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The effect of insulin upon glucose metabolism by adipose cells of different size: *Influence of cell lipid and protein content, age, and nutritional state*

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The protein content of fat cells was found to be relatively constant over a wide range of fat cell size. Thus, enlarged insulin "resistant" fat cells contained the same amount of protein as smaller insulin "sensitive" cells.

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The Effect of Insulin upon Glucose Metabolism by Adipose Cells of Different Size

INFLUENCE OF CELL LIPID AND PROTEIN CONTENT, AGE, AND NUTRITIONAL STATE

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ABSTRACT Glucose metabolism and insulin sensitivity of isolated rat epididymal fat cells and of their delipidated derivatives ("ghosts") was studied as a function of cellular lipid content (fat cell size), cellular protein content, animal age, and state of nutrition in an effort to examine the relationship of adipose cell size to adipose tissue insulin sensitivity.

In *ad libitum*-fed rats, basal rates of glucose-1-^{14}C incorporation into CO_2 and triglyceride are similar over a wide range of adipose cell size. In contrast, the insulin sensitivity of intact fat cells from rats fed *ad libitum* is inversely related to their lipid content: the larger the cell, the less the response to insulin. This "resistance" of the enlarged adipose cell to the action of insulin was demonstrated by a reduction in the per cent rise above the basal rate as well as in the absolute rate of glucose oxidation and lipogenesis caused by insulin.

The protein content of fat cells was found to be relatively constant over a wide range of fat cell size. Thus, enlarged insulin "resistant" fat cells contained the same amount of protein as smaller insulin "sensitive" cells.

These relationships between insulin sensitivity and cellular lipid or protein content were true regardless of whether cells of different sizes were obtained from animals of different body weights and ages, or from different portions of the epididymal fat pads of animals of the same weight and age.

Acute delipidation of intact fat cells did not appear to alter these relationships between basal glucose metabolism, insulin sensitivity, and cell size. "Ghosts" prepared from fat cells of widely different sizes metabolized glucose to CO_2 and triglyceride at similar rates. The insulin sensitivity of the fat cell "ghost" appeared to be inversely related to the size of the intact cell from which it was derived: the larger the intact cell the less insulin sensitive its "ghost."

Although the insulin "resistance" of adipose tissue was reversed by weight loss and reduction of fat cell size, these studies also demonstrate that the insulin sensitivity of adipose cells of similar sizes can vary widely depending upon the state of nutrition and growth of the animal. Thus, factors other than cell size can also influence the insulin sensitivity of the adipose tissue.

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INTRODUCTION

Disordered glucose metabolism is commonly observed in obesity. Kinetic studies of glucose utilization in obese patients have demonstrated a reduction in the disappearance rate and uptake of glucose from the blood (1). There is now considerable evidence in support of the concept that the disordered glucose metabolism of obesity may be related to the presence of insulin resistance. Hyperinsulinemia has been well documented in obese humans and laboratory animals (2–5) and has generally been assumed to reflect insulin resistance in the obese state. The forearm tissues of patients with spontaneous (6) and experimentally induced (7) obesity have been shown to be resistant to the action of insulin. The nature of the insulin resistance in obesity is unknown. Several recent studies have focused attention on the role of the expanded adipose tissue in the insulin resistance of obese humans (5, 8) and experimental animals (9–12).

Glucose metabolism and insulin sensitivity of adipose tissue have been shown to be influenced by the cellular

character of the tissue. Basal glucose metabolism, i. e. glucose metabolism in the absence of added insulin, was reported to be related to the number of fat cells in the tissue fragment; small and large adipose cells oxidize glucose to carbon dioxide at similar rates. In contrast, the ability of insulin to stimulate glucose oxidation in adipose tissue was found to be inversely related to the size of the fat cells in the tissue (lipid content per cell). Enlarged adipose cells from obese patients and animals were found to be relatively resistant to this action of insulin compared to smaller fat cells from nonobese controls. Weight loss in the obese and reduction of fat cell size were associated with restoration of normal adipose tissue insulin sensitivity. These changes in adipose tissue sensitivity to insulin were paralleled by similar changes in the concentration of insulin in the plasma. It was therefore postulated that the insulin resistance of the enlarged fat cell might play a role in the development or perpetuation of the glucose intolerance and hyperinsulinemia commonly seen in obesity. In this study, we have attempted to examine the relationship between the size of the adipose cell and its insulin sensitivity in terms of the lipid and protein content of the fat cell, and the age and dietary intake of rats varying widely in age and body weight.

METHODS

Animals. Male rats from the Charles River Breeding Laboratories, Wilmington, Mass., were used for all experiments. A large group of weanling animals of the same age were fed standard Purina chow diets either *ad libitum* or in restricted amounts. At various intervals during growth, rats were sacrificed and their epididymal adipose tissues were studied according to one of three protocols described below.

Experimental design. In one type of study, rats were fed *ad libitum* until the time of sacrifice. The animals were separated into experimental groups according to body weight (Table I). Each experimental group contained several subgroups of 6–12 rats. The epididymal fat from each subgroup (from 6 to 12 rats) was pooled so that sufficient amounts of adipose tissue were obtained for morphologic and metabolic studies of intact and delipidated cells. Adipose cells of different sizes were obtained from animals of widely different body weights and ages; hence, comparisons of adipose cell size and metabolism were made "between" experimental groups (Table I).

In the second type of experiment, referred to as "within" animal studies, morphologic and metabolic studies of intact and delipidated cells were made in adipose cells of different sizes obtained from *ad libitum* fed rats of similar body weight and age (Table II). In this study, adipose cells of different sizes were obtained from the distal and proximal portions of the epididymal fat pads of rats within the same experimental group; hence, comparisons were made "within" experimental groups.

The third study was in rats fed restricted diets, and only intact adipocytes were examined (Table III). Older, relatively fat rats, matched for age and weight to a control group of *ad libitum*-fed animals, were separated into three subgroups on the basis of different feeding patterns. Rats of *subgroup a* were fed approximately one-third of the caloric intake of controls maintained on an *ad libitum*-chow diet. After 5 wk of dietary restriction these animals were sacrificed and their epididymal adipose tissues were studied. Rats of *subgroup b* received a diet similar to those in *subgroup a,* but after 5 wk, these animals were allowed an additional week of *ad libitum* feedings before sacrifice and examination of their epididymal adipose tissues. Animals in *subgroup c* were fed approximately one-half the caloric intake of controls in order to maintain relatively constant body weight. After 6 wk of this restriction, these rats were sacrificed and studies identical with those in *subgroups a* and *b* were performed.

