

Glucose Metabolism and the Response to Insulin by Human Adipose Tissue in Spontaneous and Experimental Obesity

EFFECTS OF DIETARY COMPOSITION AND ADIPOSE CELL SIZE

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ABSTRACT [1-¹⁴C]glucose oxidation to CO₂ and conversion into glyceride by adipose tissue from nonobese and obese subjects has been studied in vitro in the presence of varying medium glucose and insulin concentrations as functions of adipose cell size, the composition of the diet, and antecedent weight gain or loss.

Increasing medium glucose concentrations enhance the incorporation of glucose carbons by human adipose tissue into CO₂ and glyceride-glycerol. Insulin further stimulates the conversion of glucose carbons into CO₂, but not into glyceride-glycerol. Incorporation of [1-¹⁴C]-glucose into glyceride-fatty acids by these tissues could not be demonstrated under any of the conditions tested.

Both adipose cell size and dietary composition influence the in vitro metabolism of glucose in, and the response to insulin by, human adipose tissue. During periods of ingestion of weight-maintenance isocaloric diets of similar carbohydrate, fat, and protein composition, increasing adipose cell size is associated with (a) un-

changing rates of glucose oxidation and increasing rates of glucose carbon incorporation into glyceride-glycerol in the absence of insulin, but (b) decreasing stimulation of glucose oxidation by insulin. On the other hand, when cell size is kept constant, increasing dietary carbohydrate intake is associated with an increased basal rate of glucose metabolism and response to insulin by both small and large adipose cells. Thus, the rate of glucose oxidation and the magnitude of the insulin response of large adipose cells from individuals ingesting a high carbohydrate diet may be similar to or greater than that in smaller cells from individuals ingesting an isocaloric lower carbohydrate diet.

The alterations in basal glucose metabolism and insulin response observed in adipose tissue from patients with spontaneous obesity are reproduced by weight gain induced experimentally in nonobese volunteers; these metabolic changes are reversible with weight loss. The relationships among adipose cell size, dietary composition, and the metabolism of adipose tissue are similar in spontaneous and in experimental obesity.

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INTRODUCTION

Recent investigations have focused considerable attention on the relationships among glucose intolerance, insulin resistance, and the expanded adipose tissue mass in obesity. An impairment in the ability of insulin to

stimulate glucose uptake in the forearm tissues of obese patients provides indirect evidence for the existence of such a relationship (1, 2). Indeed, the *in vitro* stimulation by insulin of glucose oxidation and incorporation of glucose carbon into glyceride has been reported to be diminished in the enlarged adipose cells from obese individuals compared to the smaller adipocytes from nonobese subjects (2-4). However, discrepancies in the results of such *in vitro* studies have been reported: some investigators find no difference between the insulin sensitivity of adipose tissue from obese and from nonobese individuals (5), and others find an increased sensitivity to insulin in adipose from obese patients (6). There is also disparity in the results of studies comparing basal, noninsulin-stimulated, glucose metabolism by adipose tissue from obese and from nonobese individuals: large adipose cells from obese subjects have been reported by some investigators (4,6-9), but not others (3, 10, 11), to oxidize glucose and incorporate [^{14}C]glucose into glyceride-glycerol and fatty acids at an increased rate relative to that observed in small adipocytes from nonobese patients.

While an explanation for these discrepancies remains to be found, recent studies in experimental animals (12) and in man (9) indicate that the state of nutrition, as well as adipose cell size, influences the pattern of basal and insulin-stimulated glucose metabolism in adipose tissue. Induction of positive caloric balance enhances the insulin sensitivity of enlarged adipose cells; negative caloric balance, on the other hand, reduces the response to insulin by small adipose cells. The disparity in the results of the various studies of basal and insulin-stimulated glucose metabolism in human adipose tissue may very well, then, reflect the varied nutritional and dietary state of the patients from whom these tissues were obtained.

The present report documents that the composition of the diet being ingested before the time tissue samples are removed significantly influences both basal glucose metabolism and the response to insulin in human adipose tissue, and that these effects of diet on the metabolism of human adipose tissue can be dissociated from those of adipose cell size.

