Cellularity of adipose depots in six strains of genetically obese mice

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Abstract Adipocyte cell size and number of three adipose depots, gonadal, subcutaneous, and retroperitoneal, were determined in several strains (aA^{y}, aA^{iy}, *dbdb*, *obob*, and NZO) of adult genetically obese mice, male and female, and in male gold thioglucose-treated mice. Epididymal pad cellularity was determined during development in yellow and viable yellow obese mice and their lean littermates, as well as in the NCS/R mouse. Cell number in the mouse epididymal pad in both lean and genetically obese animals is determined early in development, i.e., before weaning. Cell enlargement is the consistent and usually dominant morphological explanation for adipose depot enlargement in genetic and in gold thioglucose-induced mouse obesity. In some instances, hyperplasia accompanied the hypertrophy, occurring most often in the subcutaneous depot. Cell number in the subcutaneous pad of the obese–hyperglycemic female is four times that of the lean control and represents the most extreme case of hyperplasia observed. In fact, hyperplasia was consistently seen in the *obob* mouse. A classification for genetic obesity based primarily upon the cellularity characteristics of the adipose depots is proposed.

Supplementary key words adipocyte · cell size · cell number · gold thioglucose obesity

In many cases of human obesity, a large increase occurs in the total number of adipocytes in adipose depots, but this hyperplasia is at times accompanied by only moderate enlargement of adipose cells; furthermore, weight reduction in very obese humans is accomplished by a reduction in adipose cell size with little or no change in cell number (1). These observations have focused attention upon the factors which influence the development of adipose cell number, and the relative contributions of adipose cell number and cell size to the size of the adipose depots. It has recently been shown in two strains of rats that the adipose depots grow by increases in cell size and number early in life, but after 12–15 wk further growth of the depots occurs almost exclusively by changes in cell size (2, 3). Yet, in the one clearly documented case of genetic obesity in the rat, i.e., the Zucker "fatty," increase in cell number of adipose depots does occur as late as the 26th wk of life (3). Onset of obesity in the adult rat produced by overfeeding or by hypothalamic lesioning is accompanied by cell enlargement with only minor changes in cell number (2, 3). The only experimental manipulation so far available which produces significant change in the adult cell number in epididymal adipose pads is an extremely early infantile undernutrition (4). Genetically obese mice, as well as mice made obese by gold thioglucose treatment, are used extensively as model systems for the study of the obese syndrome and the metabolic defects that accompany it. Taljedal and Hellman (5) have presented evidence that the obesity exhibited by the obese–hyperglycemic mutant mouse is morphologically different from that of the New Zealand obese mouse. Herberg and coworkers (6) have shown developmental and metabolic differences in these two strains that are correlated with adipocyte cell size.

Since adipocyte number may be determined by genetic and nutritional factors operating early in the life of the animal, and since cell size is one factor that influences the metabolic responses of adipose tissue in experimental animals and in humans (7), the present study was undertaken to examine the cellularity of three adipose depots in six strains of genetically obese mice and in normal mice made obese by gold thioglucose injection.

METHODS

Three questions were posed concerning the problem of adipose tissue cellularity in genetic obesity: (*a*) what is the time course of development, (*b*) how is cellularity

Abbreviations: *aa*, C57BL/6J; aA^{y}, yellow; aA^{iy}, intermediate yellow; aA^{vy}, viable yellow; *dbdb*, diabetic; *obob*, obese–hyperglycemic; NZO, New Zealand obese; NCS/R, Swiss albino.

affected when obesity is induced in the normal mouse by gold thioglucose injection, and (*c*) what are the cellularity characteristics of adipose depots in adult genetically obese mice? To answer these questions three approaches were used as described below.

Developmental studies

The cellularity of adipose tissue was studied during development in one strain of normally lean mice and two strains of genetically obese mice. The lean mice were Swiss albino (NCS/R) obtained from the animal facility at Rockefeller University. The two strains of genetically obese mice were yellow (aA^{y}) and viable yellow (aA^{vy}) obtained from the Jackson Memorial Laboratory, Bar Harbor, Maine. These were compared with C57BL/6J (*aa*) controls. All animals in this and subsequent series were maintained on Purina Lab Chow fed ad lib. Animals were killed for cellularity determinations at various intervals between 21 and 200 days of age. For these studies the adipose depot sampled was the left epididymal pad.

Induced obesity

The normally lean strain (NCS/R) used for the developmental series was also used for the experimental production of obesity by gold thioglucose injection. Gold thioglucose was obtained from Schering Corp., Bloomfield, N.J., through the courtesy of Dr. Preston L. Perlman. 15 NCS/R mice were injected intraperitoneally at approximately 25 days of age with 0.5 mg/g of body weight. Eight animals survived, of which five became obese. Five control animals were injected with isotonic saline. All were killed at 100 days of age for cellularity determinations on the left epididymal pad. In addition, male *aa* mice were injected with gold thioglucose at 14 wk of age, and those that became obese were used as obese controls (*aaGTG*) for the adult studies.

