The Role of Adipose Cell Size and Adipose Tissue Insulin Sensitivity in the Carbohydrate Intolerance of Human Obesity

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ABSTRACT Glucose metabolism and insulin sensitivity of isolated human adipose tissue was studied as a function of adipose cell size and number. Glucose metabolism by these tissues was closely related to the number of cells in the fragment, irrespective of cell size. Adipose cells of obese individuals metabolized glucose to carbon dioxide and triglyceride at rates similar to adipose cells of nonobese subjects. In contrast, insulin responsiveness of adipose tissue was dependent upon adipose cell size. The larger its adipose cells the less insulin sensitive was the tissue. Thus, adipose tissue of obese subjects, with enlarged cells, showed a diminished response to insulin. After weight loss and reduction in adipose cell size, insulin sensitivity of the adipose tissue of obese patients was restored to normal. When adipose tissue of obese individuals showed impaired responsiveness to insulin, their plasma insulin levels, after oral glucose, were elevated. Weight loss and reduction in adipose cell size restored plasma insulin concentration to normal, concomitant with the return of normal tissue insulin sensitivity.

INTRODUCTION

Glucose intolerance is often observed in obese individuals without clinically manifest diabetes mellitus. Furthermore, excessive increase in plasma insulin after glucose ingestion has been well documented in obese patients in the presence or absence of decreased glucose tolerance (1–3). It has been postulated that "insulin resistance" of the peripheral tissues of the obese subject is responsible for these abnormalities of glucose and insulin metabolism (4–6). Such studies, however, afford little or no information as to which tissues may be "resistant" to the effect of insulin and what the nature of the tissue abnormality may be.

It is tempting to postulate that the characteristic abnormality of the obese individual, the excessive size of the adipose depot, plays an important role in the carbohydrate intolerance of obesity. The observation that weight loss and reduction in the size of this depot can restore glucose tolerance to normal (7) may support this concept. Consequently, the role of adipose tissue, particularly adipose depot size, in the carbohydrate intolerance of obesity was examined.

Techniques are now available for sampling adipose tissue from various depots in man by needle aspiration (8), for in vitro measurement of the metabolic activity of these tissue samples and their sensitivity to insulin, and for determination of adipose cell number and size (9). Development of these methods has made possible a detailed study of human adipose tissue in relation to the metabolic abnormalities described above.

In the present study these techniques have been used to examine glucose metabolism and insulin responsiveness of adipose tissue of obese and nonobese subjects. These studies indicate that the cellularity of the tissue sample is of prime im-

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TABLE I

Body Weight (kg) in Five Obese Patients before and after Weight Reduction

Patient	Sex	Age	Period I	Period III	Net loss
			kg	*kg*	*kg*
F.J.	M	21	138.4	86.9	51.5
M.P.	F	35	162.9	110.0	62.2
L.P.	F	27	147.8	96.8	51.0
F.H.	M	24	185.1	120.8	64.3
C.A.	M	20	186.6	122.6	64.0

Liquid formula diet was fed as described in the text. In Period I, sufficient calories were provided to maintain constant body weight in the obese state. In Period III, sufficient calories were provided to maintain constant body weight at the reduced level.

portance in evaluating the metabolic activity of human adipose tissue. The studies also reveal that the adipose tisssue of markedly obese patients shows a decreased sensitivity to insulin in vitro, an abnormality which is reversible by weight loss and reduction in adipose cell size.

METHODS

Subjects

Nonobese. Seven children[1] without family history of diabetes mellitus, hospitalized for reasons other than metabolic or nutritional disorders, were selected to provide a wide spectrum of age (5–13 yr) and normal body weight (19–49 kg).

12 average weight adults (49–85 kg), ranging in age from 22 to 68 yr, included eight healthy medical student volunteers without family history of diabetes mellitus, on ad libitum diets, and four in-patients at The Rockefeller University Hospital subsisting on formula diets, with normal carbohydrate tolerance and no known abnormality of adipose tissue.

Obese. Five markedly obese patients were hospitalized for 8 months on the metabolic ward of The Rockefeller University Hospital. Pertinent clinical data are given in Table I. These patients were placed on a formula diet consisting of 15% protein, 45% carbohydrate, and 40% fat, with daily supplements of iodized salt, iron, and vitamins, and studied during three dietary periods. During an initial 6 wk period of hospitalization, sufficient calories were provided to maintain constant body weight (Period I). This was followed by a 4 month period of restriction of caloric intake to 600 cal/day and weight reduction (Period II). During this time, the protein content of the formula was increased to 20%. Finally, an 8 wk period of weight maintenance, but at a lower and more nearly normal weight, followed (Period III).

[1] We are indebted to the Department of Pediatrics, New York Hospital, Cornell Medical Center, for providing the opportunity to study these children.

Adipose tissue sampling

Adipose tissue samples were obtained in all patients from the subcutaneous tissue of the buttock by the method of needle aspiration (8). In the obese patients, adipose tissue was studied weekly during each of the three dietary periods. No studies were performed during Period III until 1 wk after the reinstitution of weight maintenance. In four patients, studies were carried out from 6 to 24 months after weight reduction.

Sufficient quantities of tissue were obtained for both the metabolic studies and the determination of the number and size of adipose cells in the tissue. The tissue fragments were immediately placed in bicarbonate buffer, kept at 37°C under 95% O_2: 5% CO_2 in a thermos flask, and subsequently washed with large amounts of warm buffer to remove adherent oil droplets and blood.