METHODS

Two groups of subjects were studied: five nonobese male volunteer inmates of the Vermont State Prison without personal or family history of obesity and diabetes mellitus, and six obese patients hospitalized at the Dartmouth-Hitchcock Medical Center. The nonobese volunteers were studied before and after weight gain achieved experimentally through overeating balanced high caloric diets over a 3-4-mo period. The prison conditions under which these individuals participated in this study are described elsewhere (13). The six obese patients were studied before and after weight loss achieved through being fed a 600 cal/day

TABLE I
General Patient Characteristics

Age	Study period	Body weight	Body fat	Body fat	Adipose cell size	Adipose cell number
yr		kg	kg	%	$\mu\text{g lipid/cell}$	$\times 10^6$
Nonobese 23 \pm 2	I	62 \pm 3	10 \pm 1	15 \pm 1	0.37 \pm 0.03	29 \pm 2
	II	73 \pm 5*	17 \pm 3*	23 \pm 2*	0.63 \pm 0.04*	31 \pm 3
Obese 30 \pm 4	I	111 \pm 7	47 \pm 4	43 \pm 4	0.87 \pm 0.06	57 \pm 5
	II	74 \pm 4*	13 \pm 1*	17 \pm 3*	0.35 \pm 0.03*	60 \pm 6

Values represent the means \pm SEM for the nonobese volunteer group before (study period I) and after (study period II) weight gain, and for the obese patient group before (I) and after (II) weight loss.

* $P < 0.05$ for comparisons of I vs. II within each patient group.

formula diet containing 45, 20, and 35% (by calories) dextrose, milk protein, and corn oil respectively. Table I summarizes the pertinent clinical characteristics of each group of subjects before and after weight change.

The experimental protocol used in the study of the two groups is shown in Fig. 1. At each weight level for each patient group, serial metabolic and morphologic studies of samples of adipose tissue were carried out during the 3rd and 6th wk of successive 3-wk periods of weight-maintenance, isocaloric diets differing only in the ratio of carbohydrate to fat. The diet containing the low ratio of carbohydrate to fat (low carbohydrate diet) contained 100 g carbohydrate/ m^2 of body surface area and 25, 60, and 15% (by calories) carbohydrate, fat, and protein, respectively; the diet with the high ratio of carbohydrate to fat (high carbohydrate diet) contained 300 g carbohydrate/ m^2 of body surface area and consisted of 70% carbohydrate, 15% fat, and 15% protein. Commercial frozen meals (Swanson T.V. dinners in uniform lots) were appropriately supplied.

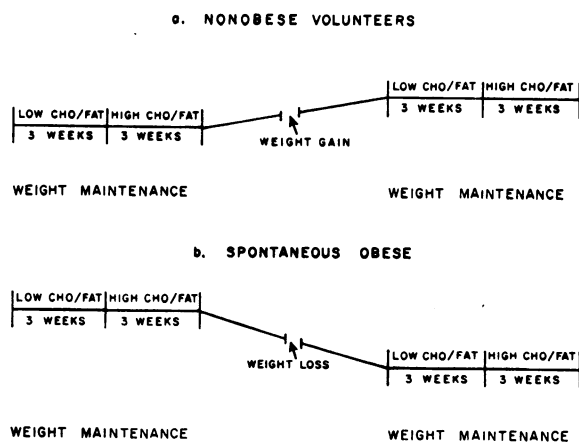


FIGURE 1 Patients in each group were fed weight-maintenance, isocaloric diets differing only in the ratio of carbohydrate to fat, for two periods of 3 wk each. Studies of adipose tissue were undertaken during each dietary period in the nonobese volunteers before and after weight gain (a) and in the obese patients before and after weight loss (b).

mented to achieve the desired caloric intake and dietary composition. Low and high carbohydrate dietary periods were random in order for the nonobese volunteers and consecutive in order for the obese patients. Although the dietary periods were not randomized for the obese patients, the results obtained from studies in the volunteers were, at each level of body weight, independent of the order in which the two diets were ingested.