Isolation and preparation of tissues. Animals were killed by a stunning blow to the head, their entire epididymal fat pads immediately removed and placed in bicarbonate buffered Krebs-Ringer medium at 37°C. The epididymal fat was then divided into proximal (adjacent to the epididymis), medial, and distal (furthest from the epididymis) segments and kept separately at 37°C. Portions of tissues were taken for determination of cell number and size, for lipid extraction, and for preparation of free cells and ghosts.

TABLE I

Body Weight, Average Triglyceride Content per Cell (Cell Size), and Protein Content per Cell in "Between Animal Studies"

Experimental group	Number of subgroups	Total rats	Body weight		Lipid/cell	Protein/cell
			Range	Mean ±SEM*		
				g	*μgTG/cell**	*mμg/cell*‡
1	5	60	88–116	96 ±6	0.053 ±0.004	0.540 ±0.012
2	5	50	124–153	136 ±5	0.087 ±0.001	0.594 ±0.004
3	5	40	207–229	215 ±8	0.148 ±0.004	0.585 ±0.012
4	5	30	485–521	504 ±4	0.305 ±0.003	0.599 ±0.010
5	5	30	533–583	552 ±6	0.425 ±0.007	0.589 ±0.008

Animals were separated into experimental groups and subgroups as detailed in the text. Mean values ±SEM represent the means of the five subgroups within each experimental group. TG = triglyceride.

* $P < 0.05$ between all experimental groups.

‡ $P > 0.05$ between all experimental groups except one, for which $P < 0.05$.

TABLE II

Body Weight, Average Triglyceride Content Per Cell (Cell Size), and Protein Content Per Cell in "Within Animal Studies"

Experimental group	Number of subgroups	Total rats	Body weight		Lipid/cell		Protein/cell	
			Range	Mean ±SEM*	Distal	Proximal	Distal‡	Proximal
			g		*μgTG/cell*		*mμg/cell*	
10	2	16	147–192	169 ±4	0.072 ±0.001§	0.151 ±0.010	0.593 ±0.007	0.577 ±0.011
11†	2	16	228–251	243 ±2	0.116 ±0.013§	0.222 ±0.013	0.601 ±0.019	0.605 ±0.010
12	2	12	408–460	429 ±9	0.244 ±0.007§	0.381 ±0.008	0.590 ±0.016	0.596 ±0.009

See text for explanation of experimental groups and subgroups. Mean values ±SEM represent the means of the two subgroups within each experimental group. TG = triglyceride.

* $P < 0.05$ between all experimental groups.

‡ $P > 0.05$ between all experimental groups, and between distal and proximal in all groups.

§ $P < 0.05$ between distal and proximal within each experimental group.

Determination of adipose cell size. Adipose cell size is expressed as the lipid content per cell and is calculated from the measurement of the total number of fat cells in a known amount of tissue lipid. The methods used in this study are those described by Hirsch and Gallian in which osmium-fixed fat cells are electronically counted in a Coulter Electronic Counter (Coulter Electronics, Inc., Hialeah, Fla. (13). In most studies, three pieces of adipose tissue (proximal, medial, and distal), were placed on a tared nylon mesh (Tobler, Ernst & Traber, Inc., New York), washed with 37°C bicarbonate buffer, blotted, and weighed; then placed in a solution of 2% osmium tetroxide in collidine buffer, pH 7.4 at 37°C. The lipid content of these tissues is determined after extraction of a known wet weight of comparable but unfixed adipose tissue in chloroform: methanol (2:1). In some studies, adipose cell number and size were determined separately for proximal and distal portions of the fat pad. Using these procedures, the average lipid content which is proportional to the average size of fat cells in the tissue can be calculated as follows:

$$\mu g \text{ lipid/cell} = \frac{\text{Wet weight of osmium fixed tissue } (\mu g)}{\text{Total number of cells in osmium fixed tissue}} \times \frac{\text{Lipid in unfixed tissue } (\mu g)}{\text{Wet weight of unfixed tissue } (\mu g)}$$

The total number of free adipose cells per incubation flask was calculated by dividing its total lipid content by the average lipid per cell determined in intact tissue. This assumes that in the preparation of free cells the lipid content per cell is not changed.

Preparation of intact cells. Free fat cells were prepared from the pooled adipose tissue. Intact adipose cells were isolated by collagenase digestion as described by Rodbell (14). The tissue was incubated in bicarbonate buffered medium, pH 7.4, 37°C, containing 1.0 mg glucose per ml, 4 mg bovine serum albumin per/ml (fraction V, Armour Laboratories, Kankakee, Ill.), and 0.33 mg bacterial collagenase/ml (Worthington Biochemicals Corporation, Freehold, N. J.). After 1 hr, the contents of the flask were shaken gently for no more than 3 min. Using this procedure, most of the tissues were converted to free fat cells with a minimum of cellular rupture. Fat cells were separated from the tissue matrix and from the remaining intact tissue by passage through a nylon mesh filter and by repeated washings with albumin-free buffer at 37°C. The cells were suspended in a lusteroid tube and gently mixed so that homogeneous portions could be removed for appropriate metabolic and chemical studies of intact adipose cells.

In order to determine the amount of collagenase protein adhering to the free cells after repeated washings, the identical procedure for preparing free fat cells was performed using tritiated collagenase (Worthington Biochemicals, prepared by Wilzbach exchange). Studies were carried out in rats of different body weights so as to obtain cells of different sizes and the amount of radioactivity remaining in a known number of cells after five washes with albumin-free buffer was determined in a Packard Liquid Scintillation Spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill.)