Incubation of tissue

Individual fragments of adipose tissue were placed into 30-ml plastic flasks containing 2 ml of Krebs-Ringer bicarbonate buffer, pH 7.4, at 37°C, with or without insulin[2] at 1000 μU/ml, to which were added glucose (1 mg/ml), μU/ml, to which were added glucose (1 mg/ml), gelatin (2 mg/ml), and a tracer amount of glucose-1-^{14}C.[3] The flasks were each capped with a rubber stopper to which was attached a small glass cup which hung suspended in the flask. The tissue was equilibrated with 95% O_2: 5% CO_2 for 5 min and incubated for 4 hr at 37°C, with gentle shaking. Roughly equivalent amounts of tissue fragments were distributed randomly in all flasks, except that very small adipose shreds (less than 1 mg in wet weight) were discarded (10). A total of 10–20 mg of tissue was placed in each flask.

At the end of the incubation period, ½ ml of Hyamine hydroxide was injected through the rubber stopper into the glass cup suspended in the flask, 1 ml of 6 N H_2SO_4 was introduced into the medium, and $^{14}CO_2$ was collected in Hyamine during an additional 1 hr of shaking.[4] The adipose tissue in the flask was thoroughly washed in saline to remove adsorbed isotope, transferred into 20 ml of isopropanol: heptane: 1 N sulfuric acid (4:1:0.1), and the total lipid was extracted overnight (11). After the addition of water and heptane, aliquots were taken from the heptane upper phase for lipid determination by measurement of carboxyl ester bonds (12) and for the determination of lipid ^{14}C content. The $^{14}CO_2$ and lipid ^{14}C derived from carbon-1 of the glucose was counted in a

[2] Glucagon-free beef zinc insulin, courtesy of Dr. R. W. Kirtley, Eli Lilly Laboratories, Indianapolis, Ind.

[3] New England Nuclear Corporation, Boston, Mass.

[4] A variable amount of counts was recovered in Hyamine in those flasks without tissue, probably indicating isotopic impurity. Although ^{14}C recovery in the absence of tissue was small compared to that recovered in the presence of tissue, all counts were corrected for this error.

Packard Liquid Scintillation spectrometer at 85% efficiency in a solution of phosphor (0.4% 2,5-diphenyloxazole, 0.01% 1,4-bis[2-(5-phenyloxazolyl)]benzene) in toluene.

Determination of adipose cell size and number

Fragments of adipose tissue were processed according to the method described by Hirsch and Gallian (9). Tissue shreds were fixed in a solution of osmium tetroxide in collidine buffer at 37°C for 48 hr. During the prolonged incubation, individual intact cells fixed with osmium tetroxide spontaneously and completely fell away from the tissue matrix and were counted in a Coulter Electric Counter. The average amount of lipid per cell (cell size) and the total number of adipose cells in the tissue were determined by this method.

Plasma insulin and blood glucose determination

Plasma immunoreactive insulin was measured by a modification[5] of the method of Herbert et al. (13). Insulin values are the means of triplicate analyses. All triplicate determinations agreed within 10%. These measurements were made before and after weight reduction, during periods of weight maintenance, after the ingestion of oral glucose (1.75 g/kg of lean body mass plus 15%). Lean body mass was estimated from the tritiated water space, as described by Lesser, Kumar, and Steele (14), and 15% was added to approximate normal body fat content. Blood glucose was determined by the glucose oxidase method.

Calculations

Glucose metabolism and insulin effect. Basal metabolic activity of adipose tissue was expressed in two ways. The rate of incorporation of glucose-1-^{14}C into CO_2 and tissue triglyceride (TG) was expressed as the number of micrograms of glucose metabolized per unit of tissue lipid per hour and was calculated as follows:

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

In addition to these calculations, which are based upon the total lipid content of the tissue, all metabolic parameters were expressed on a per cell basis. The activity per cell was calculated as follows: μg glucose incorporated/cell per hr = μg glucose/μg tissue lipid per hr × 1/number of cells per μg of lipid which is the same as 1/(1/average μg lipid per cell). Since the number of counts incorporated per cell was quite small, all data are expressed × 10^7.

[5] Mirsky, I. A. Personal communication.

The effect of insulin upon the rate of glucose-1-^{14}C incorporation into CO_2 was expressed as the percentage increase in the rate of incorporation of glucose carbon into these metabolic parameters and was calculated as follows:

$$\frac{(\mu g \text{ glucose} \rightarrow CO_2) \text{ insulin} - (\mu g \text{ glucose} \rightarrow CO_2) \text{ no insulin}}{(\mu g \text{ glucose} \rightarrow CO_2) \text{ no insulin}} \times 100 = \% \text{ increase.}$$

The same calculations were made for insulin effect upon glucose incorporation into tissue triglyceride.

Statistical analyses

The rate of incorporation of glucose carbon into CO_2 and triglyceride, and the effect of insulin upon these metabolic parameters were calculated as above for each individual subject. For the sake of clarity of presentation, however, individual data were averaged into groups, i.e., children, nonobese adults, and obese adults (Periods I, II, and III). To determine if the treatment of the data in this manner allowed for a fair and meaningful comparison of the metabolism of the adipose tissues of these groups of patients, the following statistical evaluation of the data was performed. The data were normalized by logarithmic transformation, and a two-way analysis of variance was performed (15) in order to define the variability of the metabolic activity of human adipose tissue. Significance testing of adipose tissue metabolism between groups was determined by the *t* test. A power function (16) was developed for the averaged data, for each metabolic variable, and for each patient group.