Samples of subcutaneous adipose tissue were obtained after an overnight fast (10 h) during each study period from each subject either by surgical biopsy through a small incision in the anterior abdominal wall, or by needle aspiration (14) from the same site. Both techniques were performed with 2% Xylocaine local anesthesia (Astra Pharmaceutical Products Inc., Worcester, Mass.). The tissue samples were immediately placed in a 37°C Krebs-Ringer-bicarbonate buffer under a 95% O₂:5% CO₂ gas phase. Individual fragments of tissue were taken for determination of adipose cell size by the osmic acid fixation method described by Hirsch and Gallian (15). Total body fat and adipose cell number in each subject was estimated by the method, described by Salans, Cushman and Weissmann (16), using the mean size of adipose cells obtained from the subcutaneous fat depots of the triceps, gluteal, and anterior abdominal wall regions. In the nonobese volunteers, total body fat was estimated from underwater weighing (17).

Basal glucose metabolism and the response to insulin in these adipose tissue fragments were examined as follows. 30–40 mg of tissue, removed by surgical biopsy, were incubated in plastic vials for 2 h at 37°C in 2.0 ml Krebs-Ringer bicarbonate buffer containing 40 mg/ml untreated bovine serum albumin (Bovine Albumin Powder, Fraction V, Armour Pharmaceutical Company, Chicago, Ill.), and either, (a) 0.2, 0.5, or 5.0 mg glucose/ml in the absence or presence of 400 μ U insulin/ml (courtesy of Dr. W. Kirtley, Eli Lilly and Company, Indianapolis, Ind.) or, (b) 0.5 mg glucose/ml in the presence of 0, 40, 400, or 4,000 μ U insulin/ml. [1-¹⁴C]glucose was added in tracer amounts to achieve constant medium glucose specific activity. Adipose tissue obtained by needle aspiration was incubated only in the presence of 0.5 mg glucose/ml, with or without 400 μ U insulin/ml.

After incubation, the [¹⁴C]glucose carbon incorporated by the adipose tissue fragments into CO₂ was trapped with Hyamine hydroxide by a variation of the technique described by Gliemann (18). The incubated tissue was then thoroughly washed in saline and the total lipid extracted into heptane by the method described by Dole (19). Samples of the heptane phase were analyzed for triglyceride by measuring carboxyl ester bonds (20) and for the quantity of [¹⁴C]glucose carbons incorporated into total lipid. More than 99% of the total lipid of human adipose tissue is triglyceride (14). While no attempt has been made to examine the distribution of [¹⁴C]glucose carbons among the various tissue lipid classes, one study (10) reports that as much as 23% of the label may be found in diglyceride, with 75% found in triglyceride; the present results, therefore, have been expressed as [¹⁴C]-glucose incorporation into total glyceride. ¹⁴CO₂ and [¹⁴C]-glyceride were counted in a Packard Liquid Scintillation Spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill.) at 85% 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 quenching, background, and isotopic degeneration (21). On

occasion, the distribution of [¹⁴C]glucose carbons between the glycerol and fatty acid moieties of the extracted lipid was analyzed by the method described by Rodbell (22) and modified by Cushman and Rizack (23). All results have been expressed as micrograms glucose carbon incorporated into CO₂ and glyceride-glycerol or fatty acid/cell per h, calculated as previously reported (3).

Significance testing was performed by analysis of variance and by the Tukey method of multiple comparisons (24). *P* values of 0.05 or less are considered significant. All statistical analyses were carried out on the Dartmouth Time Sharing System computer facilities.

RESULTS

Table I indicates that adipose cells from the obese patients before weight loss are significantly larger than those from the nonobese volunteers before weight gain. Induction of weight gain in the nonobese volunteers is accompanied by increases in body fat and adipose cell size, but not in total adipose cell number. Weight loss by the obese patients is associated with reductions in body fat and adipose cell size, but again, not in total adipose cell number. The magnitude of the changes in body weight, body fat, and adipose cell size in the obese patients, however, is considerably greater than that in the nonobese volunteers under the conditions of these studies. Changes in the composition of the diet at a given level of body weight, on the other hand, affect neither body fat nor adipose cell size.