Similarly, to determine how much albumin remained on the intact cells, the identical procedure for preparing the

TABLE III

Body Weight and Average Triglyceride Content per Cell (Cell Size) in Animals Fed Restricted Diets

Age	Number of animals	Body weight	Fat cell size
		g	*μg TG/cell*
Experimental group 13 *a*			
16 wk	6	498 ±5	0.297 ±0.007
21 wk	6	355 ±9	0.037 ±0.012
Experimental group 13 *b*			
16 wk	6	502 ±6	0.283 ±0.003
21 wk	6	362 ±10	
22 wk	6	454 ±12	0.218 ±0.015
Experimental group 13 *c*			
16 wk	6	513 ±6	0.304 ±0.009
21 wk	6	509 ±8	
22 wk	6	512 ±12	0.187 ±0.017
Control group 14			
16 wk	3	518 ±7	0.301 ±0.012
21 wk	3	565 ±6	0.327 ±0.009
22 wk	3	591 ±9	0.349 ±0.015

Animals were separated into groups and subgroups as detailed in the text. Mean values ±SEM represent the means of number of animals. TG = triglyceride.

cells was carried out using albumin-I^{131} (Abbott Laboratories, Chicago, Ill.). Adipose tissue was obtained from rats of different body weights and free cells prepared in bicarbonate buffer containing collagenase and albumin-I^{131}. The amount of radioactivity remaining in a known number of fat cells after five washes with albumin-free buffer was determined using a Packard Automatic Gamma Counter (Packard Instrument Co., Inc., Downers Grove, Ill.).

Preparation of fat cell ghosts. A portion of the intact fat cells was suspended in a hypotonic medium consisting of 0.1 M $CaCl_2$, 1 mM K HCO_3, 25 mM $MgCl_2$, 2.5 mM ATP, 0.1 mM NAD, 0.05 mM NADP, pH 7.2. Thereafter, the cells were kept at 4°C and all preparative procedures carried out at this temperature. Fat cells were lysed as described by Rodbell, by repeated exposure of intact cells to the hypotonic medium, centrifugation at 900 *g*, and by collection and pooling of the turbid aqueous phase (15). 40% sucrose (w/v) was added to the aqueous lysate to make a final sucrose concentration of 8.5%. The aqueous lysate, containing the delipidated cells, was subjected to a series of centrifugations at 900 *g* and the resultant sediment suspended in sufficient ice-cold bicarbonate buffer to give approximately 1 mg of ghost protein/ml, from which portions for appropriate studies could be obtained.

Metabolic studies of intact cells. From each suspension of intact cells, duplicate portions were taken for lipid extraction in isopropanol: heptane: 1 N sulfuric acid (4:1:0.1) in order to determine the triglyceride content per milliliter of the cell suspension (16). Another two portions were taken for determination of protein content per milliliter of cell suspension (17). 1 ml portions of the cell suspension were transferred to polyethylene bottles (Nalgene Sybron Corp., Rochester, N. Y.) already containing 1 ml of bicarbonate buffered medium with 2.0 mg glucose per ml, 8 mg albumin per ml, and 0.50 μCi glucose-1-^{14}C/ml. In some flasks, 200 microunits of crystalline insulin (courtesy of Dr. W. Kirtley, Eli Lilly & Company, Indianapolis, Ind.) was added. Thus, in each flash, cells were incubated in a bicarbonate buffered medium containing 1.0 mg per ml glucose, 4 mg/ml albumin, 0.25 μCi glucose-1-^{14}C/ml, with or without 100 μμ insulin/ml. The flasks were capped with rubber stoppers from which was suspended a small glass vial. After gassing the contents of the flask with 95% O_2: 5% CO_2 for 5 min, the cells were incubated for 2 hr at 37°C in a metabolic shaker. 0.5 ml of hyamine hydroxide was injected into the suspended glass vial, 1 ml of 6 N sulfuric acid introduced into the medium, and $^{14}CO_2$ was collected in hyamine during an additional 1 hr of shaking. The remaining cells and incubation buffer were carefully transferred into 20 ml of isopropanol: heptane: 1 N sulfuric acid (4:1:0.1) and total lipid extracted overnight. After the addition of water and heptane, portions were taken from the heptane upper phase for lipid determination by measurement of carboxyl ester bonds (18) and for determination of lipid-^{14}C content. The $^{14}CO_2$ and ^{14}C lipid derived from carbon-1 of the glucose, was counted in a Packard Liquid Scintillation Spectrometer at 87% efficiency in a solution of phosphor (0.4% 2.5-diphenyloxazole), 0.01% 1, 4-bis[2(-5(phenyloxazolyl)]benzene) in toluene. All counts were corrected for background and for isotopic impurity as previously reported (19).

Metabolic studies of ghost. As with intact cells, duplicate portions of each suspension of ghosts were taken for determination of protein and lipid content per milliliter of ghost suspension. 100 μl of the ghost suspension were transferred to incubation flasks containing 1.9 ml of bicarbonate buffered medium with 1.0 mg glucose/ml, 4 mg albumin/ml, and 0.75 μCi glucose-1-^{14}C/ml. Insulin, 100 μμ/ml, was added to some flasks. All incubations were carried out in triplicate. Flasks were capped with rubber stoppers as above, gassed for 5 min with 95% O_2: 5% CO_2 and incubated for 1 hr at 37°C in a metabolic shaker. Determination of $^{14}CO_2$ and lipid-^{14}C was made as described above for intact cells.

Calculations. The rate of incorporation of glucose-1-^{14}C into CO_2 and lipid by intact fat cells is calculated on a per cell basis as follows:

$$(1)\ \frac{\text{cpm in } CO_2 \text{ of lipid}}{\text{specific activity of medium glucose in cpm/}\mu\text{g}} \times \frac{1}{\text{hr}} \times \frac{1}{\mu\text{g of tissue lipid}} = \mu\text{g glucose/}\mu\text{g tissue lipid} \times \text{hr}.$$

(2) μg glucose incorporated/cell per hr = μg glucose/μg tissue lipid × hr × 1/number of cells per μg of lipid.

The rate of incorporation of glucose-1-^{14}C into CO_2 and lipid by "fat cell ghosts" was calculated per unit of ghost protein. Metabolic activity of intact cells was also calculated per unit of protein.

The insulin sensitivity of intact fat cells and of ghosts is expressed in two ways: (*a*) as the per cent increase above the basal rate of incorporation of glucose carbon into CO_2 or triglyceride (TG)[1] caused by insulin:

$$\frac{[\mu\text{g glucose to } CO_2(TG)]\text{insulin} - [\mu\text{g glucose to } CO_2(TG)]\text{basal}}{[\mu\text{g glucose to } CO_2(TG)]\text{basal}} \times 100 = \%\ \text{increase}$$

and (*b*) in terms of the absolute rate at which glucose oxidation or incorporation into triglyceride is proceeding in the presence of insulin.