RESULTS

A. Metabolic activity as a function of the cellularity of the tissue

The rate of incorporation of glucose into carbon dioxide and triglyceride by adipose tissue from individuals with adipose cells of widely different sizes is shown in Figs. 1 and 2, expressed *per unit of tissue lipid* and *per adipose cell.* When the activity was expressed per unit of lipid, the various metabolic functions proceeded at significantly different rates in these tissues. When activity was expressed per cell, these observed differences disappeared, and tissues with cells of widely different sizes metabolized glucose at similar rates per cell.

In contrast, the insulin responsiveness of the tissue was found to be closely related to the size

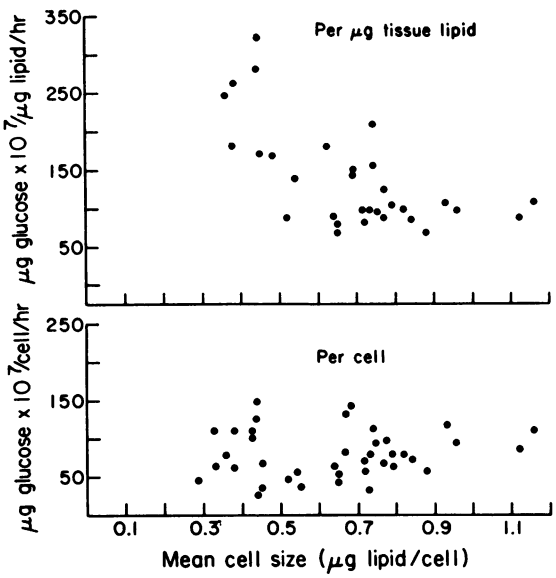

FIGURE 1 Glucose-1-^{14}C oxidation to CO_2 by human adipose tissue. Each point represents the mean of triplicate determinations for a patient.

of the adipose cells in the tissue fragment (Fig. 3). The larger the mean cell size in a tissue sample, the less responsive it was to insulin. Thus, the cellular characteristics of the tissue are of considerable metabolic importance, and all data are expressed on a per cell basis.

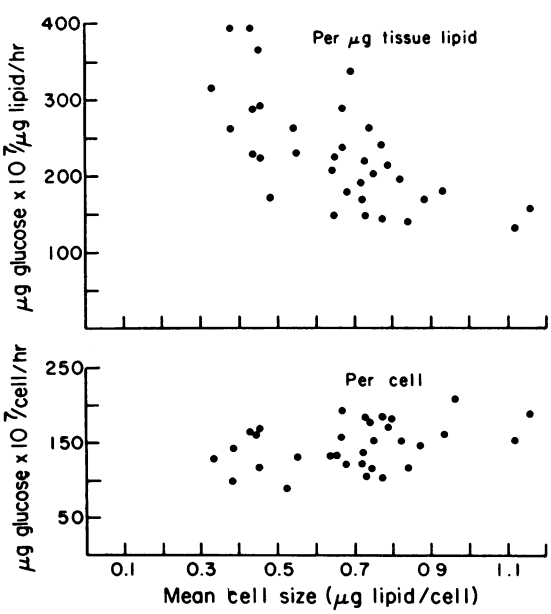

FIGURE 2 Glucose-1-^{14}C incorporation into triglycerides by human adipose tissue. Each point represents the mean of triplicate determinations for a patient.

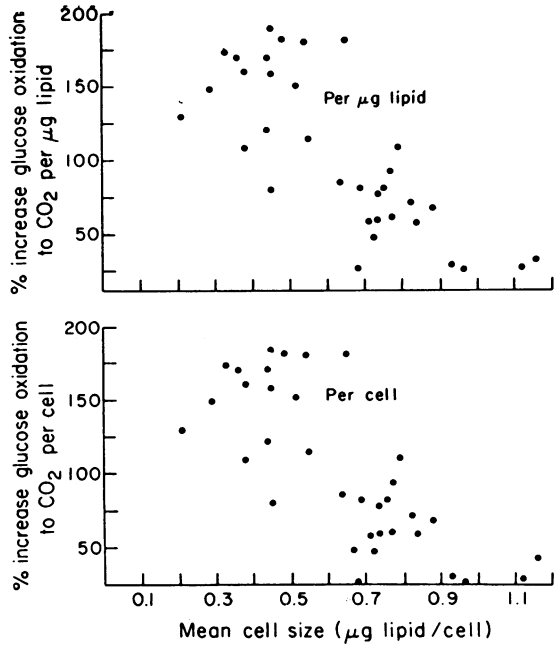

FIGURE 3 Insulin effect upon glucose-1 ^{14}C oxidation to CO_2. Each point represents the mean of triplicate determinations for a patient.

B. Glucose metabolism of adipose cells of nonobese and obese individuals

The mean size (μg lipid/cell) of adipose cells in tissue fragments obtained from children, adults of average body weight, and markedly obese patients differed significantly (Table II). In the obese patients, weight loss was associated with a

TABLE II

Adipose Cell Size of Children, Normal Adults, and Obese Adults before and after Weight Reduction

Patient group	(n)	Mean cell size ± SEM
		μg TG/cell
Children	(7)	0.4280 ± 0.0141*
Normal adults	(12)	0.6653 ± 0.0282‡
Obese adults		
I	(5)	0.9206 ± 0.1099§
III	(5)	0.5180 ± 0.0866

Cell size is based upon at least two determinations for each individual.