Influence of dietary composition and adipose cell size on basal glucose metabolism. Adipose tissue from the nonobese volunteers and obese patients incorporates [1-¹⁴C]glucose into CO₂ and glyceride-glycerol in the absence of added insulin at a rate which is influenced by the concentration of glucose in the medium, the composition of the diet, and the size of its constituent fat cells (Fig. 2). Incorporation of [¹⁴C]glucose into glyceride-fatty acids, on the other hand, is not detected at all in the adipose tissue from either patient group under any of the conditions of these studies.

Increasing medium glucose concentrations are associated with increasing basal rates of ¹⁴CO₂ and [¹⁴C]-glyceride-glycerol production in the adipose tissue of both patient groups during the ingestion of either diet, regardless of cell size. The rate of increase in these two parameters of basal glucose metabolism is significantly greater, however, when the medium glucose concentration is raised from 0.2 to 0.5 mg/ml than from 0.5 to 5 mg/ml.

Adipose tissue from both patient groups incorporates [1-¹⁴C]glucose into CO₂ at a significantly greater rate after the period of ingestion of the high than the low carbohydrate diet. This dietary effect is similar at each concentration of medium glucose, but somewhat greater in the smaller adipose cells of either group. During ingestion of the low carbohydrate diet, the basal rate of glucose oxidation is independent of adipose cell size,

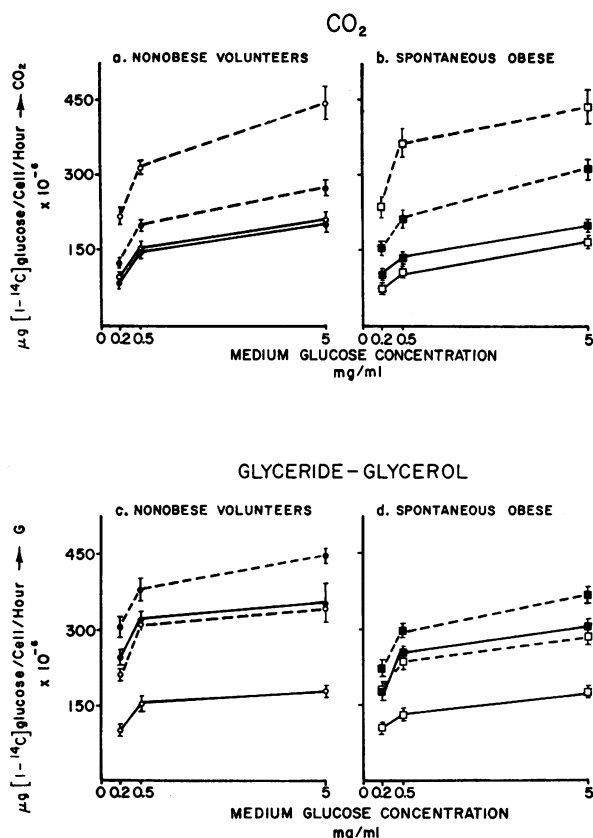


FIGURE 2 Basal rates of $[1-^{14}\text{C}]$ glucose incorporation into CO_2 (a and b) and glyceride-glycerol (c and d) by adipose tissue from nonobese volunteers and obese patients during ingestion of isocaloric low (—) and high (---) carbohydrate diets. Studies were conducted on adipose tissue from the nonobese volunteers containing small cells before weight gain (○) and large cells after weight gain (●), and in the obese patients on large cells before (■), and small cells (□) after weight loss. Tissues were incubated in the presence of one of three concentrations of medium glucose, and in the absence of insulin. Each point represents the mean \pm SEM.

both within and across groups, at a given glucose concentration; a slightly lower rate is observed, however, in the small cells from the reduced-obese patients (Fig. 2b). In contrast, during ingestion of the high carbohydrate diet, the small adipose cells from either patient group oxidize glucose at a greater rate than do the large cells at each medium glucose concentration. Dietary composition, therefore, can markedly affect the comparison between the basal rates of glucose oxidation by small and large adipose cells; the large cells obtained during high carbohydrate intake oxidize glucose at greater rates than do the small cells obtained during ingestion of the low carbohydrate diet.

