Description of obesity in the PBB/Ld mouse

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Abstract A new strain of obese mouse, the PBB/Ld, has been studied in terms of fat pad cellularity, serum insulin and blood glucose levels, and response to gold thioglucose injections. Age-matched C57Bl/6J mice were used as controls. Adipocyte size and number in the major fat depots were determined at various ages from weanling to maturity in the PBB/Ld and C57Bl/6J strains. Results indicated that obesity in the PBB/Ld was due to hypertrophy of adipocytes in retroperitoneal and subcutaneous fat depots and to hypertrophy and hyperplasia in the epididymal fat pad. PBB/Ld mice also developed hyperinsulinemia and hyperglycemia and these findings have been discussed in terms of the developmental changes in fat pad cellularity. The injection of gold thioglucose led to increased food intake in both PBB/Ld and C57Bl/6J mice. Hyperphagia was also present in the PBB/LD control group, but increased efficiency of converting calories to body weight was not observed in this group when compared to control C57Bl/6J mice. The characteristics of obesity seen in the PBB/Ld mouse are discussed and comparisons are made to similar studies in other rodent models of obesity.

Supplementary key words adipocyte number · adipocyte size · plasma insulin · hypothalamic obesity

A current approach to the study of obesity is based on the determination of the relative number and size of adipocytes in the major fat depots. By using this technique it has been shown that adipocyte number in the normal adult is fixed in certain laboratory animals (1) and in man (2). Increases in body weight following increased food intake in man (2) or VMN lesioning in rats (1) and mice (3) have been found to result in increased fat cell size without effect on cell number. Weight loss following food restriction in man (4) and laboratory animals (1) has been shown to result in reduction of fat cell size without an accompanying change in cell number. In contrast fat pads from certain rodent models of obesity such as the obese–hyperglycemic mouse (*obob*), the New Zealand obese mouse (NZO), and the Zucker rat have been found to contain an increased number of cells whose size is also abnormally increased (3, 5). Similarly, marked fat cell hyperplasia has been found to contribute to the increased adiposity of early onset obesity in humans (2). These findings have led to obese states being described as either hypertrophic (increased adipocyte size) or hypertrophic–hyperplastic (increased adipocyte size and number) (3).

Earlier studies on the nature of obesity often emphasized the role of food intake regulation. The discovery that the injection of gold thioglucose (GTG) caused hyperphagia and accompanying obesity in a number of different animal species encouraged this approach. The subsequent finding that GTG caused destruction of neurons in the ventromedial nucleus (VMN) of the hypothalamus led to the glucostatic theory of food intake regulation and suggested that a possible malfunction of this regulatory center might be involved in some natural states of obesity (6–8).

The PBB/Ld is a newly discovered rodent model of obesity (9, 10). These mice were derived from animals purchased at a pet store and subsequently studied for inheritance of coat color. A black line of obese mice emerged during inbreeding (**Fig. 1**). The PBB/Ld now has been brother–sister mated for over 21 generations and limited genetic studies indicate that the obesity is polygenic.

This report examines the cellularity of fat depots in the development of obesity in the PBB/Ld strain, with a concomitant evaluation of serum insulin and blood glucose. The response of the PBB/Ld mice to GTG, in terms of alterations in food intake and weight gain, also is evaluated. These findings are briefly discussed in terms of similar studies of other rodent models of obesity and the unique characteristics of the PBB/Ld strain are illustrated.

METHODS

Fat pad cellularity study

Male PBB/Ld mice (from a colony maintained by the Department of Comparative Medicine) and male

Abbreviations: GTG, gold thioglucose; VMN, ventromedial nucleus; NZO, New Zealand obese; *obob*, obese–hyperglycemic.

Fig. 1. Photograph of 1-year-old female PBB/Ld mouse weighing 75 grams.

C57B1/6J mice (Jackson Memorial Laboratory, Bar Harbor, ME) were used in all studies. Animals were maintained in groups of up to five in polypropylene cages with hardwood chip bedding (Ab-Sorb-Dri, Inc., Garfield, NJ) and kept in a temperature-controlled room (23°C) with a regulated light cycle (12 hr, dark/light). The mice were given free access to tap water and fed Purina Lab Chow ad libitum.