Significance levels were calculated by Student's *t* test.

T. G., triglyceride; n, number of subjects.

* $P < 0.05$ for children vs. normal adults and obese adults Periods I and III.

‡ $P < 0.05$ for normal adults vs. obese adults Period I.

§ $P < 0.05$ for obese adult Period I vs. Period III.

significant reduction in cell size, even though some patients remained overweight.

The mean rates of incorporation of carbon from glucose-1-^{14}C into CO_2 and triglyceride by adipose cells of children, adults of average body weight, and obese patients were not significantly different (Table 3).

During weight reduction (Period II) the adipose cells of obese patients showed a decrease in all metabolic parameters, and the rate of oxidation of glucose-1-^{14}C to CO_2 was significantly diminished. Adipose cells of reduced obese patients (Period III) oxidized significantly less glucose to CO_2 (66 ± 12) when compared to prereduction (102 ± 10) and nonobese rates (88 ± 9). However, the rate of incorporation of glucose carbon into triglyceride returned to normal after weight reduction (166 ± 13 vs. 155 ± 15).

In four patients, adipose tissue was obtained for metabolic studies 6–24 months after cessation of weight reduction (Table III). Weight had been maintained within 5 kg, and cell size remained reduced in all patients. Glucose-1-^{14}C oxidation to CO_2 continued to be below prereduction levels.

TABLE III

Incorporation of Glucose-1-^{14}C into CO_2 and Triglyceride by Adipose Tissue of Nonobese Subjects and Obese Patients before and after Weight Reduction

Patient group	(n)	Glucose oxidation to CO_2	Glucose incorporation into triglyceride
		μg glucose ×10⁷/cell per hr	*μg glucose ×10⁷/cell per hr*
Children	(7)	97 ± 12	143 ± 11
Nonobese adults	(12)	88 ± 9	145 ± 10
Obese adults			
I	(5)	102 ± 10	166 ± 13
II	(2)	62 ± 4	128 ± 8
III	(5)	66 ± 12	155 ± 15
6–24 months postreduction	(4)	60 ± 11	138 ± 10

All values represent the mean ± SEM of the means of triplicate determinations for individuals within that group. Significance levels were calculated by Student's *t* test.

Significant *P* values ($P < 0.05$): CO_2, children and nonobese vs. obese II and III, and 6–24 months postreduction. Obese I vs. obese II and III and 6–24 months postreduction. T.G., children and nonobese vs. obese II.

No statistically significant differences between other groups ($P > 0.05$).

TABLE IV

Variability of Adipose Tissue Metabolism

Source of variance	Log variance: Nonobese subjects	Obese subjects (Periods) I	II	III
	Glucose-1-^{14}C→CO_2; no insulin			
Within patients (triplicates)*	0.0084	0.0054	0.0154	0.0111
Between patients‡	0.0702	0.0743	0.0961	0.0806
Across weeks, same patient§	0.0309	0.0727	0.1727	0.1475
	Glucose-1-^{14}C→CO_2; insulin			
Within patients (triplicates)	0.0077	0.0060	0.0045	0.0091
Between patients	0.0573	0.0820	0.0747	0.0953
Across weeks, same patient	0.0267	0.0668	0.1305	0.0606
	Glucose-1-^{14}C→TG; no insulin			
Within patients (triplicates)	0.0026	0.0029	0.0029	0.0032
Between patients	0.0023	0.0401	0.0318	0.0384
Across weeks, same patient	0.0188	0.0582	0.0543	0.0390
	Glucose-1-^{14}C→TG; insulin			
Within patients (triplicates)	0.0022	0.0022	0.0015	0.0010
Between patients	0.0414	0.0297	0.0293	0.0438
Across weeks, same patient	0.0102	0.0256	0.0555	0.0195

All data were analyzed by two-way analysis of variance and are expressed as the logarithm of the variance of the metabolism of the tissue for each group.

* Intraindividual variance (variance within individuals of triplicate determinations).

‡ Interindividual variance (variance between individuals in a group).

§ Variance from week to week in the same individual.

Incorporation of glucose into triglyceride, however, returned to prereduction levels.

Considerable variability was found in the rate of glucose oxidation to CO_2 and incorporation into tissue lipid by the adipose tissue of these subjects. The results of the analysis of variance, shown in Table IV and Fig. 4, indicate that:

(*a*) There was greater variability in the rate of glucose oxidation to CO_2 than in that of incorporation of glucose into lipid by the adipose tissue of all groups.

(*b*) Triplicate variability for each patient was similar in the adipose tissue of all groups.

(*c*) Variability between patients in a group was of the same order in the obese patients (Period I) as in the nonobese subjects. Reduced obese subjects (Period III), however, showed increased variability, both in glucose oxidation and incorporation into tissue lipid.

(*d*) The variability of triplicate determinations for each patient in a group was in every case smaller than the variance between patients in that group.