Table II shows the results of an analysis of the sources of variance in the basal in vitro rate of glucose oxida-

tion by the adipose tissues from the volunteers and obese patients under all of the body weight and dietary conditions studied. The data are derived from studies in which the tissues were incubated at 0.5 mg glucose/ml and in the absence of insulin; similar relationships are observed at other levels of medium glucose. Dietary alterations account for more of the variability in the basal rate of glucose oxidation than does adipose cell size; there is, however, significant interaction between these two factors, indicating that this effect of the diet is influenced by adipose cell size. On the other hand, obesity per se (differences between the volunteers and obese patients, irrespective of their body weight) accounts for only a small and insignificant amount of the total variability in this metabolic function; furthermore, there is no significant interaction of this factor with diet.

The relationship between adipose cell size and the basal rate of $[1-^{14}\text{C}]$ glucose incorporation into glyceride-glycerol is also affected by the composition of the diet (Fig. 2). During the period of ingestion of diets of similar composition, the large adipose cells incorporate $[^{14}\text{C}]$ glucose carbons into glyceride-glycerol at a greater rate than do the smaller cells at each concentration of medium glucose. However, $[^{14}\text{C}]$ glyceride-glycerol production in the small adipose cells obtained during ingestion of the high carbohydrate diet is similar to that observed in the larger cells removed during ingestion of the diet low in carbohydrate. The basal rate of $[^{14}\text{C}]$ -

TABLE II
Analysis of Sources of Variance in Adipose
Tissue Metabolism

Factor	% of total variability			
	-Insulin		+Insulin	
	CO_2	Glyceride	CO_2	Glyceride
	%		%	
Diet	69*	61*	57*	62*
Cell size	11*	19*	27*	18*
Obesity	2	2	1	2
Diet-Cell Size	13*	15*	14*	14*
Diet-Obesity	3	2	<1	2

For each metabolic parameter ($[1-^{14}\text{C}]$ glucose incorporation into CO_2 and glyceride, + or - insulin) the effects of the diet (low and high carbohydrate), adipose cell size (small and large), obesity (whether tissue is obtained from the volunteer or obese patient group), and the interaction of these three factors was analyzed by a three-way complete factorial analysis of variance ($2 \times 2 \times 2$). The values shown are the percentage contribution of each factor to the total variability observed in a given metabolic function by adipose tissues from all subjects, under all conditions studied.

* $P < 0.05$.