At intervals between 5 and 34 weeks of age, selected mice of both strains were killed by exsanguination following CO_2 narcotization. Blood glucose was determined by the glucose oxidase method using Dextrostix reagent strips and an Eyetone reflectance colorimeter (Ames Company, Elkhart, IN). Serum insulin concentrations were determined in duplicate by radioassay with Sephadex-bound antibody (Pharmacia, Uppsala, Sweden) using crystalline rat insulin (Novo Terapeutisk Laboratium, Copenhagen) to establish seven-point standard curves. The epididymal fat pads from each animal were removed and weighed. Samples weighing 100 mg or less were taken from the left pad and processed for cell counting. In 5-week-old animals it was necessary to use the entire left fat pad. Corresponding sections of equivalent size from the right pad were used for estimation of lipid content. In animals 24 weeks of age subcutaneous and retroperitoneal fat pads were removed and treated similarly. Other tissues (heart, lung, kidneys, liver, spleen, and pancreas) were removed, fixed in 10% buffered formalin, and processed by routine methods for histologic study.

Fat cells were counted and sized by a slight modification in Method III of Hirsch and Gallian (11). Fat pads were fixed for a minimum of 48 hr at 37°C in 25 ml of 2% osmium tetroxide (Electron Microscopy Sciences, Fort Washington, PA) in 0.05 M collidine-HCl buffer, pH 7.4. Nylon filters (250 μm) (Tetko, Inc., Lancaster, NY) mounted on Buchner funnels were employed for final cell separation and collection of noncellular debris. Cell collection was accomplished with 25 μm nylon filters mounted in the same manner. Cells were suspended in a filtered solution of 0.85% saline–glycerol 70:30 (v/v) and counted with a model F Coulter counter (Coulter Electronics, Hialeah, FL). Cell number was calculated by using the formula:

$$\text{cell number per fat pad} = \frac{\text{wet weight of total pad}}{\text{wet weight of tissue for osmium fixation}} \times \text{number of cells obtained in osmium fixation}$$

Estimation of total lipid content was determined by a slight modification of the method described by Stauffacher and Renold (12). Preweighed fat pad samples were placed in 25-ml volumetric flasks containing 15 ml of chloroform–methanol 2:1 (v/v) and then were shaken for 24 hr. Tissue ghosts were removed and 5 ml of 0.03N H_2SO_4 was added. After aspiration of the upper phase the total volume of chloroform was increased to 25 ml. Anhydrous Na_2SO_4 was added to permit removal of remaining upper phase contaminant. Aliquots (10 ml) from each sample were dried in tared vials to a constant weight under a stream of warm, dry air. Average fat cell size was estimated by calculating the μg of lipid per cell by using the formula:

$$\mu\text{g of lipid per cell} = \frac{\text{prefixation wet weight of osmium-fixed tissue (mg)}}{\text{number of cells in osmium-fixed tissue}} \times \frac{\text{lipid in unfixed tissue } (\mu\text{g})}{\text{wet weight of unfixed tissue (mg)}}$$

Means, standard deviation, and standard error of the mean were determined in the usual way and the significance of the difference between groups was established using the Student's *t* test. Regression analysis was used to determine correlation coefficients when this was applicable (13).

Gold thioglucose study

Male mice of the PBB/Ld and C57B1/6J strains were maintained as described above except that they were housed in individual cages and fed a purified high carbohydrate diet (14) modified by the substitution of 2% agar for 2% cellulose to produce uniform agar-gel blocks.

At 3 months of age mice of each strain were randomly divided into two groups and fasted for 24 hr. Approximately half of each group was injected intraperitoneally with 0.8 g/kg of a 100 mg/ml solution of gold thioglucose (Sigma Chemical Company, St. Louis, MO) in 0.85% saline. During the next 3 months food intake, weight gain, and blood glucose were monitored at regular intervals. Two months after the injections, glucose tolerance tests were performed on animals in each of the four groups. Mice were fasted for 24 hr and blood was collected from the tail in order to establish base line blood glucose values. Each animal then received 2 g/kg of a 20% glucose solution in 0.85% saline by intraperitoneal injection; blood was collected 30, 60, 120, and 180 min post-injection. Blood glucose was determined by the glucose oxidase method (Worthington Biochemical Corporation, Freehold, NJ). At 6 months of age the animals were killed by the method previously mentioned and tissues, including brain, were removed and treated by routine histologic methods. Serum insulin concentrations were determined as previously described.