Adult genetically obese strains

Cellularity was determined in five strains of adult genetically obese mice: New Zealand (NZO), yellow (aA^{y}), intermediate yellow (aA^{iy}), diabetic (*dbdb*), and obese–hyperglycemic (*obob*). Three adipose depots, the subcutaneous, gonadal, and retroperitoneal, were sampled in both males and females at 26 wk of age. The NZO mice were obtained from the Institute for Cancer Research, Philadelphia, Pa., through the courtesy of Dr. George Wolff. NCS/R mice obtained from Rockefeller University animal facilities were used as controls for this strain. All other strains were obtained originally from the Jackson Memorial Laboratory. The yellow (aA^{y}) and intermediate yellow (aA^{iy}) were subsequently bred in the Rockefeller Laboratory. Both the aA^{iy} and the aA^{vy} mice used for the adult and the developmental studies were heterozygotes obtained from mating the yellow mutant with an *aa* littermate. Controls were black littermates (*aa*) of the aA^{y} and aA^{iy} animals and *aa* males made obese by gold thioglucose injection. All animals were maintained in the Rockefeller Laboratory on Purina Lab Chow fed ad lib. They were housed in a temperature-controlled room with a 12-hr light–dark cycle.

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 parametrial pad was cut at the midpoint of the base of the uterus and trimmed away along the length of the left horn and from around the left ovary. 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 abruptly, 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 rostrally, 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 dissected away, as well as any strips of muscle adhering to the pad. This method results in the removal of a section of the subcutaneous fat of the mouse which is anatomically reproducible.

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 tared nylon sieves, blotted, and weighed. One piece was placed in chloroform–methanol 2:1 for lipid extraction, and the other piece was fixed in 2% osmium tetroxide in a 0.05 M collidine buffer (8) for subsequent cell counting. Any remaining tissue was blotted and weighed on a tared nylon sieve, so that the total wet weight of the tissue could be determined. In the case of *aa* control mice, it was necessary to collect both left and right retroperitoneal pads in order to obtain enough tissue for the two samples. When this was done, pieces of tissue from each pad were combined for the lipid extraction sample and for the

osmium-fixed sample. After a 48-hr extraction, distilled water (0.2 vol) was added to the chloroform–methanol extract to break the phases, and aliquots for gravimetric lipid determination were taken from the chloroform phase.

Cell counting

The cells separated from the osmium-fixed 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 (8). Cell size is expressed as micrograms of lipid per cell.

RESULTS

Development of left epididymal pad in normal and genetically obese mice

During normal growth in albino Swiss mice, both body weight and epididymal pad weight increase rapidly from weaning (21 days) to approximately 60 days of age (Fig. 1). Between 60 and 100 days, the body weight and the pad weight become and remain relatively constant. The increase in wet weight of the epididymal pad during the period of rapid growth can be accounted for on the basis of increase in cell size alone. Cell number increases little, if at all, from weaning through 100 days of age in the NCS/R mouse.

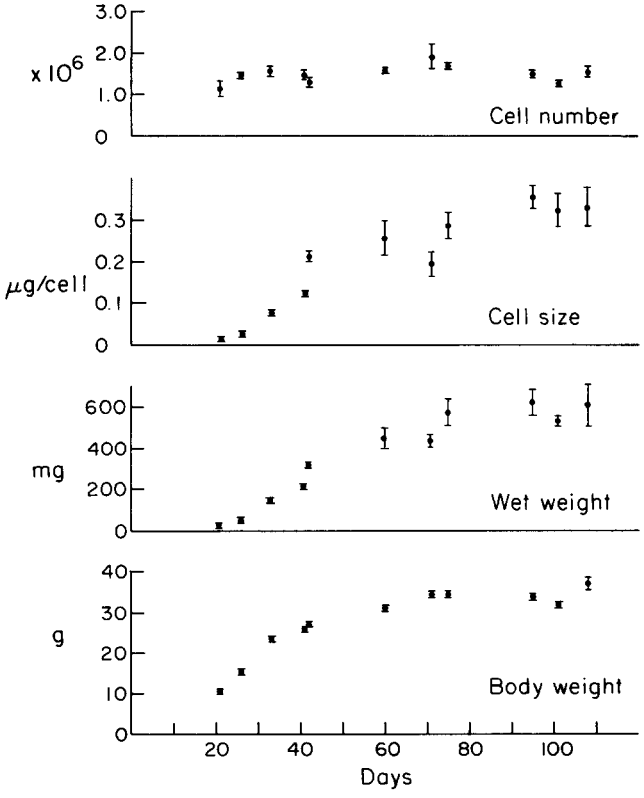

FIG. 1. Cellularity of left epididymal pad in NCS/R mice. Critical data points are presented as means ± SEM.

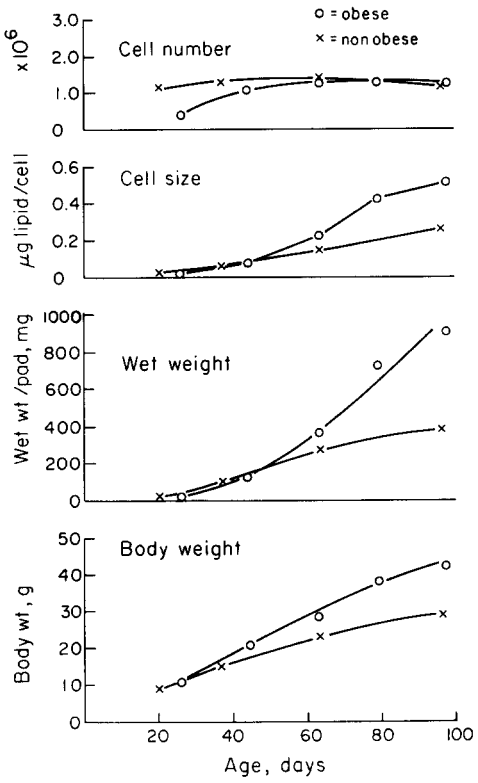

FIG. 2. Development of obesity, left epididymal pad, aA^{y} mice. Data points are means for two or three animals.