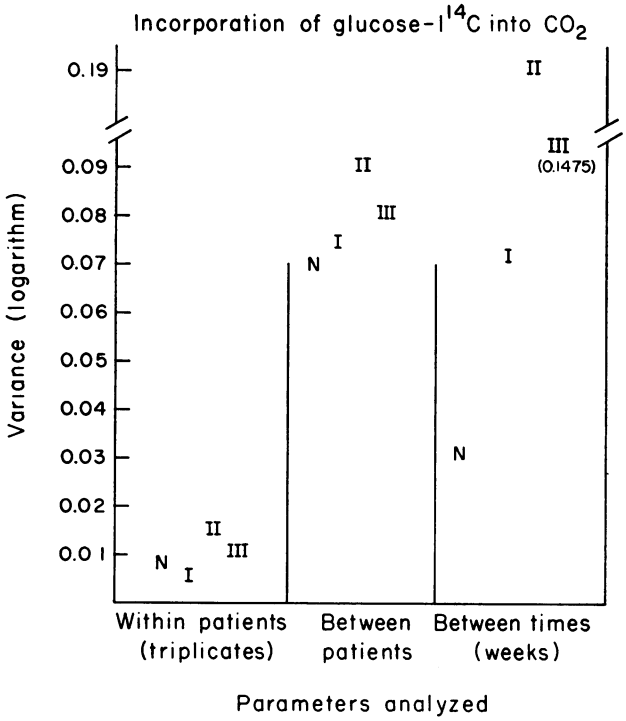

FIGURE 4 Variability of human adipose tissue metabolism. Incorporation of glucose-1-^{14}C into CO_2. *N*, normal weight subjects; I, II, III, obese subjects during dietary periods. Each point represents the mean of individuals within the respective group.

(*e*) The adipose tissue of nonobese individuals showed less week-to-week variability in glucose metabolism than did tissue of the obese subjects. During and after weight reduction, week-to-week variability within individual obese patients increased and became greater than the variability between these patients.

Since considerable variability was present between patients in each group, it was necessary to determine the sensitivity of this system to detect differences in adipose tissue metabolism between patient groups. A power function (16) was developed for the averaged data, for each metabolic variable, and for each patient group. Operating at the 95% confidence level and using the *t* test, there was a greater than 95% probability of detecting a difference of 20% or more in the mean rate of glucose carbon incorporation into tissue triglyceride, and a 95% probability of detecting at least a 40% difference in the rate of glucose oxidation to CO_2 by adipose tissue of obese (Periods I, II, III) vs. nonobese subjects.

On the basis of these analyses, the data are expressed as the means of individual observations for subjects within each group or period. Within the limits defined above, no significant differences were found in the rates of glucose metabolism by adipose cells of children, nonobese adults, and the obese subjects.

C. Insulin sensitivity of adipose tissue of normal and obese patients

When adipose tissue samples from nonobese and obese subjects were exposed to insulin, in vitro, there was a significant increase in the rate of glucose-1-^{14}C oxidation to CO_2 when compared to control tissue (Table V). A statistically significant stimulatory effect of insulin upon incorporation of glucose into tissue triglyceride was not consistently found.

The adipose tissue of children showed a 151% increase in the rate of glucose oxidation when insulin was added to the medium, compared to the tissue of nonobese adults and obese patients, which showed only 113% and 50% increases, respectively. During the period of caloric restriction (Period II), the adipose tissue of two obese subjects continued to respond to insulin as in Period I, with a 42% increase in CO_2 production.

After weight loss and during Period III, when adipose cell size was reduced and body weight was maintained at the reduced level, the adipose tissue

TABLE V

Effect of Insulin upon Glucose-1-^{14}C Incorporation into CO_2 and Triglyceride by Adipose Tissue of Nonobese Subjects and Obese Patients before and after Weight Reduction

Patient group	(n)	Glucose oxidation to CO_2			Glucose incorporation into triglyceride		
		Control	Insulin	% Increase	Control	Insulin	% Increase
		μg glucose ×10³/cell per hr ±SEM			*μg glucose ×10³/cell per hr* ±SEM		
Children	(7)	97 ± 12	239 ± 48	151 ± 19*	138 ± 11	181 ± 15	34 ± 10*
Nonobese adults	(12)	88 ± 9	194 ± 12	113 ± 16*	145 ± 10	153 ± 15	9 ± 3
Obese adults							
I	(5)	102 ± 10	144 ± 14	50 ± 8*	162 ± 15	183 ± 13	20 ± 7
II	(2)	62 ± 4	84 ± 8	42 ± 2*	128 ± 8	165 ± 5	30 ± 4*
III	(5)	66 ± 12	148 ± 27	152 ± 13*	155 ± 15	172 ± 15	15 ± 4
6–24 months postreduction	(4)	60 ± 11	141 ± 25	164 ± 29*	138 ± 10	157 ± 9	17 ± 11

All values represent the mean ± SEM of the means of triplicate determinations for individuals within that group. Significance levels were calculated by Student's *t* test.

Significant *P* values ($P < 0.05$): CO_2, children and nonobese adults vs. obese I and II; obese I and II vs. obese III; obese III vs. nonobese adults.

No statistically significant differences between other groups for glucose oxidation ($P > 0.05$).

* Indicates a statistically significant stimulatory effect of insulin ($P < 0.05$).

of these individuals was significantly more responsive to insulin than it was during the markedly obese period (I = 50%; III = 152%), and was similar to the 151% response of adipose tissue of normal children and the 113% response of the adipose tissue of nonobese adults.

The insulin response of adipose tissue from four patients studied 6–24 months after weight reduction remained in the same range as that observed during Period III and that of nonobese subjects (Table V).