Twenty-four-hour food intake was measured 13 times between days 22 and 60 of the 3-month study. The food remaining after each 24-hr period was dried to a constant weight in a drying oven (110°C) and this weight was subtracted from the calculated dry weight of the initial sample to determine the dry weight of food consumed. These weights were averaged to provide an estimate of the mean daily food intake of each mouse. Daily weight gain was calculated on the basis of difference in the weight of each animal at the beginning and end of the same 38-day period. Food efficiency was determined for each mouse by dividing average daily weight gain by average daily food intake.

RESULTS

Fat pad cellularity study

Significant increases in body weight in the C57B1/6J mice occurred only until 24 weeks of age, whereas increases in body weight in the PBB/Ld mice occurred throughout the 34-week study. The rate of increase in both strains slowed after 11 weeks, with this tendency being greater in the C57B1/6J mice (**Table 1**).

Left epididymal fat pad weight in the C57B1/6J mice also increased up to 24 weeks of age but the rate of increase slowed after the first 11 weeks. The epididymal fat pads of PBB/Ld mice increased in weight dramatically until 34 weeks of age with only a slight slowing of the rate of increase at 11 weeks (Table 1). The increases in left epididymal fat pad weight in the C57B1/6J mice appeared to result primarily from hypertrophy of adipocytes, with cell number remaining essentially unchanged after 11

TABLE 1. Developmental studies relating cellularity, blood glucose, and insulin in C57Bl/6J and PBB/Ld mice

	No. Animals	Age	Body Wt.	Blood Glucose	Insulin	Left Epididymal Fat Pad Weight	Left Epididymal Fat Pad Cell Size	Left Epididymal Fat Pad Cell No.
		wks	*g*	*mg/dl*	*μU/ml*	*mg*	*μg lipid/cell*	*×10⁶*
C57Bl/6J	3	5	15.0 ± 1.0	110.0 ± 10.0	54.8[e]	35.5 ± 8.3	0.012 ± 0.003	1.40 ± 0.16
PBB/Ld	5	5	18.5 ± 0.7[b]	123.0 ± 3.0[d]	41.8 ± 4.1	30.2 ± 3.9[d]	0.013 ± 0.003[d]	1.02 ± 0.04[c]
C57Bl/6J			N.D.[f]	N.D.	N.D.	N.D.	N.D.	N.D.
PBB/Ld	6	8	29.2 ± 0.7	180.0 ± 10.0	55.9 ± 16.0	132.9 ± 8.1	0.073 ± 0.010	1.62 ± 0.25
C57Bl/6J	3	11	24.8 ± 0.4	121.7 ± 4.4	39.8 ± 7.3	141.5 ± 22.8	0.101[e]	1.53 ± 0.22
PBB/Ld	5	11	38.6 ± 0.6[a]	158.0 ± 8.7	50.7 ± 5.0[d]	288.1 ± 21.7	0.101 ± 0.018	2.76 ± 0.31[b]
C57Bl/6J	3	16	26.1 ± 1.2	131.7 ± 9.3	38.2[e]	138.8 ± 4.8	0.074 ± 0.004	1.60 ± 0.12
PPB/Ld	5	16	39.8 ± 1.2	167.0 ± 8.6[c]	77.4 ± 8.5	435.2 ± 33.3	0.143 ± 0.011	2.88 ± 0.41[b]
C57Bl/6J	3	24	30.6 ± 0.3	135.0 ± 7.6	41.8 ± 9.3	234.2 ± 37.0	0.132 ± 0.013	1.60 ± 0.26
PBB/Ld	5	24	44.3 ± 1.7	166.0 ± 4.0	111.6 ± 9.1	590.7 ± 56.7	0.246 ± 0.024	2.14 ± 0.18[d]
C57Bl/6J	4	34	28.0 ± 0.2	157.5 ± 2.5	N.D.	242.3 ± 10.4	0.105 ± 0.000	2.13 ± 0.06
PBB/Ld	3	34	50.3 ± 1.8	198.3 ± 33.4[d]	N.D.	801.7 ± 90.5	0.188 ± 0.019	3.97 ± 0.66[c]

[a] All values are significantly different from C57Bl/6J mice of the same age at $P < 0.01$ except as indicated otherwise. Values represent group means ± SEM (second value in each pair of numbers).
[b] $P < 0.025$.
[c] $P < 0.050$.
[d] Not significant.
[e] Two animals only.
[f] Not determined.

