentage of total body fat. The head was severed at the first cervical vertebra, the tail at its point of connection to the body, and the forepaws at the wrist joint, and these parts were discarded. All skin, all viscera, and all remaining visceral fat deposits were removed and discarded. The stripped carcass, representing the musculature and a large and anatomically defined portion of the skeleton, was thoroughly washed, blotted dry, weighed, and analyzed for water, fat, and nitrogen content as described above.

In the fourth experiment, the carcass was divided into skin and fur, total subcutaneous fat (procedure described above), mesenteric plus omental fat, other dissectable abdominal fat (principally gonadal and retroperitoneal), pooled abdominal organs, and residual carcass containing most of the blood. In this case, nothing was discarded except intestinal contents; the total weight of the six fractions was within 5–8 g of the original cleaned carcass. Each of the six fractions was analyzed for lipid content by the method described above.

Lesioning

Obesity was produced by bilateral electrolytic lesions of the ventromedial nuclei at 13–15 wk of age. With the animal under sodium pentobarbital anesthetic, its head was positioned in the stereotaxic unit so that the bregma and lamboid sutures were in the same horizontal plane. Coordinates were 2.6 mm posterior to the bregma, 0.5 mm lateral to the mid-sagittal sinus, and 8.5 mm below the surface of the brain. 2 ma of anodal direct current was passed through the positioned electrode for 15 sec.

RESULTS

The data on adipose tissue characteristics were obtained by sampling three adipose depots in each of 4–6 animals in the developmental series and 6–13 animals in the study on adults. The sites sampled, left epididymal pad, left retroperitoneal pad, and dorsal scapular subcutaneous pad, were examined as to lipid content, adipose cell size, and adipose cell number. Critical data points are presented as the mean ± SEM. Other data reported include body weight, tibia length, fatness, nitrogen, and fat-free solids. The carcass analysis data were generally obtained on representative smaller samples, with 2–4 rats per group.

Growth and Fat Distribution

The growth curves of male "fatties" and their lean littermates from weaning (3 wk) to 26 wk of age are presented in Fig. 1. Although there is no appreciable weight difference in the two groups at weaning, there is a greater percentage of body fat in the obese animal, and this condition contributes to a barely detectable difference in appearance of the "fatties" and their nonobese littermates. By 5 wk of age, as previously reported (5, 6), a decided weight difference is present,

[1] An exception occurs in the data for 3-wk-old animals; these data include values determined by the second method for four animals. The two methods are in exact agreement when the cells to be measured are fairly small, i.e., less than 0.135 μg of lipid. In an extensive comparison of the two methods (unpublished data, to be reported separately), Zucker, Andersen, and Hirsch found that in 32 comparisons made on a variety of tissues from young rats, mean cell size was 0.0775 for the osmium fixation method and 0.0750 for the collagenase method. The average of the individual differences was 0.025 with a SEM of 0.0033.

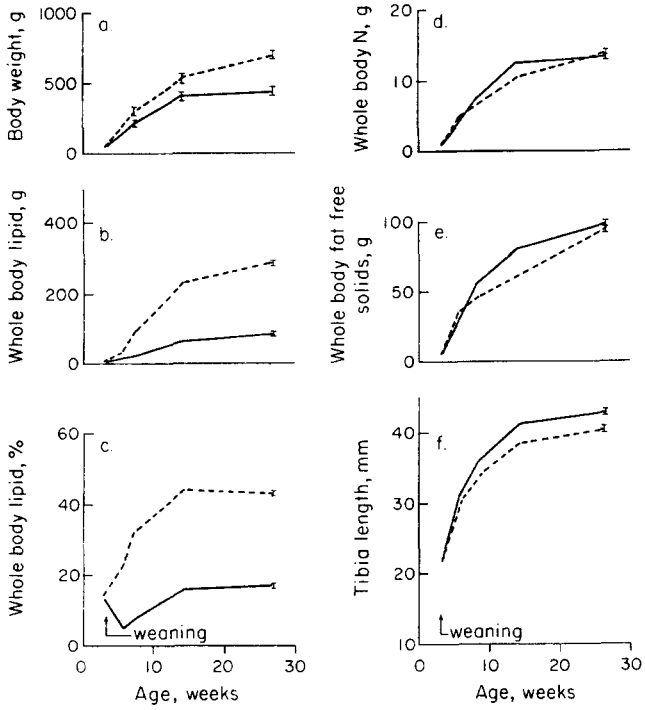

FIG. 1. Body weight (*a*), whole body lipid (*b*), whole body lipid % (*c*), whole body N (*d*), whole body fat-free solids (*e*), and tibia length (*f*) in Zucker obese (*fa/fa*) (– – –) and nonobese (*Fa/-*) (——) rats during growth. Each data point is derived from two rats.