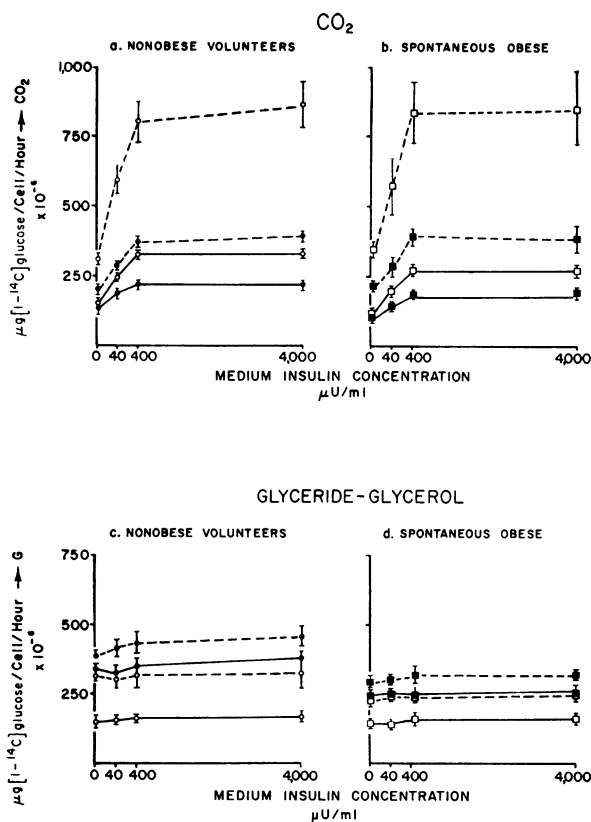


FIGURE 3 Effect of insulin on the rate of $[1-^{14}\text{C}]$ glucose incorporation into CO_2 (a and b) and glyceride-glycerol (c and d) by adipose tissue from nonobese volunteers and obese patients during ingestion of isocaloric low (—) and high (---) carbohydrate diets. Studies were conducted on adipose tissue from the nonobese volunteers, containing small adipose cells before weight gain (\circ) and larger cells after weight gain (\bullet), and in the obese patients on large cells before (\blacksquare) and small cells (\square) after weight loss. Tissues were incubated in the presence of 0.5 mg glucose/ml and 0–4,000 μU insulin/ml. Each point represents the mean \pm SEM.

glyceride-glycerol production by the enlarged adipose cells from the nonobese subjects after weight gain (Fig. 2c) is greater than that observed in the even larger cells of the spontaneously obese subjects (Fig. 2d). The contribution of cell size, diet, and obesity per se to the variability observed in this parameter of glucose metabolism, shown in Table II, is similar to that previously discussed for glucose oxidation. Adipose cell size, however, accounts for more of the total variability in this basal metabolic parameter than in glucose oxidation.

Influence of dietary composition and adipose cell size on insulin-stimulated glucose metabolism. Insulin stimulates the rate of incorporation of $[1-^{14}\text{C}]$ glucose into CO_2 in the adipose tissue from both the nonobese volunteers and the obese patients. The magnitude of this

stimulatory effect of insulin is influenced by the concentrations of insulin and glucose in the medium, the composition of the diet, and adipose cell size (Fig. 3 and 4). Under the conditions of these studies, however, a consistent stimulatory effect of insulin on $[^{14}\text{C}]$ glucose incorporation into glyceride-glycerol (Figs. 3c and d) or fatty acids could not be demonstrated in the adipose tissue of either patient group.

Increasing medium insulin concentrations from 40 to 400 $\mu\text{U}/\text{ml}$ are associated with increasing rates of glucose oxidation by adipose tissue, independent of cell size and dietary composition (Figs. 3a and b). In addition, the stimulatory effect of 400 μU of insulin/ml upon glucose oxidation is significantly enhanced by increasing medium glucose concentrations from 0.2 to 0.5 mg/ml; a near maximal effect is observed, however, at 0.5 mg glucose/ml, regardless of adipose cell size or the composition of the diet (Figs. 4a and b).

The in vitro effect of insulin on glucose oxidation by adipose tissue obtained from individuals ingesting the low carbohydrate diet, although statistically significant, is quite small, particularly in the large cells from the obese patients (27–67% increase above basal). However, during ingestion of the high carbohydrate diet, the response to each concentration of insulin (Figs. 3a and b) and glucose (Figs. 4a and b) is considerably increased; this dietary effect is more marked in the smaller than in the larger adipose cells within each group.

During ingestion of diets of similar composition, the stimulatory effect of insulin on glucose oxidation is significantly greater in the smaller than in the larger cells, both within and between patient groups, at each medium concentration of insulin (Figs. 3a and b) and glucose (Figs. 4a and b). This difference in the insulin response between small and large adipose cells is most marked during ingestion of the high carbohydrate diet. Furthermore, even when the medium insulin concentrations is 4,000 $\mu\text{U}/\text{ml}$, the rate of glucose oxidation by the larger adipose cells remains less than that observed in the smaller cells exposed to only 40 or 400 $\mu\text{U}/\text{ml}$ during periods of comparable dietary intake (Figs. 3a and b). The insulin response of the small adipose cells from the reduced obese patients is slightly less than that of cells of similar size obtained from the nonobese volunteers.