TABLE 2. Cellularity and cell size in three fat depots in adult (24 wk.) C57Bl/6J and PBB/Ld mice

	Body Wt	Epididymal Fat Pad			Subcutaneous Fat Pad			Retroperitoneal Fat Pad		
		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*
C57Bl/6J										
no. 25	30.5	171.1	0.137	1.07	161.1	0.050	2.52	94.7	0.038	2.07
no. 28	31.2	299.2	0.152	1.80	257.8	0.054	3.85	109.4	0.149	0.62
no. 30	30.0	232.4	0.106	1.95	203.7	0.058	2.53	112.3	0.038	2.53
mean of	30.6	234.2	0.132	1.60	207.5	0.054	2.97	105.5	0.075	1.74
group	± 0.3	± 37.0	± .013	± 0.26	± 28.0	± .000	± 0.44	± 5.4	± .037	± 0.57
PBB/Ld										
no. 23	50.8	690.0	0.260	2.34	355.5	0.158	1.71	394.6	0.216	1.48
no. 24	43.8	634.6	0.320	1.74	375.4	0.217	1.49	283.5	0.277	0.88
no. 27	42.3	644.7	0.263	2.19	563.9	0.107	4.59	335.5	0.301	0.96
no. 29	43.7	614.9	0.205	2.74	499.5	0.129	3.02	315.3	0.150	1.84
no. 31	41.1	369.2	0.180	1.85	135.7	0.059	1.64	193.9	0.112	1.46
mean of	44.3[a]	590.7	0.246	2.14[d]	386.0[d]	0.134[b]	2.49[d]	304.6	0.211[c]	1.32[d]
group	± 1.7	± 56.7	± .024	± 0.18	± 73.6	± .026	± 0.59	± 33.1	± .036	± 0.18

[a] All values are significantly different from C57Bl/6J at $P < 0.01$ except as indicated otherwise. Values represent group means ± SEM (second value in each pair of numbers).
[b] $P < 0.025$.
[c] $P < 0.050$.
[d] Not significant.

weeks of age. Increased weight of this fat pad in the PBB/Ld mice could be accounted for by increases in both adipocyte number and size (Table 1).

Retroperitoneal and subcutaneous fat pad weights of 24week-old PBB/Ld mice generally exceeded those of C57B1/6J mice of the same age. Cell size was found to be markedly larger in these fat depots in the PBB/Ld mice but fat cell number was generally less than that of the C57B1/6J mice (**Table 2**).

Increases in the mean blood glucose concentration were observed in both strains as age increased, with the levels generally being greater in the adult PBB/Ld mice (Table 1). Whereas the insulin concentration was not observed to increase with age in the C57B1/6J strain, a progressive increase was observed in the PBB/Ld mice (Table 1). Regression analysis indicates that fat cell size and insulin concentration correlate significantly in the PBB/Ld mouse (**Table 3**).

Gold thioglucose study

Hypothalami from the GTG-injected mice contained lesions of variable extent consisting of collapse, pigment (lipofuscin?) accumulation, and glial scarring in the area of the ventromedial nuclei. Although disruption of the nucleus occurred both unilaterally and bilaterally, complete bilateral VMN destruction was not seen. The ventral portions of the VMN were more commonly affected with the lesion often extending to the area of the infundibulum. There were no discernable light microscopic differences in the lesions of GTG-injected PBB/Ld and C57B1/6J mice, or between the hypothalami of the untreated PBB/Ld and C57B1/6J controls. Varying degrees of fatty change and glycogen accumulation were observed in the livers of GTG-treated and untreated PBB/Ld mice. Similar but less extensive changes were seen in GTG-treated C57B1/6J animals whereas the livers of C57B1/6J control animals

TABLE 3. Relationship between fat cell size and number and plasma insulin and glucose concentration

	Strain	Regression Equation	Correlation Coefficient	Significance of Correlation
Fat cell size vs. plasma insulin	PBB	$y = 272.9x + 36.5$	0.7	$P < 0.01$
	C57BL	$y = -78.4x + 48.9$	0.3	N.S.[a]
Fat cell number vs. plasma insulin	PBB	$y = 57.2x + 5.2$	0.1	N.S.
	C57BL	$y = 34.5x + 5.5$	0.1	N.S.
Blood glucose vs. plasma insulin	PBB	$y = 144.6x + 0.2$	0.3	N.S.
	C57BL	$y = 127.7x + 0.1$	0.1	N.S.