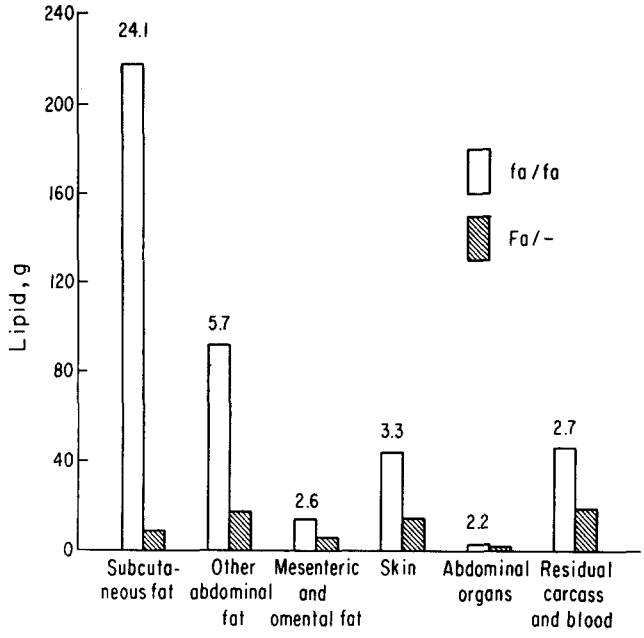

FIG. 2. Fat distribution in obese (*fa/fa*) and nonobese (*Fa/-*) Zucker rats. The data are derived from year-old female rats (n = 2 for each group). Maximal obesity occurs at 1 yr in the female obese rat. The numbers at the tops of columns represent the ratios of obese to nonobese.

and the obese condition is clearly observable. At 12 wk the difference is striking. By 26 wk the weight differential is more than 200 g, and may often continue to increase as long as the animal remains reasonably free of severe pathology (12). The dramatic accumulation of adipose tissue is demonstrated by the curves for body lipid and body lipid as a percentage of body weight, also shown in Fig. 1. The lipid distribution data presented in Fig. 2 and Table 1 reveal that the greatest accumulation of fat occurs in the subcutaneous depots, with an impressive, but lesser, accumulation in the abdominal pads, i.e., the retroperitoneal area and the gonadal pads. The dissectable subcutaneous fat of the obese animal represents almost 30% of its total body weight as compared with 5% for the nonobese littermate (Table 1). The data confirm an earlier report that much of the excess fat is located in the subcutaneous depots. A more modest expansion of retroperitoneal fat occurs, while the epididymal fat pad expands least of all (6).

Tibia length measurements (Fig. 1 and Table 1), stripped carcass weight, and carcass content of fat-free solids and nitrogen (Table 1) confirm the earlier reports that the "fatty" has a smaller skeleton than its normal littermate (12–14). The size depression at 26 wk is about 30%. The stunting of the "fatty" is most clearly revealed by analysis of stripped carcass (Table 1), and not by whole body determinations of fat-free solids and nitrogen as shown in Fig. 1, since these data include contributions from adipose tissue nonfat solids, from increased blood volume, and possibly from enlarged skin.

Ventromedial hypothalamically (VMH)-lesioned Zucker rats show no significant growth depression except in stripped carcass nitrogen (Table 1). They develop adiposity in the retroperitoneal and gonadal sites comparable to that seen in the "fatty" (Table 1), but the deposit of lipid in the subcutaneous site does not reach the magnitude seen in the "fatty."

Growth and Cellularity of Adipose Depots in the Normal Zucker Rat

At weaning there is a decrease in percentage of lipid for two of the three depots sampled (Figs. 3 and 4) as well as for the whole body (Fig. 1). Cell size shows a downward trend at this time, but the decrease is not significant. A similar delay in body fattening immediately after weaning was previously reported for another rat strain (6), and this delay in fat deposition could be minimized by raising the rats on a diet calorically denser than the conventional rat stock diet.