In the yellow obese mouse, body weight increases continually up to 100 days of age, in contrast to the NCS/R, and the epididymal pad continues to grow as well (Fig. 2). During this entire period, the epididymal pad grows by cell enlargement. Cell numbers appear constant throughout this period for both the yellow mouse and its lean black littermate. A similar pattern for the period from 60 to 200 days of age is seen in viable yellow mice when they are compared with lean black littermates (Fig. 3). (Data are not available for this strain for the period from weaning to 60 days.) The body weights of aA^{vy} mice average 8–10 g more than their lean black littermates and remain relatively constant between 60 and 200 days of age (Fig. 3). The difference in adiposity between the aA^{vy} mouse and its lean littermate can be explained on the basis of cell size difference alone. Cell number is the same in the obese and lean littermates, and it remains constant over the 60–200-day period. Thus, at no point after the first month of life is there any evidence of an increase in cell number in either normal or yellow obese mice.

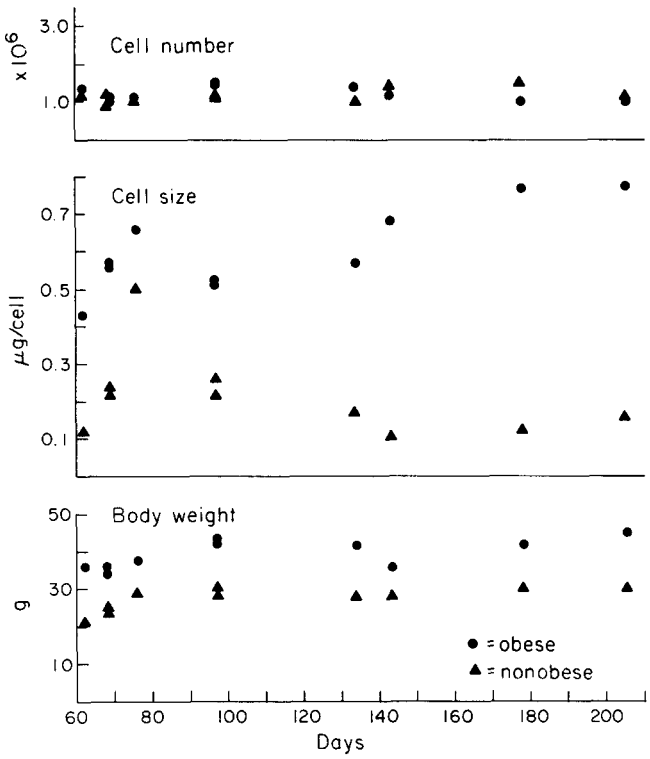

FIG. 3. Development of obesity, left epididymal pad, aA^{vy} mice. Data points are means for two or three animals.

Cellularity in gold thioglucose-induced obesity

NCS/R mice made obese by gold thioglucose injection reach a body weight almost twice that of saline-injected controls by 100 days of age (Fig. 4). The lipid content of the epididymal pad is more than doubled. The increased fat deposition in the epididymal depot is clearly the result of the enlargement of adipocytes. Cell number in the obese animals is the same as in the controls.

In *aaGTG* mice (see Table 1), a significant increase in cell number of the retroperitoneal pad does occur, but no change occurs in cell number of the subcutaneous or epididymal pad.

Cellularity in adult genetically obese mice of the strains aA^{y}, aA^{ty}, *dbdb*, and *obob*

A summary of body weights, pad weights, and cell size and cell number of three adipose depots in males and females of four strains of genetically obese mice and their lean controls and GTG obese controls is presented in Table 1. The mice were all 26 wk of age when cellularity was determined.

In all the strains studied in which the obesity is the result of an established single gene mutation, the adult genetically obese mice, both male and female, have significantly larger cells than lean controls in all three depots. In general, the greater the body weight of the obese mouse, the greater the pad weight and the larger the cell size in all three depots. The *obob* mouse is an exception; it is the heaviest of the strains studied, but it does not have the largest adipose cells at any site.

On the other hand, *obob* mice have a significantly larger number of adipose cells than controls in all three depots, and the increase in number of the subcutaneous depot in the female is striking (Table 1). Cell numbers are not significantly different from control values for the other obese strains, with the exception of aA^{y} and aA^{ty} males, which show a small increase in number in the retroperitoneal pad. If the cell number comparison is made with *aaGTG* male controls, the increases seen in the retroperitoneal pad disappear. The GTG mouse in this instance is viewed as a control in which the adipose depots have expanded under the pressure of hyperphagia, and any small cells that might have been below the limits for counting by osmium fixation are now being measured as part of the depot. The relative contributions of adipose cell number and cell size to the size of the adipose depots can be seen in Fig. 5. The contribution of increased cell number to the fatty depots of the *obob* mouse is consistently greater than in the other strains, and is particularly pronounced in the female. These block diagrams also indicate the relative contributions of the three depots to the adiposity of the animal.