[a] Not significant.

TABLE 4. Comparative biochemical and dietary data on the GTG-injected and control C57Bl/6J AND PBB/Ld mice

	No. Animals	Blood glucose	Insulin	Average Food Intake	Food Efficiency
		mg/dl	*μU/ml*	*g/day*	$\times 10^{-2}$
I. C57Bl/6J (saline injected)	8	174.6 ± 4.6[a]	120.7 ± 25.6[b]	2.26 ± 0.07	2.96 ± 0.22
II. C57Bl/6J (GTG injected) (vs. I)	10	205.7 ± 3.1 ($P < 0.001$)	N.D.[d]	2.72 ± 0.04 ($P < 0.001$)	6.46 ± 0.59 ($P < 0.001$)
III. PBB/Ld (saline injected) (vs. I)	10	203.1 ± 7.8 ($P < 0.010$)	182.1 ± 27.7[c] (N.S.)[e]	3.53 ± 0.10 ($P < 0.001$)	3.73 ± 0.68 (N.S.)
IV. PBB/Ld (GTG injected) (vs. I) (vs. III)	10	227.9 ± 10.6 ($P < 0.001$) (N.S.)	N.D.	3.98 ± 0.16 ($P < 0.001$) ($P < 0.050$)	4.34 ± 0.58 ($P < 0.025$) (N.S.)

[a] All values represent group means ± SEM.
[b] Only seven animals represented
[c] Only eight animals represented.
[d] Not determined.
[e] Not significant.

appeared to be normal. Sections of heart, lung, pancreas, spleen, and kidneys from the four groups appeared normal.

PBB/Ld mice consumed significantly more food than control C57B1/6J mice, but the efficiency of converting calories to body weight was not significantly different in the two groups. Average daily food intake and food efficiency of C57B1/6J mice injected with GTG were significantly greater than those of control mice. While the GTG-treated PBB/Ld mice consumed more food than any of the other groups, their food efficiency did not differ significantly from that of control PBB/Ld mice (**Table 4**). Average weight gain for the GTG-injected animals of both strains was about twice that of the control animals, and a few of the GTG-injected C57B1/6J mice achieved body weights exceeding those of PBB/Ld controls.

The mean blood glucose concentration was significantly higher in PBB/Ld mice when compared with C57B1/6J controls, but did not differ from that of the C57B1/6J GTG-injected group (Table 4). Glucose tolerance tests at 5 months of age were abnormal in both GTG-treated and untreated PBB/Ld mice as well as in the C57B1/6J GTG-injected group when compared with the C57B1/6J controls (**Fig. 2**). Serum insulin concentrations were slightly higher in the untreated PBB/Ld mice when compared with the C57B1/6J controls, but this difference was not statistically significant (Table 4).

DISCUSSION

The results presented here indicate that obesity in the PBB/Ld mouse is due primarily to hypertrophy of adipocytes in the epididymal, subcutaneous, and retroperitoneal fat pads. Additionally, fat cell hyperplasia appears to occur in the epididymal pad. Obesity resulting from hypertrophy of adipocytes in epididymal, subcutaneous and retroperitoneal fat pads has also been described in the yellow (aA^y), intermediate