Both cell size and number increase during post-weaning growth. Results in the normal are much like those previously reported for Sprague-Dawley rats (2); in that study, fat cell number in both epididymal and retroperitoneal pads stabilized between 12 and 14 wk of age. In the subcutaneous depot of the Zucker rat,

TABLE 1 BODY WEIGHT, GROSS ADIPOSE DEPOT WEIGHTS, STRIPPED CARCASS CONTENT, AND TIBIA LENGTH IN ZUCKER OBESE, NONOBESE, AND VMH-LESIONED NONOBESE RATS

		Total Subcutaneous		Total Epididymal + Retroperitoneal		Stripped Carcass					
	Body Wt	Wt	% Body Wt	Wt	% Body Wt	Wt	% Body Wt	Lipid	Fat-free Solids	N	Tibia Length
	g	*g*		*g*		*g*		*g*	*g*	*g*	*mm*
Nonobese *Fa/-* (6)	432.0 ± 22.0	24.9 ± 1.4	5.4	8.2 ± 0.9	1.9	204.7 ± 11.6	46.4	10.0 ± 0.6	49.5 ± 0.2	6.58 ± 0.03	43.0 ± 0.1
Obese *fa/fa* (6)	705.0* ± 11.3	201.6* ± 8.7	28.7	48.6* ± 3.1	6.7	179.2† ± 3.8	25.2	39.3* ± 1.6	33.8* ± 0.7	4.64* ± 0.19	38.7* ± 0.3
Nonobese, lesioned *Fa/-* (6)	592.7* ± 18.8	103.2* ± 7.3	17.0	34.2* ± 2.1	6.0	197.1 ± 5.3	33.2	31.4* ± 0.6	41.5 ± 2.9	5.44‡ ± 0.25	41.5 ± 0.6

Values are means ± SEM.
* Significantly different from nonobese *Fa/-*, $P < 0.01$.
† Significantly different from nonobese *Fa/-*, $P < 0.05$.
‡ Significantly different from nonobese *Fa/-*, $P < 0.02$.

however, there is some indication that fat cell number may continue to increase after 14 wk. At 14 wk the mean cell number in this depot was 10.70×10^6 (Fig. 4), whereas at 26 wk the combined mean from experiment 2 (Fig. 4) and from experiment 3 (Table 2) was 16.70×10^6. The increase in cell size in this depot site is much less impressive than in the other sites studied. It is noteworthy that adult fat cell size is characteristically different in each fat depot. When the data for the 26-wk-old adults from experiment 2 (Figs. 3–5) and experiment 3 (Table 2) are combined, the means for epididymal, retroperitoneal, and subcutaneous fat cell size are 0.352 ± 0.026, 0.594 ± 0.056, and 0.259 ± 0.015 μg of lipid per cell, respectively. The relative contributions of increasing cell size and number to the growth of the fat pads can be assessed easily by comparing the values at 3 wk with the combined data for 26 wk. Compared in this manner, the ratios for cell size and number, respectively, are 8.5 and 6.5 in the epididymal pad, 8 and 5 in the retroperitoneal pad, and 2.5 and 5 in the subcutaneous site.

Growth and Cellularity of Adipose Depots in the Obese Zucker Rat

Lipid percentage increases without a break at weaning (Fig. 1), in contrast to the normal. Both cell size and number increase during growth to a much greater extent than in the normal (Figs. 3–5). There is a tendency for the very young "fatty" to have *fewer* fat cells than the normal. This tendency is most clearly seen in the epididymal pad where the cell number is below normal from 3 to 14 wk. Cell number is also low in the retroperitoneal pad at 3 and 5.5 wk, and in the subcutaneous pad at 3 wk. Subsequently, cell numbers in the "fatty" overtake those of the control and continue to increase through the 26th wk in the retroperitoneal and subcutaneous depots.

In contrast to the observation in the normal animal, then, cell number does not stabilize around 14 wk in the obese rat, but continues to rise throughout the period of observation. Also, quite in contrast to the normal, the adult obese Zucker rat has cells of the same size in all three adipose depots studied. The combined means for the 16 animals sampled at 26 wk are 1.14 ± 0.06, 1.20

TABLE 2 CELL SIZE AND NUMBER IN THREE ADIPOSE DEPOTS OF ADULT ZUCKER RATS

	Subcutaneous		Epididymal		Retroperitoneal	
	Cell Size	Cell Number	Cell Size	Cell Number	Cell Size	Cell number
	μg lipid/cell	*× 10⁶*	*μg lipid/cell*	*× 10⁶*	*μg lipid/cell*	*× 10⁶*
Nonobese *Fa/-* (n = 13)	0.1621 ± 0.0141	16.380 ± 1.750	0.3440 ± 0.0316	5.359 ± 0.393	0.4876 ± 0.0538	3.653 ± 0.279
Obese *fa/fa* (n = 12)	1.2633* ± 0.0959	41.042* ± 9.000	1.2251* ± 0.0547	7.386† ± 0.673	1.1820* ± 0.0761	11.186* ± 1.728
VMH-lesioned *Fa/-* (n = 6)	1.030* ± 0.136	18.22 ± 1.82	1.251* ± 0.170	5.90 ± 0.66	1.527* ± 0.265	4.38 ± 0.59

* Significantly different from nonobese *Fa/-*, $P < 0.01$.
† Significantly different from nonobese *Fa/-*, $P < 0.02$.