The subcutaneous site, which has the smallest cells in normal males and females, appears to have the greatest capacity for expansion; next is the gonadal, and least of all, the retroperitoneal. The parametrial pad in the female contributes more to the fat deposits in the animal than does the epididymal pad in the male.

Cellularity in the New Zealand obese mouse

The New Zealand obese mouse is considered separately, since the inherited obesity has not been established as a defect traceable to a single mutant gene, but rather is a condition that occurs in a highly inbred strain. In the case of such an inbred strain, there is no true control to which the obese animal can be compared. We have chosen to compare the NZO to the NCS/R. The data are presented in Table 2. The adipose depots are greatly enlarged in the NZO mouse. Cell enlargement and increased cell numbers contribute significantly to all three depots, with the one exception of the subcutaneous depot in the female (Fig. 6). In the female subcutaneous pad, there is a fourfold increase in cell number, but the cell size is small relative to the NCS/R.

Comparison of adiposity in males and females

Male mice weigh the same or slightly more than females of the same age, except in the case of obese–hyperglycemic mutants; in this strain the females are heavier than the males at 26 wk of age (Fig. 7). The female of the species is fatter, however, even in those cases where the

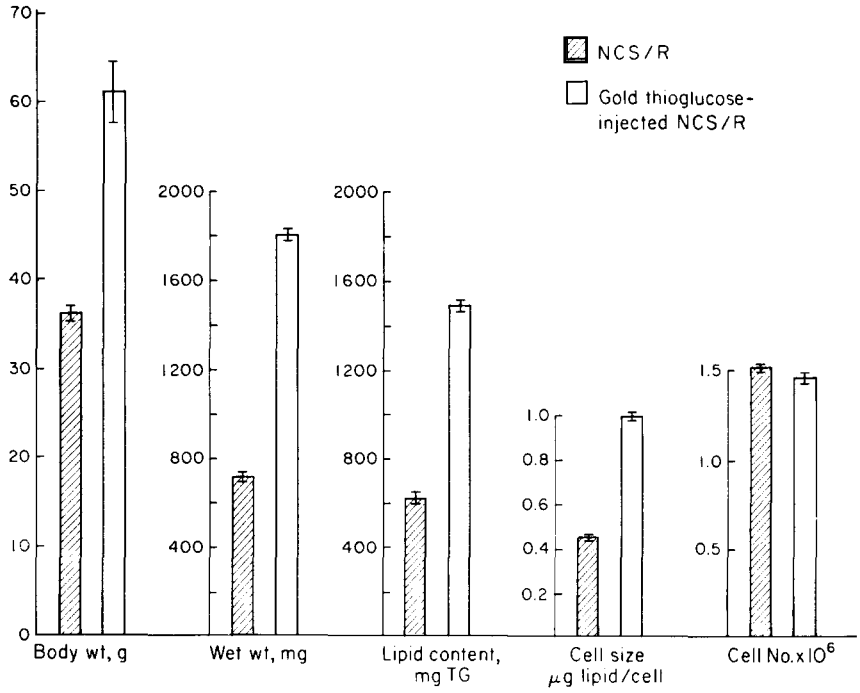

FIG. 4. Body weight, wet weight, lipid content, cell size, and cell number of left epididymal pad in NCS/R and gold thioglucose-injected NCS/R mice. Values are means ± SEM.

TABLE 1. Cellularity of three adipose depots in four strains of genetically obese mice