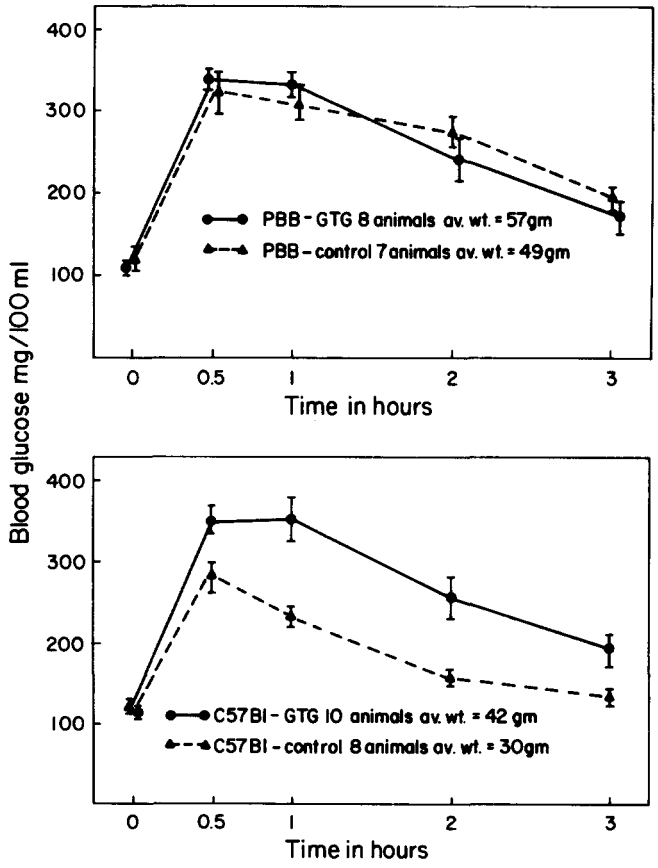

Fig. 2. Glucose tolerance curves for 5-month-old GTG-lesioned and control groups of C57B1/6J and PBB/Ld mice. Data points are ±SEM.

(aA^{iy}), viable yellow (aA^{vy}), and diabetic (*dbdb*) mutant mouse. Obesity involving hypertrophy and hyperplasia of adipocytes has been reported in the obese–hyperglycemic mouse (*obob*) and the obese Zucker rat (3, 5). Obesity in these strains is thought to be the result of single gene mutations (3). Obesity in the New Zealand obese (NZO) mouse, which, like the PBB/Ld, is generally considered to be the result of a polygenic inheritance, is associated with both hypertrophy and hyperplasia of fat cells (3). A detailed comparison of the obesity in the PBB/Ld and other rodent models of obesity has recently been published (10).

Comparison of data from individual adult animals in the cellularity study is useful in an attempt to determine the contributions of adipcoyte hypertrophy and hyperplasia to the PBB/Ld obesity. Table 2 shows fat cell number and size data from individual PBB/Ld and C57B1/6J mice at 24 weeks of age and indicates the degree of variation that can occur in the fat pads of PBB/Ld mice. For example, two animals (nos. 24 and 31) illustrate that PBB/Ld obesity need not involve significant hyperplasia as obesity in these mice was due only to hypertrophy of fat cells. Similarly, even though hyperplasia was most conspicuous in the epididymal pads of the PBB/Ld, increased cellularity could occur elsewhere, as evidenced by the subcutaneous fat pad of one mouse (no. 27).

When comparing the epididymal fat pads of the two groups of 24-week-old mice, hyperplasia of adipocytes in the PBB/Ld strain was not significantly different than that of the C57B1/6J mice (Table 2). However, at 5, 11, 16, and 34 weeks of age, the PBB/Ld mice did show adipocyte hyperplasia in the epididymal pad when compared to the C57B1/6J strain. Hypertrophy showed less variation and fat cells of the PBB/Ld mice were significantly larger than those of the C57B1/6J mice at 16 weeks of age and this difference continued to the end of the study, at 34 weeks of age.

Adult PBB/Ld mice proved susceptible to the effects of GTG and their response was similar to that reported for other mouse strains. Hypothalamic lesions appeared identical to those of the earlier studies (e.g., see 15) and also were similar to those of the C57B1/6J mice injected in the present study. Food intake studies on both control and GTG-injected PBB/Ld and C57B1/6J mice demonstrated that control PBB/Ld mice were hyperphagic but not more efficient in converting food to body weight compared to lean C57B1/6J mice. Increased food efficiency was found to occur, however, in C57B1/6J mice made obese by GTG (Table 4). PBB/Ld mice injected with GTG also were more efficient than control (saline-injected) C57B1/6J mice but not more efficient than control PBB/Ld mice. The explanation for this difference is unclear. Increased food efficiency has also been reported in rats made obese by GTG, as well as in some rodent models of inherited obesity (16).