RESULTS

Body weight and adipose cell size. The body weight and mean epididymal adipose cell size of the animals studied is summarized in Tables I, II, and III. In studies in which comparisons were made "between animals," rats were grouped according to their body weights; the data from five such groups are shown in Table I. Five experiments were carried out within each weight range, on subgroups of 6–12 animals each. Mean body weight and mean adipose cell size is significantly different between experimental groups but not within each group (subgroups).

Table II summarizes the data on body weight and epididymal adipose cell size in the three groups of animals in whom the distal and proximal portions of the epididymal fat pads within each group were studied separately. Within each group, the mean size of the adipose cells in the proximal portion of the fat pad is significantly larger than the cells of the distal portion. Both proximal and distal fat pad adipocytes significantly increase in size as the body weight of the animals increases. However, there is overlapping of size of adipose cells from the proximal fat pad of one group with cells

[1] *Abbreviation used in this paper:* TG, triglyceride.

from the distal portion of another (10 and 11, 11 and 12).

Table III compares body weight and fat cell size between animals fed restricted diets (experimental group 13) and *ad libitum* fed rats (control group 14). Restriction of food intake by one-third for 5 wk resulted in a mean weight loss of 30% in subgroup 13 *a* and 28% in 13 *b*. No weight change was noted in subgroup 13 *c* in which caloric intake was approximately one-half that of controls. After weight loss, these animals weighed 63% of the *ad libitum* fed controls of the same age. Refeeding resulted in a regaining of 66% of the lost weight in subgroup 13 *b*, but these animals were still only 77% of the weight of the controls of the same age. Rats in subgroup 13 *c* whose body weight was maintained at a relatively constant level for 6 wk weighed 13% less than the same age controls fed *ad libitum*.

Adipose cell size decreased to 14% of original size in the animals reduced for 5 wk (subgroup 13 *a*), and at that time cell size was only 10% of that in the epididymal pads of *ad libitum*-fed controls of the same age. At this low cellular lipid content the accuracy of cell sizes derived from the counting technique using osmium-fixed cells is undoubtedly limited. Nevertheless, it is clear that there has been a marked reduction in cell size. Rats reduced for 5 wk and then refed (subgroup 13 *b*) had epididymal fat cells which were 23% smaller than before reduction, 38% smaller than *ad libitum*-fed controls, but six times larger than those of reduced rats in subgroup 13 *a*. Cell size in the animals fed just enough calories to maintain constant weight, but not enough to grow (subgroup 13 *c*) was reduced by 65%, a value which is 53% of that found in the control animals of the same age.

Protein content of intact fat cells. The mean protein content of intact adipose cells of different sizes is shown in Tables I and II. Protein content per cell has been corrected for adherent collagenase and albumin. It was found that 33 ±1% of the cell protein measured was due to adherent collagenase, regardless of cell size. The amount of albumin adhering to cells was quite small and related to cell size: 0.11% of the measured protein in the smallest (0.06 μg TG/cell) to 0.61% in the largest cells (0.51 μg TG/cell).

The protein content of adipose cells from very young rats, group 1 (0.540 mμg/cell) is less than that in the cells of older animals with larger cells (0.589 — 0.599 mμg/cell). However, there is no significant difference in the mean protein content of adipose cells from groups two through five, even though the lipid content of these cells varies considerably. Thus, the protein content of adipose cells remains relatively constant regardless of size, except in the very small cells of young rats. The same relationships are noted in adipose cells of different sizes obtained from animals of similar age and weight (Table II).

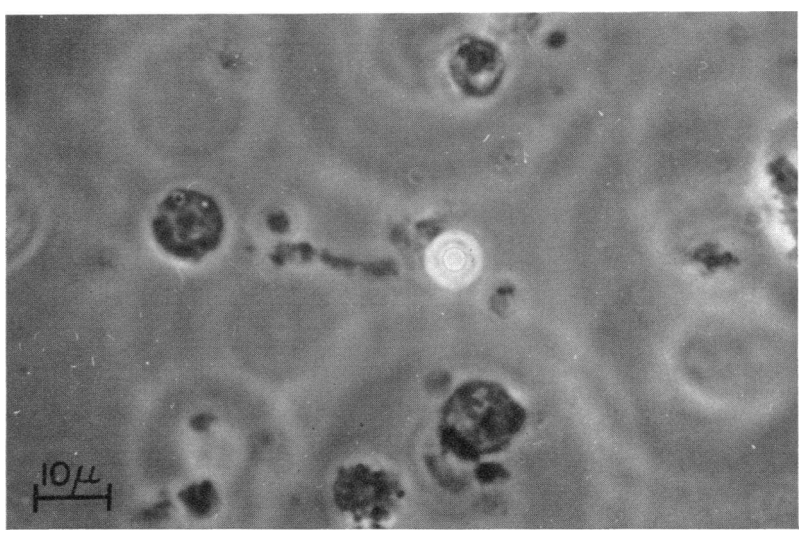

FIGURE 1 Phase micrograph of "ghosts" of intact adipose cells (× 1000). "Ghosts" were suspended in Krebs-Ringer bicarbonate medium and sized under a phase microscope using a micrometer lens. This view shows the typical delipidated cells in which particulate and vesicular material in the cytoplasm is contained within a rounded plasma membrane. This particulate matter possesses brownian movement and hence some blurring occurs in the photomicrograph. In isotonic medium the particulate structures tend to aggregate.

TABLE IV

Measurements of Diameter of Delipidated Cells (Fat Cell "Ghosts")

Experimental group	Number of animals	Mean intact cell size	Range	Mean
		*μgTG/cell**	*"Ghost" diameter, μ*	
1	200	0.053 ±0.004	3–10	6.66 ±1.49
2	200	0.087 ±0.001	3–12	6.60 ±1.64
3	200	0.148 ±0.004	4–12	6.75 ±1.42
4	200	0.305 ±0.003	5–14	7.35 ±1.76
5	200	0.475 ±0.011	5–16	8.04 ±1.73

"Ghosts" suspended in Krebs-Ringer bicarbonate buffer were sized under a phase contrast microscope using a micrometer lens. 40 fat cell "ghosts" were sized from each subgroup within each experimental group. The values for "ghost" diameter represent the range and mean ±SD for all "ghosts" sized in that group. Triglyceride, TG. Significance testing of mean "ghost" diameter performed after logarithmic transformation of data.