D. Insulin effect on free cells

These studies of insulin effect upon human adipose tissue were performed upon isolated tissue fragments. Since adipose tissue in the organism is supplied with a rich vascular network through which insulin is delivered to its cells, it is conceiv-

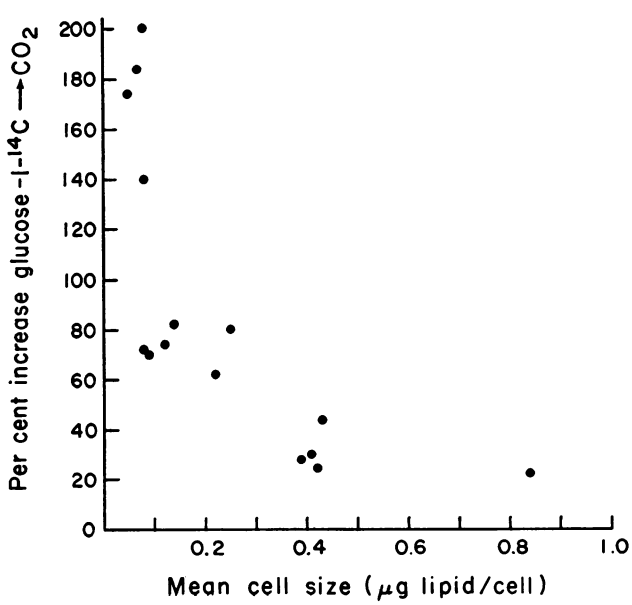

FIGURE 5 Insulin effect upon rat free adipose cells. Glucose-1-^{14}C incorporation into CO_2. Each point represents the mean of triplicate determinations for an animal.

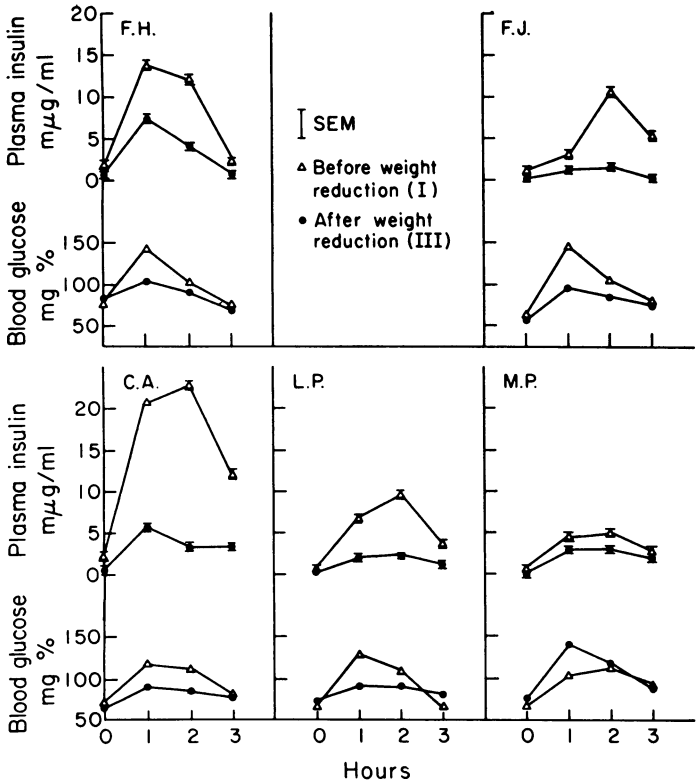

FIGURE 6 Glucose tolerance and plasma insulin response to oral glucose of obese subjects before and after weight reduction. 1 mμg insulin = 25 μU.

able that, in the in vitro system used in this study, the adipose cells of the tissue fragment were inadequately exposed to the hormone. Poor diffusion of the insulin molecule to adipose cells in the center of the tissue fragment may occur. Moreover, the differences in the insulin sensitivity of these adipose tissues could be a reflection of the relatively fewer number of adipose cells at the surface of tissue with large cells compared to the surface of the tissue with small cells. Studies utilizing free adipose cells prepared from epididymal fat pads of Sprague-Dawley rats by the method of Rodbell (17), however, indicated that this was not the case. Rat adipose tissue rather than human adipose tissue was used because, in our hands, collagenase produced a large amount of rupture of human adipose cells. Rats were selected so that adipose cells of widely different sizes were obtained. The isolated cells were incubated under the identical conditions used for intact human tissue shreds, except that 5% albumin was substituted for gelatin. As indicated in Fig. 5, there is an inverse relationship between cell size and insulin responsiveness in the free cell preparations, identical with that found in intact human tissue shreds; i.e., the larger the adipose cell, the less sensitive it is to the effect of insulin.

E. Blood glucose and plasma insulin

Blood glucose and plasma immunoreactive insulin levels of the five obese patients in response to oral glucose are shown in Fig. 6. Glucose tolerance to a glucose load based on lean body mass plus 15% was not impaired in these obese patients, according to the criteria of Conn and Fajans (18). Lower blood glucose concentrations, however, were noted after weight reduction.

In each obese patient, the plasma insulin response to glucose was greater than normal. After weight reduction, at each hour after glucose administration, the levels of insulin in all patients were significantly lower than the prereduction levels ($P < 0.01$). Fasting levels of insulin were significantly increased in only two of the five obese patients, when compared with nonobese individuals; furthermore, there was no significant change in the fasting plasma insulin concentrations of the obese patients as a group after weight reduction.