Early nutritional status is now thought to influence adipose tissue cellularity in an irreversible fashion (1, 17). Fat cell hyperplasia has been induced in young mice that were allowed to suckle mothers being maintained on a high fat diet (18). Similarly, high fat diets fed to Naval Medical Research Institute (NMRI) mice for 11 weeks have been found to result in hyperplasia of fat cells in the epididymal and subcutaneous depots (19). As Hahn and Novak point out in a recent review (20), the development of hyperplasia or hypertrophy may well be decided by the age at which the animal initially is exposed to overnutrition. Early postnatal overnutrition may initiate hyperplasia whereas overnutrition later in life may produce only hypertrophy of fat cells.

Elevated serum insulin at an early age also has been thought to play a role in the development of adipocyte hyperplasia (21), although there is not general agreement on this possibility (22). Recent work with transplantation of islets of Langerhans from normal mouse pancreas into the *obob* and NZO strains with subsequent normalization of blood glucose and insulin levels has led to the conclusion that a primary abnormality responsible for the development of obesity in both these strains lies within the islets (23, 24, 25). The finding that islets from normal animals treated with streptozotocin are equally effective in producing this normalization suggest that the beta cell may not be involved directly in this process (26). It has been proposed that a yet unidentified factor may be involved in the normal regulation of insulin release, and that resultant hypersecretion of insulin may be the abnormality responsible for the development of obesity in the *obob* and NZO strains (26).

Blood glucose and insulin levels were measured in PBB/Ld and C57B1/6J mice concurrently with the study of cell size and number in an attempt to determine whether a relationship could be found. Summary data in Table 1 show that insulin increased slowly with age in the PBB/Ld, while levels in the C57B1/6J were relatively constant. It should be noted, however, that insulin concentrations in PBB/Ld mice were not elevated when compared to C57B1/6J mice until after conspicuous hyperplasia had already occurred in the epididymal fat pad. Insulin levels in the PBB/Ld also can be shown to correlate best with fat cell size rather than with cell number or blood glucose (Table 3) and this correlation has also been reported to occur in other obese states (27, 28, 29).

Hyperinsulinemia in the PBB/Ld may therefore be a secondary phenomenon related to increased adiposity. Blood glucose and insulin levels were also measured in the GTG study (Table 4) and both were elevated when compared to the data of the cellularity study. The high calorie diet consumed by animals in the GTG study was thought to be a likely reason for this elevation.

Analysis of the cellularity data from 5-week-old PBB/Ld and C57B1/6J mice (Table 1) reveals that plasma insulin was lower and adiposity less marked in the PBB/Ld mice than in the age-matched lean C57B1/6J mice. The only suggestion that obesity might subsequently develop is the observation that the PBB/Ld weanling mice are slightly heavier. Overnutrition resulting from hyperphagia could account for this early headstart in body weight and would suggest that increased adiposity, including fat cell hyperplasia and hyperinsulinemia, are secondary factors, at least in the PBB/Ld strain. The primary cause of the hyperphagia remains to be established.