As is the case with basal glucose metabolism, dietary composition appears to influence markedly the results of comparisons of the insulin-stimulated rate of glucose oxidation between small and large adipose cells; at each insulin concentration $^{14}\text{CO}_2$ production by the large adipose cells from individuals ingesting the high carbohydrate diet is similar or greater than that observed in the small cells removed during the period of ingestion

of low carbohydrate diet, both within and between groups (Figs. 3a and b). Similarly, at each medium glucose concentration (Figs. 4a and b), the response to insulin of the large cells obtained during ingestion of the high carbohydrate diet is greater than that observed in the smaller cells during intake of the low carbohydrate diet. Table II summarizes the relative contribution of cell size, diet, and obesity per se to the total variability of insulin-stimulated glucose oxidation and [14 C]glyceride-glycerol production. The data are derived from studies in which tissues were incubated in the presence of 0.5 mg/ml glucose and 400 μ U insulin/ml; similar relationships are observed at the other concentrations of medium glucose and insulin. The contribution of each factor is similar to that previously discussed for basal glucose oxidation. It should be noted, however, that cell size accounts for more of the total variability in the rate of insulin stimulated than basal glucose metabolism by these tissues. As with basal metabolism, diet and cell size interact to influence the insulin response of these tissues.

The effect of insulin on glucose oxidation expressed as the percent increase above the corresponding basal rate is shown in Figs. 4c and d. The relationships among adipose cell size, composition of the diet, and insulin sensitivity, when expressed in this fashion, are similar, but not identical, to those observed when the effects of insulin are expressed as absolute values. For example, the stimulation of $^{14}\text{CO}_2$ production of insulin is diminished by the highest glucose concentration when the response is expressed as a percent increase, but is increased slightly or is unchanged when expressed in absolute values.

Comparisons of biopsied and aspirated adipose tissue samples. Fig. 5 indicates that both basal and insulin-stimulated rates of glucose metabolism by subcutaneous abdominal adipose tissue obtained from the spontaneously obese patients by needle aspiration are qualitatively similar to those observed when adipose tissue from the same site is obtained by open biopsy and studied under identical conditions of medium glucose and insulin concentration, and dietary composition. Similar observations have been made in adipose tissue from the reduced-obese patients and from the nonobese subjects before and after weight gain. Thus, in both aspirated and biopsied adipose tissue during ingestion of a given diet, the basal rate of glucose carbon incorporation into glyceride-glycerol is enhanced, and the stimulation of glucose oxidation by insulin is diminished by increased adiposity and adipose cell size. Ingestion of a high carbohydrate diet enhances both basal and insulin-stimulated rates of glucose oxidation and [14 C]glyceride production in tissue obtained by both techniques. A consistent stimulatory effect of insulin on [14 C]glucose incorporation into gly-

ceride is not observed either in the biopsied tissue or in the smaller aspirated fragments. The rate of insulin-stimulated glucose oxidation by the biopsied adipose tissue is slightly, but significantly ($P < 0.05$) greater than in the aspirated tissue, when studied during consumption of the high carbohydrate diet. This observation is consistent with the earlier findings of Hirsch and Goldrick, in which the stimulatory effect of insulin on human adipose tissue aspirates was found to diminish with decreasing size of the tissue fragments (10).