The plasma insulin response to oral glucose in

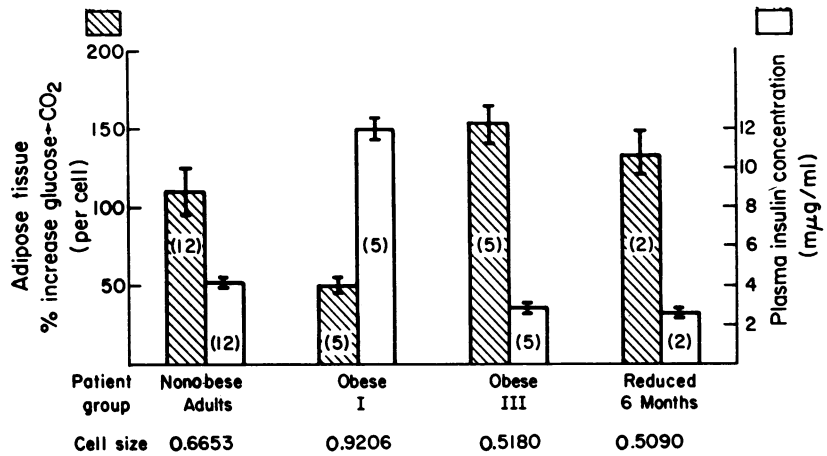

FIGURE 7 Relationship of adipose tissue insulin response and plasma insulin response to oral glucose. The plasma insulin levels are those measured 2 hr after glucose ingestion. The tissue effect of insulin was measured in adipose tissue obtained from patients in the fasting state. Data represent means of (n) individuals in each patient group. 1 mμg insulin = 25 μU.

nonobese individuals and the five obese patients, before and after weight loss, is shown in relation to the insulin responsiveness of the adipose tissue of these subjects in Fig. 7. A significant negative correlation ($r = -0.60$) between the sensitivity of adipose tissue to insulin, in vitro, and the level of plasma insulin was found. When the adipose tissue of the obese patients showed a diminished response to insulin, in vitro, plasma insulin response to glucose was three times greater than that of nonobese individuals. When the obese patients lost weight and their cell size was reduced, plasma insulin levels returned to "normal" at the same time that adipose tissue became normally sensitive to insulin, in vitro. Similar data for two reduced obese subjects studied 6 months after weight reduction are also shown in this figure.

DISCUSSION

The present investigation has made use of several recently developed techniques to examine some specific aspects of glucose metabolism and insulin sensitivity of adipose tissue in obese and nonobese subjects. By examining tissue from children, nonobese adults, and obese individuals before and after weight loss, these methods provided an opportunity to study human adipose tissue metabolism as a function of adipose cell size and number. Furthermore, in vitro studies of glucose metabolism and insulin sensitivity of human adiopse tissue were correlated with in vivo measurement of glucose tolerance and plasma insulin levels.

Considerable variability in the metabolic activity of human adipose tissue was found. In spite of the variability between patients, the in vitro methods employed in this study were sufficiently sensitive to detect small differences in glucose metabolism by adipose tissue of nonobese and obese individuals. There was no greater than a 20% difference in the mean rate of glucose carbon incorporation into tissue triglyceride and 40% in the mean rate of glucose oxidation to CO_2 in the tissues of these patients. A smaller difference in these metabolic functions between adipose tissue of nonobese and obese subjects could not have been detected by these methods. Within these limits of sensitivity, the methods described above led to the following general observations.

Glucose metabolism by human adipose tissue fragments was found to be dependent upon the number of cells in the tissue. When expressed in the customary manner, i.e. μg glucose incorporated per unit of tissue lipid, adipose tissue of obese patients, with enlarged cells, appeared to be least metabolically active. However, on a per cell basis, the adipose cells of these obese individuals (0.9206 μg TG/cell) performed these metabolic functions at rates similar to cells of children (0.4280 μg TG/cell) and of adults of average body weight (0.6653 μg TG/cell). Thus, calculation of tissue glucose

metabolism per unit of tissue lipid or per unit of tissue wet weight may result in misleading information about the metabolic potential of the tissue. On the basis of this observation, it appears that studies in which differences in the metabolic activity of the adipose tissue of human subjects (19, 20) or animals (21–23) have been reported could profitably be reexamined in terms of the cellularity of the tissue.

In contrast to noninsulin-stimulated glucose metabolism which is dependent upon the number of adipose cells in the tissue, adipose sensitivity to insulin, as measured by its effect upon the rate of glucose oxidation to CO_2, was closely related to the size of the adipose cells in the tissue sample. The small adipose cells of children were the most responsive to insulin, while the enlarged cells of obese patients showed a diminished insulin response compared to nonobese adults and children. Moreover, after weight loss and consequent reduction in adipose cell size (0.5180 μg TG/cell), the adipose tissue of reduced obese individuals became "normally" sensitive to insulin.

These findings differ from those of other studies in which adipose cell metabolism has been examined. Björntorp (24) has reported that adipose cells of obese nondiabetic subjects incorporated more glucose into CO_2 and lipid than cells of nonobese subjects. Measurements of adipose cellularity were made microscopically upon appropriate intact histologic preparations. Thus, the method for measuring adipose cellularity differs greatly from that used in the present study, and this technical difference may account for the dissimilar results obtained. Supporting this is Björntorp's observation that, per unit of tissue DNA, no differences in glucose metabolism could be found between adipose tissue of nonobese and obese subjects. However, the exceedingly high DNA content of 110×10^{-6} μg/fat cell reported by Björntorp and Martinsson (25) far exceeds the generally accepted value of 7×10^{-6} μg DNA/cell (26), suggesting contamination with stromal DNA. It is, therefore, difficult to evaluate adipose cellular metabolic activity when DNA content is used as an index of the cellularity of the tissue. Björntorp also found no differences in the insulin sensitivity of adipose cells of obese and nonobese individuals. Tissue fragments were incubated in the presence of 10,000 μU of insulin/ml, a level which may be sufficient to overcome cellular "resistance" and thus prevent the demonstration of differences in tissue sensitivity apparent when smaller amounts of insulin are used. It is likely, therefore, that the discrepancy in the results of Björntorp and those of the present study reflect differences in methodology.