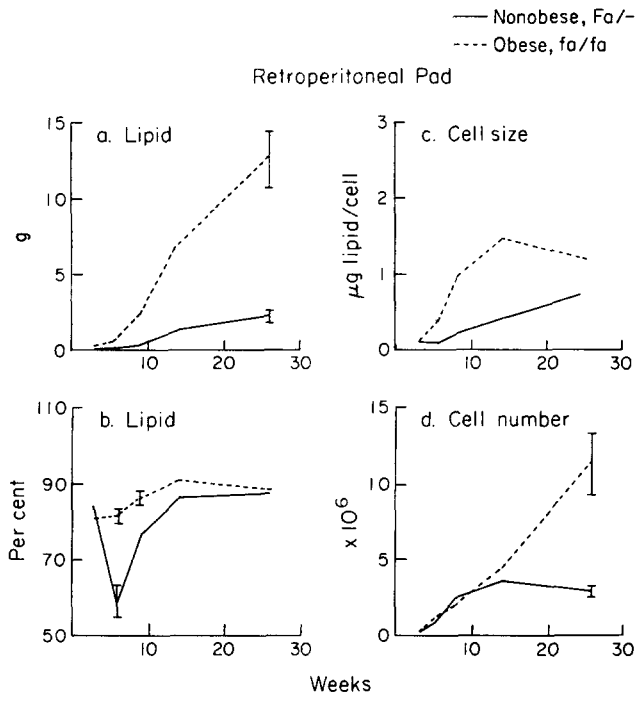

FIG. 3. Grams of lipid (*a*), percentage of lipid (*b*), cell size (*c*), and cell number (*d*) of the retroperitoneal fat pad in Zucker obese (*fa/fa*) and nonobese (*Fa/-*) rats. For all groups n = 6. Critical data points are plotted as mean values ± SEM. In some cases the SEM was too small to be accurately indicated on the scale used.

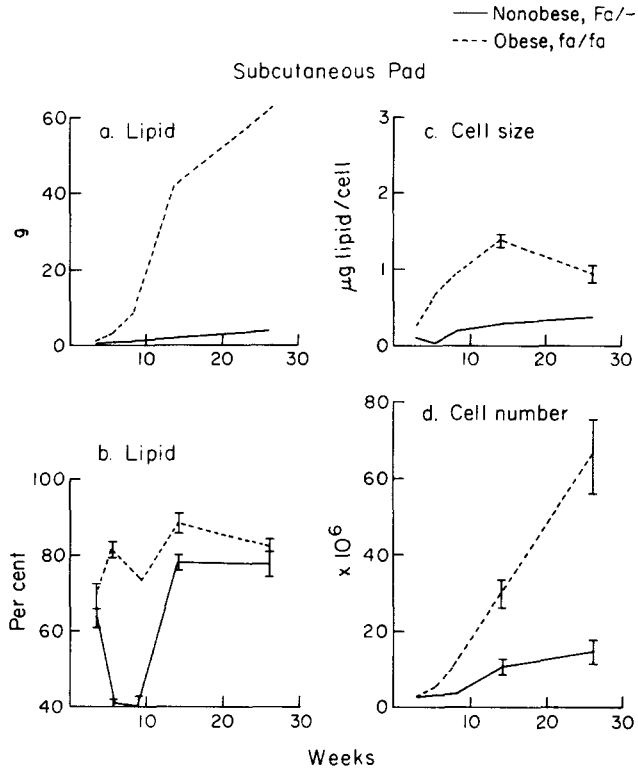

FIG. 4. Grams of lipid (*a*), percentage of lipid (*b*), cell size (*c*), and cell number (*d*) of the subcutaneous fat depot in Zucker obese (*fa/fa*) and nonobese (*Fa/-*) rats. For all groups n = 6. Critical data points are plotted as mean values ± SEM. In some cases the SEM was too small to be accurately indicated on the scale used.