		Subcutaneous Pad			Gonadal Pad			Retroperitoneal Pad		
	Body Wt	Wet Wt	Cell Size	Cell No.	Wet Wt	Cell Size	Cell No.	Wet Wt	Cell Size	Cell No.
	g	*mg*	*μg lipid/cell*	*× 10⁶*	*mg*	*μg lipid/cell*	*× 10⁶*	*mg*	*μg lipid/cell*	*× 10⁶*
Males										
aa	29.57	243.5	0.0671	2.051	205.8	0.1737	1.016	59.7	0.1421	0.324
	1.37	72.9	0.0195	0.244	43.7	0.0475	0.066	20.6	0.0536	0.030
aaGTG	40.67	1340.3	0.3467	2.852[a]	852.7	0.6385	0.936[a]	386.4	0.5404	0.563
	1.44	155.7	0.0336	0.293	63.2	0.0429	0.177	35.0	0.0452	0.044
aA^v	42.50	1065.8	0.2809	3.109[a]	711.9	0.5633	1.034[a]	281.8	0.3794	0.668
	0.70	54.2	0.0314	0.503	88.8	0.0618	0.108	25.9	0.0247	0.095
aA^iv	37.93	789.9[b]	0.2350	2.119[a]	674.0	0.4661	1.113[a]	247.1	0.4169[c]	0.460[c]
	1.25	213.4	0.0902	0.427	149.1	0.0763	0.099	44.8	0.0720	0.033
dbdb	50.26	2128.9	0.9256	1.934[a]	1211.5	1.2909	0.834[a]	427.0	0.8973	0.428[a]
	1.67	106.7	0.0624	0.192	64.3	0.0447	0.074	27.6	0.1245	0.046
obob	53.65	2407.7	0.7024	2.801[b]	1776.9	1.0952	1.350	633.8	0.8342	0.668
	1.01	128.8	0.0496	0.350	72.7	0.0713	0.057	44.4	0.0557	0.082
Females										
aa	24.13	275.3	0.0830	2.025	235.5	0.1333	1.342	48.4	0.1192	0.333
	0.51	30.0	0.0084	0.142	29.0	0.0170	0.077	1.7	0.0259	0.053
aA^v	40.46	1547.0	0.4853	2.327[a]	1532.6	0.7945	1.633[a]	289.0	0.6737	0.349[a]
	1.67	88.5	0.0488	0.382	114.0	0.0496	0.130	34.2	0.0344	0.051
aA^iv	35.20	1123.7	0.3951	2.159[a]	1205.9	0.6289	1.596[a]	283.3	0.6093	0.365[a]
	1.70	124.0	0.0427	0.094	138.2	0.0776	0.053	34.0	0.0560	0.033
dbdb	54.26	2400.0	1.0193	2.064[a]	2029.6	1.2168	1.393[a]	464.7	1.0920	0.334[a]
	1.94	171.2	0.1118	0.375	103.8	0.0616	0.063	69.1	0.0670	0.032
obob	67.08	5103.7	0.4603	8.640	2781.4	0.8467	2.643	781.8	0.8046	0.769
	1.96	292.2	0.0399	0.871	148.1	0.0330	0.110	85.0	0.0549	0.066

All values are significantly different from *aa* at $P < 0.01$ except as indicated. Values are the means of determinations on six animals ± SEM (second value in each pair of numbers).

[a] Not significant.

[b] $P < 0.05$.

[c] $P < 0.02$.

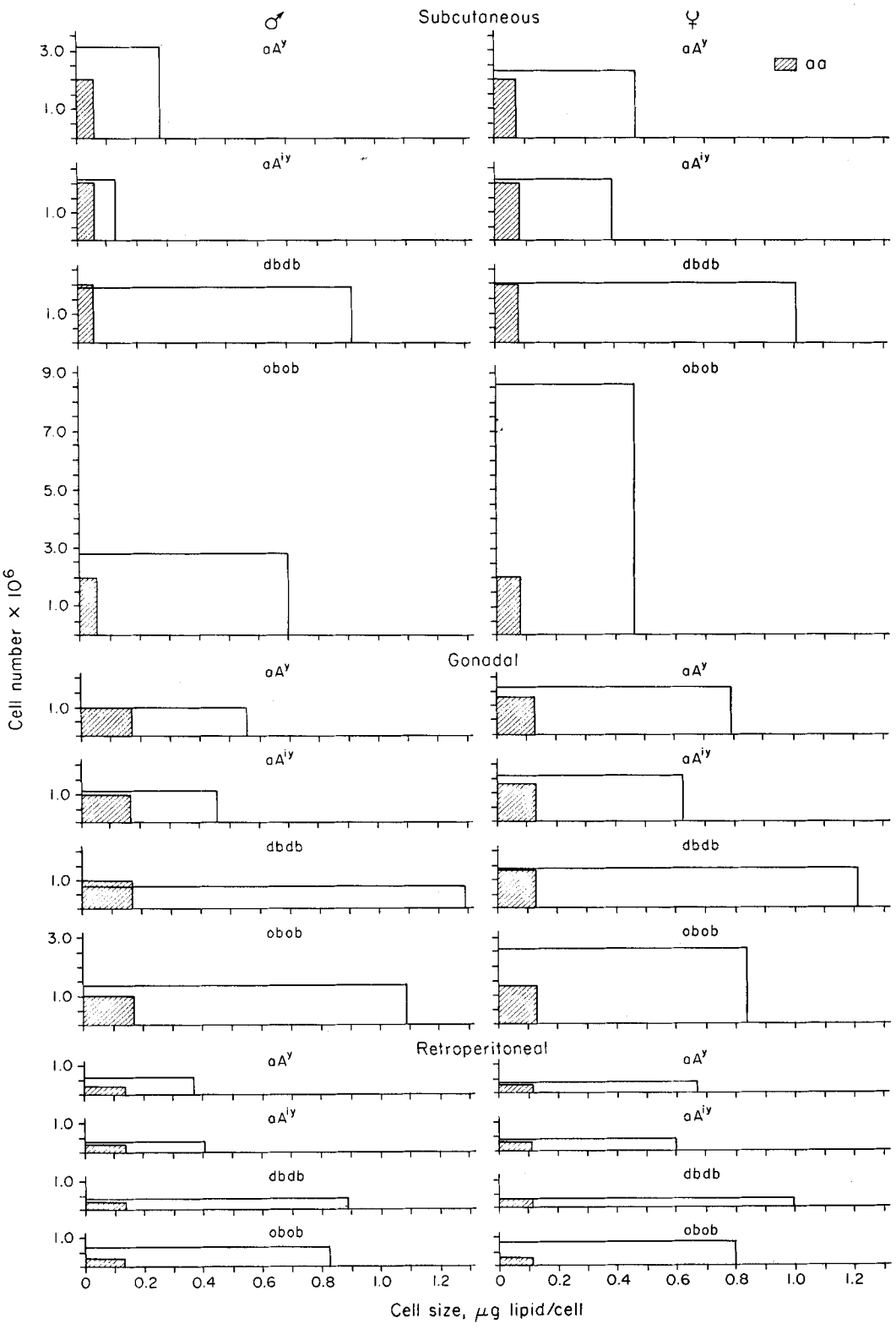

FIG. 5. Relative contributions of cell size and cell number to adiposity of the subcutaneous, gonadal, and retroperitoneal depots in male and female mice of the strains aA^{y}, aA^{iy}, *dbdb*, and *obob*.