Tucker and coworkers (27) reported that the effect of insulin upon the glucose uptake of parametrial adipose tissue of rats made obese by lesions of the ventro-medial hypothalamic nuclei was similar to that of adipose tissue from control rats, when activity was expressed per unit of tissue DNA or nitrogen. However, these studies were performed upon tissue from animals fasted for 20 hr, a manipulation known to decrease the insulin response of adipose tissue in vitro. Indeed, a relatively small insulin effect upon glucose uptake in all animals was reported by Tucker, suggesting that the ability of these adipose tissues to respond to insulin was blunted by starvation. Thus, comparison of tissue responsiveness to the hormone may be less meaningful.

The demonstration of an impairment in the action of insulin upon the glucose metabolism of adipose tissue of obese subjects provides direct evidence of insulin "resistance" in a specific tissue. The mechanisms responsible for diminished insulin sensitivity of this tissue in obese subjects are not known. Interference with the action of insulin upon glucose metabolism in adipose tissue could result from the presence of circulating antagonists to insulin (28), or from an abnormal tissue responsiveness to the form in which insulin circulates (29–31). However, these do not explain the observations of the present study. The synalbumin antagonist does not interfere with the action of insulin upon adipose tissue (32, 33) and it has been postulated that adipose tissue dissociates inactive "complexed" insulin to active "free" insulin. Furthermore, in the present investigation, adipose tissue was isolated from the organism and its circulation. It remains possible, however, that some other insulin "antagonist" was bound to the cell and not removed by repeated washing. It seems more probable that the impaired response of the adipose tissue of obese patients to insulin was related to a primary tissue defect (3, 34, 35). With weight loss and consequent reduction of adipose cell size, tissue sensitivity returned to "normal."

This observation may provide a clue into the nature of the tissue defect, i.e., the size of the adipose cell may be involved in this phenomenon. It is of interest that Di Girolamo and Rudman (36) have recently demonstrated that the adipose tissue of large, old rats became more sensitive to insulin, in vitro, after weight reduction, suggesting that similar relationships may be operating in adipose tissue of the rat subjected to weight reduction. The observation that the larger the adipose cell the less responsive the tissue to insulin may indicate that the amount of lipid within the cell may influence its metabolic efficiency and its responsiveness to insulin. "Stuffing" the cell with lipid may cause distortion of the cell membrane, resulting in a change in some receptor site(s) with which insulin interacts, or a change in some membrane constituent, such as adenyl cyclase, which may be involved in the mediation of the intracellular actions of insulin (37). On the other hand, the rate of insulin degradation by the tissue could be excessive because of the presence of an enzyme system in the enlarged adipose cell. Rudman and coworkers (38), working with rat adipose tissue, have reported the presence of an insoluble enzyme system, characterized as a dipeptidase, which degrades insulin. The large amount of insulin present in the incubation media would seem to exclude this possibility in the present studies.

The findings that the state of insulin responsiveness of adipose tissue observed in vitro is reflected in the plasma insulin response to glucose observed in vivo suggests that this tissue may play a role in the abnormalities of glucose and insulin metabolism of obesity. When the adipose tissue of obese patients showed a diminished response to insulin, in vitro, plasma insulin response to oral glucose was excessive. Furthermore, when these obese patients lost weight and their cell size was reduced, plasma insulin levels returned to normal as adipose tissue insulin sensitivity was restored. Thus, it is possible that in these obese subjects, in response to a glucose load, an increased amount of insulin is required to overcome the "resistance" to insulin-stimulated glucose metabolism in their adipose tissue. When adipose tissue becomes "normally" sensitive to insulin, the need for increased levels of the hormone is eliminated. The mechanism by which resistance of the adipose tissue to the action of insulin leads to impairment of glucose tolerance and excessive plasma insulin response to glucose can only be speculated upon at this time. The relationship between tissue sensitivity to the hormone and its concentration in the plasma suggests that some feedback mechanism between the adipose tissue and the pancreas may exist. The factors operating in such a system are unknown. For blood glucose to play such a role, it would be necessary to postulate that the relatively small changes in blood glucose concentration resulting from weight reduction were responsible for the large changes in insulin secretion. Thus, other factors may have been important in the regulation of insulin secretion in these patients. It is also possible that the elevated levels of plasma insulin are the cause rather than the result of the "insulin resistance" of the adipose cell. Under the influence of prolonged, high concentrations of insulin, fatty acid esterification and triglyceride deposition in the adipose cell may proceed at an increased rate, resulting in enlarged, fat-filled adipose cells with diminished sensitivity to insulin. Thus, the insulin resistance of the adipose tissue may be a secondary phenomenon and not causal in the plasma insulin abnormalities of these individuals. That the adipose tissue is a primary factor in these abnormalities, however, is supported by the observation that plasma insulin concentration returns to normal after weight loss and reduction in adipose depot and cell size.

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