± 0.08, and 1.10 ± 0.09 µg of lipid per cell for the epididymal, retroperitoneal, and subcutaneous pads, respectively. Perhaps this value approaches a maximal size for the Zucker rat. Hirsch and Han (2) reported cells with as much as 2 µg of lipid per cell in VMH-lesioned Sprague-Dawley rats. Comparing the relative contributions to growth of cell size and increases in cell number between 3 and 26 wk, the ratios for size and number, respectively, are 16.5 and 12.2 in the epididymal pad, 6.5 and 45.0 in the retroperitoneal pad, and 5.5 and 19.5 in the subcutaneous pad. It should be noted that the values for subcutaneous cell number varied between experiments 2 and 3, but in both cases an increase in cell number is a prominent feature of subcutaneous fat deposition in the obese animal. Cell number increase is less important than cellular enlargement in the epididymal pad, but it is the overwhelming feature of growth at the retroperitoneal site.

Comparison of Cellularity in the Genetically Obese and the Hypothalamically Lesioned Obese

The VMH-lesioned Zucker rat develops adiposity in the retroperitoneal and gonadal sites comparable to that seen in the Zucker "fatty" (Table 1), but the deposit in the subcutaneous site does not reach the magnitude seen in the "fatty." The lesioned animal develops very large adipose cells in all three sites studied (Table 2).

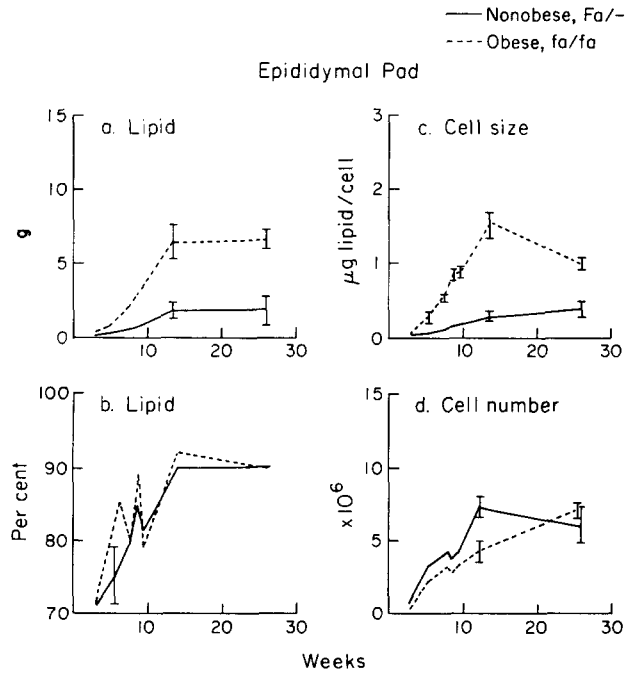

FIG. 5. Grams of lipid (*a*), percentage of lipid (*b*), cell size (*c*), and cell number (*d*) of the epididymal pad in Zucker obese (*fa/fa*) and nonobese (*Fa/-*) rats. For all groups n = 6. Critical data points are plotted as mean values ± SEM. In some cases the SEM was too small to be accurately indicated on the scale used.

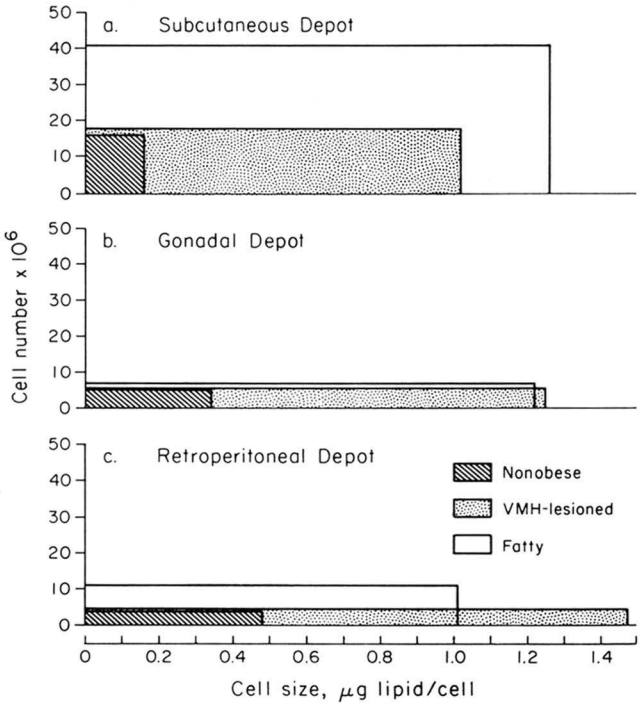

FIG. 6. Relative contributions of cell size and cell number to the subcutaneous pad (*a*), gonadal pad (*b*), and retroperitoneal pad (*c*) in Zucker obese (*fa/fa*), nonobese (*Fa/-*), and VMH-lesioned nonobese (*Fa/-*) rats.