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REFERENCES

1. Hirsch, J., and P. W. Han. 1969. Cellularity of rat adipose tissue: effects of growth, starvation, and obesity. *J. Lipid Res.* **10:** 77–82.
2. Salans, L. B., E. S. Horton, and E. A. H. Sims. 1971. Experimental obesity in man: Cellular character of the adipose tissue. *J. Clin. Invest.* **50:** 1005–1011.
3. Johnson, P. R., and J. Hirsch. 1972. Cellularity of adipose depots in six strains of genetically obese mice. *J. Lipid Res.* **13:** 2–11.
4. Hirsch, J., and J. L. Knittle. 1970. Cellularity of obese and nonobese human adipose tissue. *Federation Proc.* **29:** 1516–1521.
5. Johnson, P. R., L. M. Zucker, J. A. F. Cruce, and J. Hirsch. 1971. Cellularity of adipose depots in the genetically obese Zucker rat. *J. Lipid Res.* **12:** 706–714.
6. Mayer, J., and D. W. Thomas. 1967. Regulation of food intake and obesity. *Science.* **156:** 328–337.
7. Mayer, J. 1965. The ventromedial glucostatic mechanism as a component of satiety. *Postgrad. Med.* **38:** A101–A109.
8. Mayer, J., and N. B. Marshall. 1956. Specificity of gold thioglucose for ventromedial hypothalamic lesions and hyperphagia. *Nature.* **178:** 1399–1400.
9. Hunt, C. E., J. R. Lindsey, L. M. Maxfield, and O. J. Fox. 1972. Obesity in a new strain of mouse. *Federation Proc.* **31:** 244 (Abstr.)
10. Hunt, C. E., J. R. Lindsey, and S. U. Walkley. 1976. Animal models of diabetes and obesity, including the PBB/Ld mouse. *Federation Proc.* **35:** 1206–1217.
11. Hirsch, J., and E. Gallian. 1968. Methods for the determination of adipose cell size in man and animals. *J. Lipid Res.* **9:** 110–119.
12. Stauffacher, W., and A. E. Renold. 1969. Effect of insulin in vivo on diaphragm and adipose tissue of obese mice. *Amer. J. Physiol.* **216:** 98–105.
13. Snedecor, G. W., and W. G. Cochran. 1967. Statistical Methods. Iowa State University Press, Ames, Iowa. 6th Edition.
14. Navia, J. M., H. Lopez, and R. S. Harris. 1969. Purified diet for dental caries research with rats. *J. Nutr.* **97:** 133–140.
15. Liebelt, R. A., and J. H. Perry. 1967. Action of gold thioglucose on the central nervous system. *In* Handbook of Physiology, Sect. 6, Alimentary Canal, Vol. 1, Food and Water Intake. C. F. Code and W. Heidel, editors. 271–285.
16. Bray, G. A., and D. A. York. 1971. Genetically transmitted obesity in rodents. *Physiol. Rev.* **51:** 598–646.
17. 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.
18. Lemmonier, D. 1972. Effect of age, sex and site on the cellularity of the adipose tissue in mice and rats rendered obese by a high-fat diet. *J. Clin. Invest.* **51:** 2907–2915.
19. Herberg, L., W. Döppen, E. Major, and F. A. Gries. 1974. Dietary-induced hypertrophic–hyperplastic obesity in mice. *J. Lipid Res.* **15:** 580–585.
20. Hahn, P., and M. Novak. 1975. Development of brown and white adipose tissue. *J. Lipid Res.* **16:** 79–91.
21. Kazdová, L., P. Fábry, and A. Vrána. 1974. Effect of small doses of insulin in vivo on the proliferation and cellularity of adipose tissue. *Diabetologia.* **10:** 77–83.
22. Salans, L. B., M. J. Zarnowski, and R. Segal. 1972. Effect of insulin upon the cellular character of rat adipose tissue. *J. Lipid Res.* **13:** 616– 623.
23. Strautz, R. L. 1968. Islet implants: Reduction of glucose levels in the hereditary obese mouse. *Endocrinology.* **83:** 975–978.
24. Strautz, R. L. 1970. Studies of hereditary-obese mice (*obob*) after implantation of pancreatic islets in millipore filter capsules. *Diabetologia.* **6:** 306– 312.
25. Gates, R. J., M. I. Hunt, R. Smith, and N. R. Lazarus. 1972. Studies on implanted islets of Langerhans: normalization of blood glucose concentration, blood insulin concentration and weight gain in New Zealand obese mice. *Proc. Biochem. Soc.* **130:** 26–27.
26. Gates, R. J., M. I. Hunt, and N. R. Lazarus. 1974. Further studies on the amelioration of the characteristics of New Zealand obese (NZO) mice following implantation of islets of Langerhans. *Diabetologia.* **10:** 401–406.
27. 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.
28. Stern, J. S., B. R. Batchelor, N. Hollander, and C. K. Cohn. 1972. Adipose cell size and immunoreactive insulin levels in obese and normal-weight adults. *Lancet.* **2:** 948–951.
29. Lemonnier, D., J. P. Suquet, R. Aubert, and G. Rosselin. 1973. Long term effect of mouse neonate food intake on adult body composition, insulin and glucose serum levels. *Horm. Metab. Res.* **5:** 223–224.