TABLE 2. Cellularity of three adipose depots in New Zealand obese (NZO) and Swiss albino (NCS/R) mice

		Subcutaneous Pad			Gonadal Pad			Retroperitoneal Pad		
	Body Wt	Wet Wt	Cell Size	Cell No.	Wet Wt	Cell Size	Cell No.	Wet Wt	Cell Size	Cell No.
	g	*mg*	*μg lipid/cell*	*× 10⁶*	*mg*	*μg lipid/cell*	*× 10⁶*	*mg*	*μg lipid/cell*	*× 10⁶*
Males										
NCS/R	31.27	521.9	0.0878	4.127	339.5	0.2161	1.336	57.7	0.1614	0.383
	0.34	64.1	0.0141	0.651	28.0	0.0200	0.046	10.7	0.0458	0.068
NZO	51.22	1753.4	0.1617	8.521	944.4	0.3462	2.359	398.9	0.3180[a]	1.090
	0.59	148.7	0.0141	0.374	65.9	0.0244	0.127	35.8	0.0424	0.106
Females										
NCS/R	31.98	801.5	0.1692	3.912	660.8	0.2230	2.841	124.5	0.2054	0.548
	0.83	94.4	0.0360	0.531	71.7	0.0500	0.329	17.5	0.0300	0.080
NZO	45.73	2078.1	0.1310[b]	12.801	2546.2	0.4012[a]	5.552	538.7	0.3212[b]	1.415
	0.68	80.9	0.0100	0.978	80.4	0.0469	0.320	32.2	0.0479	0.120

All values are significantly different from NCS/R at $P < 0.01$ except as indicated. Values are the means of determinations on six animals ± SEM (second value in each pair of numbers).

[a] $P < 0.05$.

[b] Not significant.

male is heavier. Wet weights of subcutaneous, gonadal, and retroperitoneal pads are significantly greater in females than in males of each strain studied. There are variations among the strains with respect to the contribution that cell size makes to the enlarged depots in the female (Fig. 8). In the two normally lean strains, *aa* and NCS/R, cell sizes are the same in the gonadal and retroperitoneal depots, but tend to be slightly larger at the subcutaneous site in the female. Intermediate yellow and yellow females have larger cells than the males at all three sites. There are no significant sex differences in cell size in the NZO and *dbdb* strains. Only in the *obob* strain do larger fat cells occur in the male. This is true even though *obob* females are fatter, and the cell number comparison reflects this fact; *obob* females have three times as many cells as males in the subcutaneous depot, twice as many in the gonadal pad, and perhaps a few more in the retroperitoneal pad (Table 1).

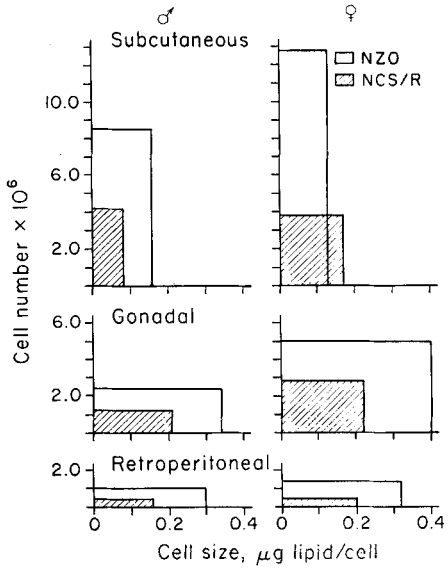

FIG. 6. Relative contributions of cell size and cell number to adiposity of the subcutaneous, gonadal, and retroperitoneal depots in male and female NZO mice compared with NCS/R controls.