Fig. 6 shows the contributions of increase in cell size and in cell number to the enlarged adipose depots in the two types of obesity.

The depot enlargement at all three sites in the VMH-lesioned Zucker rat is clearly the result of cell enlargement. On the other hand, the Zucker "fatty" has more adipose cells at all those sites than the lean control. The "fatty" has an enormously enlarged subcutaneous depot, and a highly significant part of the enlargement is due to an increased number of adipose cells: 2.5 times as many as in the nonobese and 2.3 times as many as in the VMH-lesioned obese. Hyperplasia of a similar magnitude occurs at the retroperitoneal site (3.0 × the nonobese and 2.5 × the VMH-obese). The cell number increase of the gonadal depot, while statistically significant (Table 2), is much less impressive (1.3 × the nonobese and 1.2 × the VMH-obese). If the lesioned normal Zucker rat is considered as a second control in which the fat depots have been expanded by the addition of lipid to already existing adipocytes, and if the "fatty" is compared with this obese control, the hyperplastic response of the "fatty" is unmistakable. Two morphologically distinguishable types of obesity are thus clearly demonstrated. The one, produced by the pressure of hyperphagia that results from destruction of the ventromedial satiety center, is characterized by the filling up of existing adipocytes with lipid; no new cells are added to the adipose depots. The other, produced by a genetic mutation, is characterized not only by hypertrophy of existing cells, but also by the addition and filling of more cells in the adipose depots, well into the adult phase of the rat's life.

DISCUSSION

The growth of the adipose depots in the nonobese Zucker rat is quite similar to that reported for the Sprague-Dawley animal (2). From the time of weaning (3 wk) to approximately the 14th wk of life, the epididymal, retroperitoneal, and subcutaneous depots grow by increases in both cell number and cell size. At about the 14th wk, the cell number of the epididymal and retroperitoneal pads becomes fixed. The subcutaneous pad continues to add cells through the 26th wk, although the increment is small. Since the cells of the subcutaneous depots are small, appearance of additional cells at 26 wk may be explained on the basis of cell enlargement. Small cells, i.e., less than 25 μm in diameter, are not detected by the counting method used, and thus a slight increase in size of very small cells could result in new cells being detected.

There is a temporary reduction in fat accretion in the Zucker nonobese rat in the period immediately after weaning (3–6 wk) which is reflected by slight decreases in cell size. This reduction in fat deposition follows the caloric restriction that results from weaning the animal from a diet of high caloric density (raw milk) to the less calorically dense pelleted stock diet. Such a reduction might be expected from consideration of the report by Hirsch and Han (2) that caloric restriction operates to reduce cell size.

Certain differences that appear between adipose depot sites in the lean Zucker rat should be noted. The subcutaneous depot in the dorsal scapular region is characterized by much smaller cells than the other depots, and shows increased cell numbers through the 26th wk of life. Similar differences in cell size were also noted by Bray (10). Hausberger (15) and Schemmel, Mickelsen, and Mostosky (16) pointed to differences in growth characteristics between subcutaneous fat depots and those of the genital and perirenal areas in mice and rats. These differences in cellularity may necessitate a reevaluation of the common assumption that the epididymal fat pad is representative of all adipose depots.

Unlike its lean littermate, the Zucker "fatty" does not show a fixed number of adipose cells by the 14th wk of age in any of the three depots sampled. Cell number is increased at 26 wk over that observed at 14 wk in all three sites, suggesting that new adipocytes are being added to the depot. This phenomenon of an increasing