* $P < 0.05$ between all groups, mean intact cell size.

‡ $P > 0.05$ between all groups, mean "ghost" diameter.

Morphologic character of ghosts. Phase microscopic examination of the 900 *g* sediment revealed delipidated cells and other particulate matter as previously described by Rodbell (Fig. 1). In our studies, however, considerable difficulty was encountered in separating intact "ghosts" from broken fragments. The cells vary in size, possess intracellular particulate material with brownian movement, and for the most part are devoid of nuclei. "Ghost" diameter was estimated with the aid of a measuring lens as indicated in Fig. 1. The mean diameter of "fat cell ghosts" obtained from intact adipose cells is not different in spite of the fact that they were derived from adipose cells of widely different sizes (Table IV). However, as adipose cell size increased, the range of "ghost" size also increased slightly.

Glucose metabolism of intact fat cells from ad libitum-fed rats. The glucose metabolism of epididymal fat cells is expressed *per adipose cell* and *per unit of protein.*

Table V summarizes the basal rates (no insulin added) of glucose oxidation and glucose lipogenesis by epididymal adipose cells isolated from groups of rats varying widely in body weight. Since there were no differences between the five subgroups within each experimental group, only the group means are shown. The mean rate of glucose-1^{14}C incorporation into CO_2 and triglyceride by the adipose cells from the youngest animals (experimental group 1) is less than in adipose cells of older rats. This correlates with the observation that the protein content of these cells is less. However, in the cells from older animals, where protein content is constant, the rate of glucose oxidation and lipogenesis is similar in spite of wide variation in size, when expressed per cell or per unit of protein. The largest fat cells from the oldest and fattest animals (experimental group 5) incorporate glucose into triglyceride at a greater rate than smaller cells from the other groups. (33 vs. 19–21 mg glucose × 10^{-6}/cell per hr, and 56 vs. 32–34 mg glucose × 10^{-6}/mμg protein per hr).

Similarly (Table VI), small adipose cells obtained from the distal portion of the epididymal fat pad oxidize glucose to CO_2 at the same rate as larger proximal cells from the same animals. The mean rate of glucose carbon incorporation into lipid is similar in small and large fat cells obtained from different portions of the epididymal fat pads of the same rats, except for the largest cells in group 12 in which glucose lipogenesis is proceeding at an increased rate (23 vs. 14–17 mg glucose × 10^{-6}/cell per hr and 36 vs. 23–28 mg glucose × 10^{-6} per mμg protein per hr).

Insulin sensitivity of intact fat cells from ad libitum-fed rats. The insulin sensitivity of intact, free adipose cells is expressed in Tables IV and V, in two ways: as the per cent increase above the basal rate caused by the addition of insulin into the medium, and in terms of the absolute rate at which glucose is being metabolized when the cells are exposed to insulin. The ability of insulin to stimulate glucose-1-^{14}C incorporation into CO_2 and lipid is inversely related to the lipid content of the intact fat cells. Insulin sensitivity decreases as fat cell size increases: the smallest adipocytes of young nonobese rats were the most insulin sensitive and the enlarged cells of older rats the least response to insulin, regardless of whether insulin sensitivity is expressed as per cent increase or in terms of the absolute rate of insulin stimulated glucose metabolism. This relationship was true when data were expressed on a per cell or per millimicrogram of protein basis.

Moreover, in animals of the same age and body weight (Table VI), the smaller, distal epididymal fat cells were more insulin sensitive than the larger, proximal cells, both in terms of insulin's effect on glucose oxidation and lipogenesis, irrespective of whether the data were expressed on a per cent increase or absolute rate basis.

Glucose metabolism of adipose cells from caloric restricted animals. The adipocytes of animals, reduced by underfeeding and studied before refeeding, had a marked decline in the basal rate of glucose metabolism (Table VII, subgroup 13 *a*). Glucose oxidation was 19% and glucose lipogenesis 13% of that observed in the adipose tissue of control rats of group 14 who were the same age but fed *ad libitum.*

Refeeding for 1 wk after reduction (subgroup 13 *b*) raised basal glucose metabolism to rates from one and one-half to two times greater than those observed in *ad libitum*-fed control animals.

The adipose cells of underfed animals in whom weight was maintained over a 6-wk period (subgroup 13 *c*) also showed a reduction in the basal rate of glu-

cose oxidation and glucose lipogenesis when compared to *ad libitum*-fed control animals of the same age, but the decline was less pronounced than in the rats who lost weight.

Insulin sensitivity of adipose cells from caloric restricted animals. The adipose cells of the underfed, reduced rats studied before refeeding (subgroup 13 *a*, Table VII), showed no response to insulin in spite of the fact that these cells were much smaller than the insulin resistant cells of the control animals of subgroup 14. This was true of the effect of insulin on glucose oxidation and lipogenesis.

Refeeding for 1 wk (subgroup 13 *b*) increased the insulin responsiveness of the adipose tissue. Not only were these cells more insulin sensitive than the larger cells of the *ad libitum*-fed control rats, but insulin enhanced the rate of glucose oxidation and lipogenesis to a level equal to (on a per cent increase basis), or greater than (on an absolute basis) that seen in the very small adipose cells of *ad libitum*-fed animals of group 3 (Table V).

The adipose tissues from animals underfed so as to maintain constant body weight for 6 wk (subgroup 13 *c*) did not respond to insulin even though the cells were small.

Glucose metabolism of "fat cell ghosts." Glucose metabolism to CO_2 and lipid by "fat cell ghosts" is expressed *per millimicrograms of protein.* Delipidation of adipose cells by the method used here profoundly reduces the metabolic capacity of the cell. The basal rate at which glucose-1-^{14}C incorporation into CO_2 and lipid is proceeding in "fat cell ghosts" is reduced to approximately one thousandth of that observed in the intact cells from which they are derived (Tables V and VI).