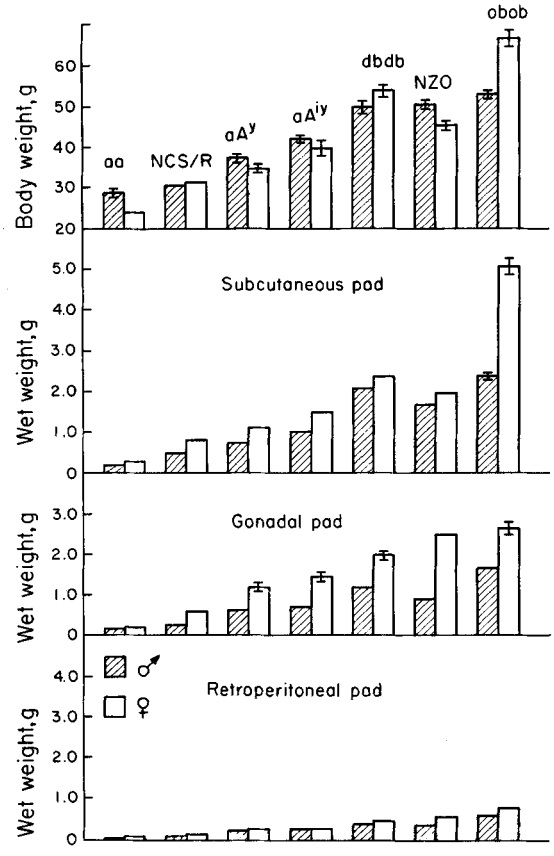

FIG. 7. Comparison of body weights and adipose depot weights in male and female mice. Strains used were *aa*, NCS/R, *aA^y*, *aA^iy*, *dbdb*, NZO, and *obob*.

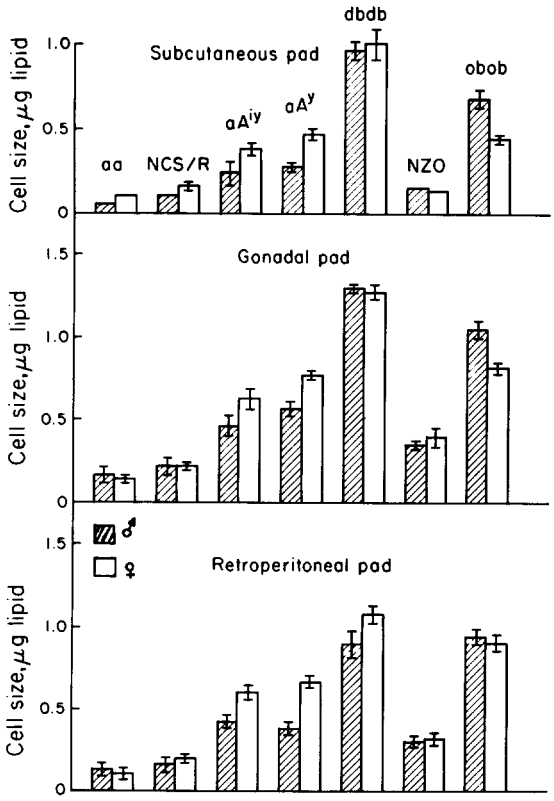

FIG. 8. Comparison of adipocyte size in male and female mice. Strains used were *aa*, NCS/R, *aA^iy*, *aA^y*, *dbdb*, NZO, and *obob*.

DISCUSSION

The Swiss albino mouse, like the Sprague-Dawley rat and the lean Zucker rat, lays down its complement of adipose cells in the adipose depots at an early stage in its growth and development. For the NCS/R mouse, the time is clearly before weaning. It is somewhat later, 12–14 wk, in the rat (2, 3). Any further enlargement of adipose depots in these animals comes about by cell enlargement; lipid is deposited in already existing adipocytes. In the two genetically obese mouse strains (*aA^y* and *aA^vy*) studied during development, there is no indication of change in cell number from shortly after weaning to 100 days of age in *aA^y* mice and 200 days of age in *aA^vy* mice. Growth of the epididymal depot is by cell enlargement only. It is not possible to fix absolutely the time at which new adipose cells cease to appear in the depots, using the osmium fixation method, since the method depends upon a minimum cell size (25 μ) for cell detection. Preadipocytes that are present at weaning may only accumulate enough lipid to be detected at some time after weaning. Thus it is possible that in all of the mouse strains studied, cell number is determined before weaning. The fact that cell number is normally fixed early in development is further substantiated when one looks at the normal NCS/R mouse made obese postweaning by gold thioglucose injection. Even though the epididymal pad has doubled in weight and in lipid content by 100 days of age in mice so treated, cell number has not changed. The enlarged depot size is the result of cell enlargement. In *aaGTG* mice as well, cell numbers of epididymal and subcutaneous pads are the same as those of lean controls, but the retroperitoneal pad does show a cell number increase. The data from these mice suggest again that site-to-site variations do occur, and that caution must be exercised in considering the epididymal pad as representative of all depots. The striking feature of adipose depot growth during postweaning development, however, is cell enlargement.

The case becomes clear, then, that in the growth of adipose depots in normally lean mice and in some obese mice, cell number is determined at some early stage in development. Attempts to manipulate cell number after the critical developmental stage are relatively unsuccessful. With few exceptions, gold thioglucose injection in the mouse and hypothalamic lesions in the rat (2, 3) do not result in the appearance of more fat-laden cells in adipose depots, but rather enhance the deposition of lipid in cells already present in the depot. The only report in the literature that suggests an increase in adipose cell number by an experimental manipulation in the adult animal is the work of Kazdova and Vrana (9). These authors report an increase in DNA synthesis and a decrease in mean adipose cell size in adipose tissue of adult male rats treated with insulin. It is not clear from these studies, however, that adipocyte cell number was changed. Hollenberg, Vost, and Patten (10) examined the effects of insulin on thymidine incorporation into the DNA of stromal and fat cell precursor pools of epididymal adipose tissue from normal adult rats. Their data suggest that insulin enhances proliferation of stromal elements only, but that it nearly doubles triglyceride content of fat cells.