cell number during the development of obesity is in sharp contrast to the way in which obesity develops in the hypothalamically lesioned animal. Hirsch and Han (2) reported that in Sprague-Dawley rats lesioned at either 7 or 13 wk of age, the epididymal and retroperitoneal fat depots grew by marked adipose cell enlargement, while the cell number remained fixed. In the Zucker rat lesioned at 13 wk of age, large increases in the cell size of all three depots were observed at 26 wk, but cell numbers had not changed. Apparently then, the great pressure for fat deposition in the lesioned rat, which is brought to bear by the animal's hyperphagic behavior, results in the filling of already existing adipocytes with lipid. Therefore, if comparison is made between the Zucker "fatty" and a Zucker "obese control" (the lesioned normal, with its normal complement of adipocytes filled), the cell number increases seen in the Zucker "fatty" are impressive. On the basis of such a comparison, it may be argued that the increased cell number in the "fatty" is not due simply to enlargement of existing small and undetectable fat cells. It is more likely that a true hyperplasia is being observed. These findings lead to the general conclusion that the obesity in the Zucker rat strain produced by the *fa* gene may be sharply differentiated from the obesity that results from hypothalamic lesions. Bray (10) also found cell enlargement of the same magnitude in adipocytes from Zucker "fatties" and VMH-lesioned Zucker rats as determined by measurement of cell diameters. He did not, however, directly determine cell number. He concluded that obesity in the two cases was the result of a similar mechanism, i.e., the increase in cell size and number is secondary to a need to store more triglyceride. Should that "need" develop at an early age in the Zucker "fatty," it might exert a developmental influence on whatever cells are serving as adipocyte precursors. Bray (17) has suggested that the primary defect in the Zucker obese rat is in the hypothalamus, since he finds a number of abnormalities in the "fatty" that may be related to hypothalamic functions, i.e., increased food intake, high urine volume, aberrant estrus cycles, and diminished thyroid function. In contrast, our data clearly show that hyperplasia as well as hypertrophy is important in the genetically obese animal, whereas only hypertrophy contributes significantly to adiposity in lesioned animals, suggesting that the mechanism is not the same in the two types of obesity. The lesioned animal can respond in only one way to its need to store more triglyceride, i.e., it fills up existing adipocytes with lipid. The "fatty" has many more cells to fill, either because they were laid down in the depot sites as preadipocytes at an early developmental stage, or because they are continually appearing in the depot as a result of mitotic activity. In fact, it may be that the primary defect in these animals is an inability to shut down cell proliferation in adipose tissue. The obesity seen in the Zucker "fatty" may parallel more closely certain types of extreme human obesity in which adiposity is related to increased cell numbers (4) than does experimental obesity produced by hypothalamic lesions. Thus, the Zucker "fatty" may prove to be an excellent model for the study of some forms of human obesity.

The Zucker obese rat has a smaller skeleton than its lean counterpart. The data presented here confirm earlier reports of stunting of true growth based on skeletal measurements and carcass analyses (12–14). Stunting has already begun by 14 days of age.[2] Han and Liu (18) found some growth depression in VMH-lesioned Sprague-Dawley rats and suggested that the impairment was due to a reduced growth hormone output. Zucker (14) suggested an impairment in growth hormone production, mediated through the hypothalamic growth hormone releasing factor, as a consideration in the generation of the stunting seen in the Zucker "fatty," and perhaps in the development of obesity as well. Insulin might also be involved in the development of obesity in these animals. We have recently confirmed the observation of Zucker and Antoniades (19) that the Zucker obese rat has elevated plasma insulin levels, and furthermore that release of insulin from isolated pancreatic islets is several times that of the nonobese Zucker rat.[3] Hyperinsulinemia has been reported in many obese mouse strains (20, 21) and is often one of the earliest observable characteristics of the obese animal (19, 22). Further investigation of both growth hormone and insulin in the etiology of obesity is obviously necessary.

The findings presented here point once again to the general conclusion that adipose cellularity is determined early in the life of the animal. The lean Zucker rat shows a pattern of development of adipose tissue quite like that reported for the Sprague-Dawley rat; it is only in the presence of the mutant gene that a different pattern emerges. One may hypothesize several ways in which the gene might exert its effects: (*a*) on the hypothalamic control of feeding behavior with secondary effects on metabolism and growth of the adipose depots, (*b*) on fat metabolism with secondary effects on feeding behavior and upon the growth of the adipose depots, and (*c*) a direct effect on the development of adipose precursor cells with secondary effects on fat metabolism and feeding behavior. The data reported to date do not argue strongly for any one of these hypotheses, although the distribution of fat in the Zucker obese animal is suggestive of a morphogenetic effect. A pattern

[2] Zucker, L. M., unpublished observation.
[3] Stern, J., personal communication.

of cellular proliferation and differentiation very distinct from that of the normal animal is observable. Continuing investigations into the developmental, metabolic, and behavioral aspects of genetically determined obesity in the Zucker rat are being conducted.