TABLE V

Glucose 1 ^{14}C Incorporation into CO_2 and Triglyceride, "Between Animal Studies"

Experimental group (n)	Intact adipose cells		Intact adipose cells		Adipose cell "ghosts"	
	CO_2	Triglyceride	CO_2	Triglyceride	CO_2	Triglyceride
	mg glucose $\times 10^{-6}$*/cell hr*		*mg glucose* $\times 10^{-6}$*/ mμg protein per hr*		*mg glucose* $\times 10^{-3}$*/ mμg protein per hr*	
1 (60)						
Basal	11 ±1	11 ±2	20 ±2	20 ±3	33 ±2	36 ±2
Insulin	91 ±3	50 ±2	168 ±6	93 ±4	61 ±2	62 ±2
% increase	764 ±70	413 ±72	769 ±66	408 ±71	73 ±3	72 ±5
2 (40)						
Basal	17 ±2	19 ±3	29 ±3	32 ±5	35 ±1	39 ±1
Insulin	70 ±2	40 ±2	118 ±5	68 ±5	53 ±2	56 ±2
% increase	341 ±39	125 ±19	371 ±40	124 ±20	55 ±3	44 ±1
3 (40)						
Basal	14 ±1	19 ±2	25 ±2	32 ±3	37 ±2	39 ±2
Insulin	46 ±3	31 ±2	78 ±5	52 ±5	47 ±2	51 ±1
% increase	223 ±25	66 ±6	224 ±26	65 ±6	29 ±2	30 ±3
4 (30)						
Basal	14 ±1	21 ±1	23 ±1	34 ±3	39 ±2	36 ±2
Insulin	18 ±1	23 ±1	29 ±2	38 ±2	38 ±1	37 ±2
% increase	27 ±7	11 ±3	26 ±7	11 ±3	2 ±1	6 ±1
5 (30)						
Basal	16 ±2	33 ±2	28 ±3	56 ±3	35 ±2	42 ±2
Insulin	19 ±2	32 ±2	32 ±3	55 ±3	39 ±2	40 ±2
% increase	16 ±7	1 ±1	16 ±6	1 ±1	6 ±2	1 ±1

(*n*) = Number of animals in each group.

Values for each group represent the mean ±SEM of the means of triplicate determinations of the pooled adipose tissues of its component subgroups. There were no differences between the five subgroups within each group. Significance levels were determined from *F* ratios calculated by one way analysis of variance. Significant differences: $P < 0.05$, per cell and per mμg protein; Basal CO_2: group 1 vs. groups 2, 3, 4, and 5 (intact cells only); Basal TG: group 1 vs. groups 2, 3, 4, and 5, and group 5 vs. groups 1, 2, 3, and 4 (intact cells only); Insulin CO_2: group 1 vs. 2 vs. 3 vs. 4 and 5 (intact cells and ghosts); and Insulin TG: group 1 vs. 2 vs. 3 vs. 4 and 5, intact cells and ghosts.

The reduction in basal glucose oxidation and lipogenesis is similar in all cells regardless of size except for the largest cells in group 5 where lipogenic capacity is reduced to a greater degree. The mean basal rate of glucose incorporation into both CO_2 and lipid in "ghosts" of small fat cells is not statistically different from that observed in ghosts of larger cells. This is true whether "ghosts" were derived from animals of different body weights and ages (Table V) or from different portions of the epididymal fat pads of animals of the same body weights and ages (Table VI).

Insulin sensitivity of "fat cell ghosts." The insulin sensitivity of ghosts is expressed as the absolute rate at which glucose oxidation or glucose lipogenesis is proceeding in the tissue in the presence of added insulin, and as the per cent increase above the basal rate caused

TABLE VI

Glucose 1 ^{14}C Incorporation into CO_2 and Triglyceride, "Within Animal Studies"

Experimental group (n)	Intact adipose cells		Intact adipose cells		Adipose cell "ghosts"	
	CO_2	Triglyceride	CO_2	Triglyceride	CO_2	Triglyceride
	mg glucose × 10^{-6}/cell hr		*mg glucose × 10^{-6}/ mμg protein per hr*		*mg glucose × 10^{-6}/ mμg protein per hr*	
10 (16)						
Distal						
Basal	16 ±1	14 ±1	26 ±3	23 ±2	37 ±2	41 ±4
Insulin	92 ±3	50 ±1	153 ±10	81 ±3	61 ±4	63 ±6
% increase	479 ±71	261 ±21	481 ±68	257 ±19	65 ±3	55 ±6
Proximal						
Basal	15 ±1	15 ±1	27 ±2	26 ±3	24 ±3	40 ±3
Insulin	46 ±3	31 ±2	86 ±11	50 ±4	48 ±3	45 ±2
% increase	194 ±13	90 ±2	221 ±18	95 ±3	40 ±2	15 ±2
11 (16)						
Distal						
Basal	15 ±1	17 ±1	24 ±2	27 ±3	35 ±1	39 ±1
Insulin	65 ±4	35 ±2	105 ±5	57 ±5	53 ±1	45 ±2
% increase	340 ±27	115 ±27	340 ±29	109 ±5	50 ±3	11 ±1
Proximal						
Basal	15 ±1	16 ±1	25 ±1	25 ±1	35 ±2	40 ±1
Insulin	28 ±2	24 ±1	47 ±3	38 ±2	40 ±2	41 ±1
% increase	89 ±7	47 ±7	87 ±6	51 ±6	13 ±2	3 ±1
12 (12)						
Distal						
Basal	16 ±1	18 ±2	27 ±2	28 ±5	36 ±1	40 ±2
Insulin	29 ±2	25 ±2	51 ±3	39 ±3	36 ±3	38 ±2
% increase	87 ±1	41 ±3	87 ±3	41 ±3	0	0
Proximal						
Basal	15 ±2	22 ±2	26 ±5	36 ±1	37 ±3	40 ±2
Insulin	14 ±1	21 ±2	26 ±3	37 ±2	38 ±4	39 ±1
% increase	5 ±5	2 ±2	5 ±5	3 ±2	2 ±1	0

(*n*) = Number of animals in each group.

Values for each group represent the means ±SEM of triplicate determinations of the pooled distal or proximal pad segments of animals. Significance levels were determined from *F* ratios calculated by one way analysis of variance.