An hypothesis that might be advanced in partial explanation of genetic forms of obesity is that adipose cell number does not become fixed at an early developmental stage; but, under genetic influence, new adipocytes continue to appear in the depots even into adulthood. Such a phenomenon does occur in the genetically obese Zucker rat (3). In genetically obese mice, however, the more common form of obesity appears to involve hypertrophy of existing cells, rather than hyperplasia. In those mouse obesities investigated which are the result of an established single gene mutation, the common pattern of adipose depot enlargement is hypertrophy. Only in the obese–hyperglycemic mouse is there a significant hyperplastic response; and yet even here, cell enlargement is the major effect except in the female subcutaneous pad. It would be tempting to speculate that the high plasma immunoreactive insulin levels that occur around 3 wk of age (11) in female *obob* mice are a causative factor in the

hyperplastic response of the adipose tissue. However, even higher plasma insulin levels have been reported in *dbdb* mice (12) at 4 wk of age, and according to Coleman and Hummel (13), increased plasma insulin is the earliest observable symptom in the *dbdb* mouse and occurs by 2 wk of age. Thus, if insulin is involved in the development of obesity, its mode of action must differ in these two instances.

The New Zealand obese mouse, a highly inbred strain, has significantly more adipose cells in all three depots than does the Swiss albino, to which it was compared in this study. Hypertrophy is a contributing factor in its obesity as well, however. Herberg and coworkers (6) have compared the obese syndrome in the NZO and *obob* mouse. They report a strong correlation between body weight and cell size in the NZO and describe the condition as hypertrophic obesity. In contrast, they find that epididymal fat cell size does not correlate well with body weight in the *obob* mouse. They suggest that cell numbers must increase in other sites, such as the subcutaneous depot, and describe the obese conditions of the *obob* mouse as hyperplastic obesity. Our data adequately confirm the predicted hyperplastic response in the subcutaneous site of the *obob* mouse. The response is particularly striking in the female. The hyperplasia is not restricted to the subcutaneous site, however; it occurs as well in the retroperitoneal depot, and to a lesser extent in the gonadal depot. The picture in the NZO is not so clear. Certainly, in the 26-wk-old animals which we studied, there were enlarged cells if cell sizes were compared with those of lean mice. However, the size differences were not so striking as those seen in all of the other genetically obese strains, including the *obob*. Cell numbers, on the other hand, were at least double those of lean controls at all sites. Taljedal and Hellman (5) found no enlargement of fat cells in the subcutaneous depot of NZO mice if they compared the size with that of the lean littermates of obese yellows. Our data show an increase in size in the males but not in the females at this site. While it may be true that the NZO and *obob* obesities are of different types with respect to cellularity, the contrast between *obob* and the other single gene obese mutants seems more clearly apparent. Yellow, intermediate yellow, viable yellow, and diabetic mutants are characterized by hypertrophy of all adipose depots; the obese–hyperglycemic is characterized by hyperplasia as well as hypertrophy.

The tendency to characterize an animal obesity on the basis of cellularity data derived from the epididymal pad alone may oversimplify, if not distort, the conclusions drawn. Cells from the subcutaneous tissue are somewhat smaller than those from gonadal and retroperitoneal sites in both lean and obese mice. When hyperplasia is observed, as in the *obob* mouse and the Zucker obese rat (3), it is most striking in the subcutaneous and retroperitoneal depots. The gonadal pad behaves more as if it were a fixed organ with a fixed complement of cells.

SUMMARY

In summary, we wish to suggest the classification given below for genetic obesity in mice and rats:

Hypertrophic obesity. The obesity is the result of an established single gene mutation. Cell number is determined before the animal reaches sexual maturity (approximately 60 days in the mouse). The adiposity of the adult animal is the result of a marked cell enlargement in subcutaneous, gonadal, and retroperitoneal depots. The yellow, intermediate yellow, viable yellow, and diabetic mutant mice are examples.

Hypertrophic–hyperplastic obesity. The obesity is the result of an established single gene mutation. Adipose cell number may not be fixed at a clearly definable age. Further developmental studies are needed to clarify this point. Adiposity in the adult is the result of both hyperplasia and hypertrophy of varying degrees in subcutaneous, gonadal, and retroperitoneal depots. The obese–hyperglycemic mouse (6) and the Zucker obese rat (3) are examples.

The New Zealand obese mouse does not fall easily into either category. The genetic origin of its obesity is undetermined (14). Although it has adipose cells of intermediate sizes, the cell size does correlate well with body weight (6). Adult cell number, however, is much greater than that of the lean mice to which it has been compared.

Considering our current understanding of adipose depot cellularity in obese humans, it is suggested that animal obesity of the hypertrophic–hyperplastic type may present the most suitable model for studies directed at understanding the human condition. In fact, none of the animal obesities so far studied remotely approaches the condition seen in the human with respect to cellularity, except for the subcutaneous depot in the *obob* female. It is recognized that these various forms of genetic obesity do not share similar metabolic characteristics in all cases, and the determination of an animal model for specific purposes must take into account the metabolic abnormalities associated with the obesity. Perhaps the age of onset of the phenotypic expression of obesity is a crucial factor, and perhaps it occurs at an earlier time in the *obob* mouse. Developmental studies with this strain should receive a high priority in the continuing effort to unravel the etiology of obesity.

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