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References

1. Salans, L. B., J. L. Knittle, and J. Hirsch. 1968. The role of adipose cell size and adipose tissue insulin sensitivity in the carbohydrate intolerance of human obesity. *J. Clin. Invest.* **47:** 153–165.
2. Hirsch, J., and P. W. Han. 1969. Cellularity of rat adipose tissue: effects of growth, starvation, and obesity. *J. Lipid Res.* **10:** 77–82.
3. Knittle, J. L., and J. Hirsch. 1968. Effect of early nutrition on the development of rat epididymal fat pads: cellularity and metabolism. *J. Clin. Invest.* **47:** 2091–2098.
4. Hirsch, J., J. L. Knittle, and L. B. Salans. 1966. Cell lipid content and cell number in obese and non-obese human adipose tissue. *J. Clin. Invest.* **45:** 1023. (Abstr.)
5. Zucker, L. M., and T. F. Zucker. 1961. Fatty, a new mutation in the rat. *J. Hered.* **52:** 275–278.
6. Zucker, T. F., and L. M. Zucker. 1963. Fat accretion and growth in the rat. *J. Nutr.* **80:** 6–19.
7. Hirsch, J., and E. Gallian. 1968. Methods for the determination of adipose cell size in man and animals. *J. Lipid Res.* **9:** 110–119.
8. Hellman, B., I. Taljedal, and S. Westman. 1962. Morphological characteristics of the epididymal adipose tissue in normal and obese-hyperglycemic mice. *Acta Morphol. Neer.-Scand.* **5:** 182–189.
9. Zinder, O., R. Arad, and B. Shapiro. 1967. Effect of cell size on the metabolism of isolated fat cells. *Israel J. Med. Sci.* **3:** 787–791.
10. Bray, G. A. 1969. Studies on the composition of adipose tissue from the genetically obese rats. *Proc. Soc. Exp. Biol. Med.* **131:** 1111–1114.
11. Herberg, L., F. A. Gries, and Ch. Hesse-Wortmann. 1970. Effect of weight and cell size on hormone-induced lipolysis in New Zealand obese mice and American obese-hyperglycemic mice. *Diabetologia.* **6:** 300–305.
12. Zucker, T. F., and L. M. Zucker. 1962. Hereditary obesity in the rat associated with high serum fat and cholesterol. *Proc. Soc. Exp. Biol. Med.* **110:** 165–171.
13. Zucker, L. M. 1965. Hereditary obesity in the rat associated with hyperlipemia. *Ann. N.Y. Acad. Sci. U.S.A.* **131:** 447–458.
14. Zucker, L. M. 1967. Some effects of caloric restriction and deprivation on the obese hyperlipemic rat. *J. Nutr.* **91:** 247–254.
15. Hausberger, F. X. 1966. Pathological changes in adipose tissue of obese mice. *Anat. Rec.* **154:** 651–660.
16. Schemmel, R., O. Mickelsen, and U. Mostosky. 1970. Influence of body weight, age, diet and sex on fat depots in rats. *Anat. Rec.* **166:** 437–445.
17. Bray, G. A. 1970. Metabolic and regulatory obesity in rats and man. *In* Adipose Tissue Regulation and Metabolic Functions. B. Jeanrenaud and D. Hepp, editors. Academic Press Inc., New York. 175–180.
18. Han, P. W., and A. C. Liu. 1966. Obesity and impaired growth of rats force fed 40 days after hypothalamic lesions. *Amer. J. Physiol.* **211:** 229–231.
19. Zucker, L. M., and H. N. Antoniades. 1970. Insulin and fattening in the genetically obese hyperlipemic rat "Fatty." *Federation Proc.* **29:** 379. (Abstr.)
20. Stauffacher, W., A. E. Lambert, D. Vecchio, and A. E. Renold. 1967. Measurements of insulin activities in pancreas and serum of mice with spontaneous ("Obese" and "New Zealand Obese") and induced (goldthioglucose) obesity and hyperglycemia, with considerations on the pathogenesis of the spontaneous syndrome. *Diabetologia.* **3:** 230–237.
21. Wyse, B. M., and W. E. Dulin. 1970. Influence of age and dietary conditions on diabetes in the *db* mouse. *Diabetologia.* **6:** 268–273.
22. Chlouverakis, C., E. F. Dade, and R. A. L. Batt. 1970. Glucose tolerance and time sequence of adiposity, hyperinsulinemia and hyperglycemia in obese-hyperglycemic mice (obob). *Metab. Clin. Exp.* **19:** 687–693.