Significant differences: $P < 0.05$, cells and ghosts, per cell and per millimicrogram of protein.–Basal CO_2: none; Basal TG: group 12, proximal vs. distal; group 12, proximal vs. proximal of groups 10, 11 cells only; Insulin CO_2: proximal vs. distal within each group except "ghosts" of group 12, proximal vs. proximal and distal vs. distal between all groups except "ghosts" of group 12; Insulin TG: proximal vs. distal within group 10, proximal vs. distal within group 11 (intact cells only), distal vs. distal between each group and proximal vs. proximal between groups 10, 11.

TABLE VII

Glucose 1 ^{14}C Incorporation into CO_2 and Triglyceride by Adipose Cells of Rats Fed Restricted Diets

Age	Number of animals	CO_2			Triglyceride		
		Basal	Insulin	Increase	Basal	Insulin	Increase
				%			%
Experimental group 13 *a*							
16 wk	3	14 ±2	17 ±2	22 ±7	23 ±3	25 ±1	9 ±4
21 wk	6	3 ±1	2 ±1	2 ±1	3 ±2	4 ±1	0
Experimental group 13 *b*							
16 wk	3	14 ±1	16 ±1	14 ±5	25 ±1	25 ±1	0
22 wk	6	27 ±3	93 ±3	244 ±29	35 ±1	60 ±3	71 ±2
Experimental group 13 *c*							
16 wk	3	16 ±2	17 ±1	10 ±3	25 ±3	26 ±2	2 ±1
22 wk	6	6 ±1	8 ±1	5 ±2	9 ±3	9 ±1	0
Control group 14							
16 wk	3	16 ±2	19 ±1	19 ±5	23 ±2	23 ±1	3 ±1
21 wk	3	14 ±3	14 ±3	2 ±1	34 ±1	32 ±2	0
22 wk	3	14 ±1	15 ±2	4 ±2	37 ±2	37 ±1	0

Values for each subgroup represent the means ±SEM of the means of triplicate determinations in number of rats and are expressed as mg glucose × 10^{-6}/cell per hr.

Significance levels were determined from *F* ratios calculated by a one way analysis of variance. $P < 0.05$: Control vs all experimental groups at age 21 or 22 weeks for, basal CO_2, triglyceride, insulin stimulated CO_2 and triglyceride and per cent increase. $P > 0.05$: Control vs. all experimental groups at age 16 wk for all parameters.

by the addition of insulin to the medium (Tables V and VI). The ability of insulin to stimulate glucose-1-^{14}C incorporation into CO_2 and triglyceride in "fat cell ghosts" is approximately one thousandth of that observed in intact cells, with greater reduction in the smaller cells. In spite of the diminished insulin effect, insulin significantly enhanced glucose metabolism in the "fat cell ghosts" derived from groups of animals containing small cells (groups 1, 2, and 3). The "ghosts" derived from animals containing larger fat cells were not influenced by insulin in terms of the parameters measured. Thus, the larger the fat cell from which it is derived the less insulin sensitive the "ghosts" irrespective of whether insulin sensitivity is expressed as the absolute rate of glucose metabolism or on a per cent increase basis.

The same relationships were observed when "ghosts" were prepared from small and large adipose cells of different sizes obtained from different portions of the epididymal fat pads of animals of similar body weights (Table VI). The larger the intact fat cells, the less effect insulin had upon the glucose metabolism of the ghosts.

DISCUSSION

Previous studies have indicated that the insulin sensitivity of adipose tissue from obese humans is impaired (5, 8). With increased weight and age, rats have shown a diminished responsiveness of their adipose tissues to the effect of insulin upon glucose oxidation and lipogenesis (9–11), fatty acid esterification, and lipoprotein lipase activity (20). Insulin resistance has also been demonstrated in the adipose tissue of experimentally obese mice (12). The nature of the insulin resistance of this tissue in obesity is not known.

One possibility is that the insulin sensitivity of the adipose tissue is related to its cellular character. This was suggested by the observation that diminished insulin responsiveness of human adipose tissue was associated with the presence of enlarged fat cells, an abnormality reversible by weight loss and reduction of fat cell size (5). Recently, Smith has demonstrated a similar reduction in the ability of insulin to stimulate glucose oxidation and lipogenesis in enlarged human fat cells (21). In the current study of adipose tissue from rats fed *ad libitum* and allowed to grow normally, insulin sensitivity appears to be related to the size of the fat cells in the tissue—the larger its fat cells, the less insulin sensitive the adipose tissue. When the large fat cells of these animals are reduced by weight loss, their insulin responsiveness increases after recovery from starvation (Group 13, subgroup *b*). These studies confirm the earlier observations in *ad libitum*-fed humans in which the cellular character of the adipose tissue was shown to have an important bearing upon its metabolic potential. However, the mechanism by which the

enlarged fat cell becomes insulin resistant remains unknown.

The observation that the protein content per cell remains relatively constant over a wide range of fat cell size and that the lipid-protein ratio increases with increasing cell size suggests that diminished insulin sensitivity is unlikely to be due to loss of cellular protein at the expense of added fat. In this regard, it is of interest that the smallest adipose cells from the youngest animals contain significantly less protein per cell than the larger cells but are the most insulin sensitive. These data may mitigate against the concept that the insulin sensitivity of fat cells of different sizes depends upon differences in protein content per cell. However, qualitative changes in cellular protein may exist in fat cells of different sizes and may play a role in varying insulin sensitivity. Nevertheless, these studies indicate a relative constancy of protein content per cell. The reduced protein content of the smallest fat cells from the youngest animals may be a reflection of the continued development of their adipose depots, since cellular multiplication is still occurring (22). An incidental finding of these studies is that adipose cells retain a significant amount of collagenase protein when prepared by the method of Rodbell, even after repeated washings. Thus, in order to determine accurately the protein content of cells, correction for adherent collagenase protein is necessary.

The observation that insulin sensitivity of adipose tissue from *ad libitum*-fed rats is inversely related to the size of its constituent fat cells, regardless of whether sensitivity is expressed as the per cent increase above the basal level or in terms of the absolute rate of glucose metabolism, establishes that differences in insulin sensitivity between cells of different sizes are not a function of differences in basal levels. Similar data have now been derived from studies in human adipose tissue (8).

The current study also indicates that diminished insulin responsiveness of enlarged fat cells is not a function of the age of the animal from which the cells were derived. Adipose tissue from rats of the same age and body weight showed widely different sensitivity to insulin, depending upon the size of its constituent fat cells. Although it is possible that the smaller insulin-sensitive cells represent the "younger" more actively metabolizing component of the tissue, the fact that basal glucose metabolism is similar in large and small cells may mitigate against this concept.

Another possible explanation for the insulin resistance of the enlarged fat cell is that the intracellular accumulation of a large amount of fat may distort the structural and functional integrity of the cell membrane or subcellular material such that the ability of insulin to initiate and propagate its normal sequence of events is impaired. In this study we have attempted to evaluate this possibility by comparing the insulin sensitivity of intact fat cells of widely different sizes, and their delipidated derivatives prepared by the method of Rodbell. These cells were essentially devoid of lipid (20 ± 3 μg of triglyceride/mg of ghost protein) and there were no observable differences in the lipid content of "ghosts" derived from small and large cells. There is a profound reduction in the metabolic capacity and insulin sensitivity of these delipidated cells as compared to intact cells, and thus, it is clear that the physiologic significance of these studies may be extremely limited. Nevertheless, the relationship between the size of the original cell and the basal metabolism and insulin responsiveness in the ghosts is qualitatively similar to that observed in the intact cells. As in the intact cells, the basal rate of glucose oxidation and lipogenesis in fat cell ghosts is similar, irrespective of the size of the cells from which they were derived. Like the whole cells from which they were derived, the insulin sensitivity of fat cell ghosts progressively decreased as the size of the original cell increased; thus, acute in vitro removal of the large central fat globule did not in itself appear to reverse the defect of insulin insensitivity of the enlarged cell. Furthermore, the observation that the preparative procedure reduces the insulin sensitivity of ghosts derived from small cells to a greater degree than those derived from large cells may suggest that the difference in insulin sensitivity between ghosts may be more marked than the data indicate. Although the lipid content of fat cell ghosts appeared to be similar, irrespective of the size of the cell from which it was derived, it remains possible that ghosts prepared from large cells retain slightly more lipid than those from small cells, a difference our methods were unable to detect. If this were the case, apparent differences in insulin responsiveness might still reflect differences in lipid content.

Since fat cell ghosts retain such a small fraction of their original activity, these data cannot be used as the basis for quantitative interpretation. Their possible significance and meaning in terms of adipose cellular insulin resistance can only be speculated upon at this time. One possibility suggested by these data is that the factor(s) mediating differences in insulin sensitivity between fat cells of different sizes is retained in the ghosts derived from these cells. Whether this is most compatible with the concept that the diminished insulin sensitivity of the enlarged cell is due to interference with the normal action of the hormone at the cell membrane or due to some intracellular alteration cannot be answered by this study. Preparation of "ghosts" which retain a more significant fraction of the original cell activity than so

far possible would permit a more detailed examination of these and other factors.

The studies in *ad libitum*-fed animals support the concept that the insulin sensitivity of adipose tissue is influenced by the size of its constituent fat cells. However, the studies in rats fed restricted diets, as well as those of Huber, Gershoff, and Antoinades (23), Bray, Mothon, and Cohen (24) and Di Girolomo and Rudman (9) clearly indicate that under the appropriate conditions, the insulin sensitivity of adipose tissue can be influenced by factors other than the size of its constituent fat cells. These studies establish that the insulin sensitivity of adipose cells of similar size can vary widely depending upon the state of nutrition and state of growth of the animal. The fat cells of rats who were reduced but not refed (subgroup 13*a*) were no more insulin sensitive than before weight reduction, even though these cells were of smaller size. Refeeding increased the insulin sensitivity of the adipose tissue of the reduced animals (subgroup 13 *b*) to levels equal to (when expressed as per cent increase) or greater than (when expressed in absolute rates) that observed in *ad libitum*-fed rats with smaller cells (Group I, II, Table V). Finally, rats fed sufficient calories to maintain constant body weight but not enough to grow, had small, but relatively insulin-resistant adipocytes (subgroup 13 *c*). Thus, factors other than the absolute size of the fat cells can be important in determining adipose tissue sensitivity to insulin. Di Girolomo and Rudman, in studies of fasted and refed rats (9), found that the insulin responsiveness of rat adipose tissue could be correlated with the rate at which it was metabolizing glucose to glyceride glycerol and fatty acid: increased conversion to glycerol and decreased conversion to fatty acid were associated with insulin resistance. From these data they inferred an increased rate of cellular lipolysis and postulated that this might lead to insulin resistance. Theoretically, this possibility receives some support from the recent observations of Nestel and Whyte indicating that the rate of lipolysis is increased in the obese (25) and of Cushman and Rizack who have reported that intracellular free fatty acid influences the metabolism of adipose cells (26). However, the studies of Di Girolomo and Rudman do not exclude the possibility that these metabolic alterations in glucose metabolism are the result of adipose cellular insulin resistance rather than the cause, and that loss of insulin sensitivity may be due to some other cellular derangement. Furthermore, although these studies indicate that profound changes in nutrition and growth influence the insulin sensitivity of the animal regardless of fat cell size, they do not negate the relationship of adipose cell insulin sensitivity and adipose cell size under conditions of normal intake and growth. In animals fed *ad libitum* and allowed to grow normally, adipose tissue insulin responsiveness appears to be related to fat cell size. This is further illustrated by some recent human studies indicating that the carbohydrate content of isocaloric diets significantly inffuences the insulin sensitivity of isolated adipose tissue (8). Although high carbohydrate intake increases the insulin responsiveness of enlarged adipose cells, the level of response remains below that observed when an identical diet is ingested by nonobese controls with smaller cells. In this respect, the recent speculation by Grey, Goldring, and Kipnis (27) that insulin resistance in obesity represents an adaptation to hyperinsulinemia induced by high carbohydrate intake, may be of interest. These studies suggest that it may be important to distinguish between the enlarging adipocyte and the enlarged fat cell; between the state of becoming obese from that of being obese.

All of these studies clearly indicate that a variety of factors, including cell size, nutrition, and state of growth influence the insulin sensitivity of the adipose tissue. These studies do not, as of this time, allow one to distinguish between which, if any, of the various factors examined are of primary importance in the normal and abnormal state. The mechanism by which these factors interact to determine the insulin sensitivity of adipose tissue and of the whole organism (28) requires continued investigation.

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