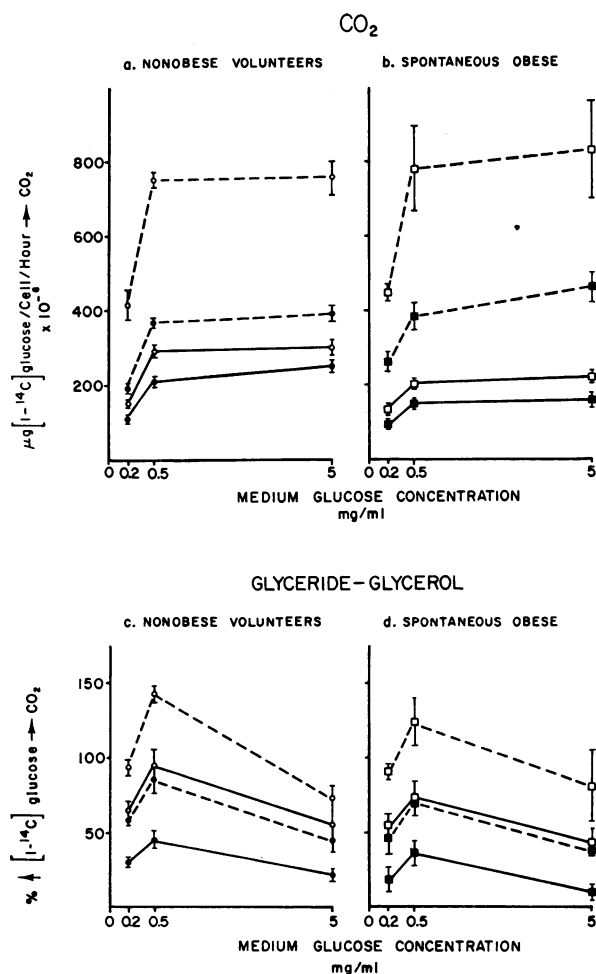


FIGURE 4 Effect of varying medium glucose concentration on the insulin-stimulated rate of glucose oxidation (a and b) and percent increase above basal glucose oxidation caused by insulin (c and d) in adipose tissue from non-obese volunteers and obese patients during ingestion of isocaloric low (—) and high (---) carbohydrate diets. Studies were conducted on adipose tissue from the non-obese volunteers containing small adipose cells before weight gain (○) and larger cells after weight gain (●), and in the obese patients on large cells before (■) and small cells (□) after weight loss. Tissues were incubated in the presence of 400 μ U insulin/ml and 0.5–5 mg/ml glucose. Each point represents the mean \pm SEM.

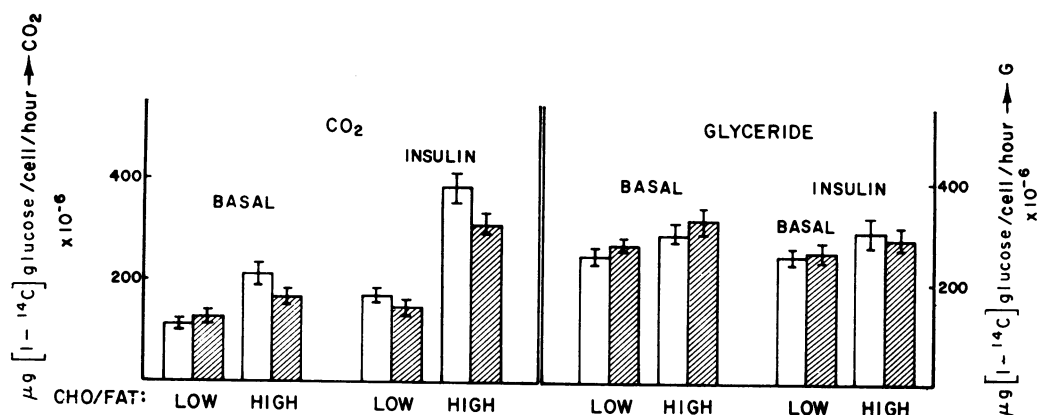


FIGURE 5 Comparison of basal and insulin-stimulated glucose metabolism by adipose tissue from six obese subjects obtained by biopsy (□) and needle aspiration (▨) techniques. Tissues were incubated under identical conditions in medium containing 0.5 mg glucose/ml and in the presence or absence of 400 $\mu\text{U/ml}$. Values represent the mean \pm SEM.

DISCUSSION

The present study indicates that both the composition of the diet and adipose cell size influence the *in vitro* metabolic activity of human adipose tissue, and that these dietary and morphologic effects can, to some extent, be dissociated. The same relationships are observed whether adipose tissue is obtained from individuals with spontaneous or experimental obesity. Finally, the results of this series of studies are essentially independent of the experimental method, needle aspiration or surgical biopsy, by which adipose tissue samples are obtained.

A change from a weight-maintenance, low carbohydrate diet to an isocaloric diet containing a high ratio of carbohydrate to fat, at a time when adipose cell size remains constant, is associated with an increase in the basal rate of $[1\text{-}^{14}\text{C}]$ glucose incorporation into CO_2 and glyceride-glycerol (Fig. 2), as well as an enhanced rate of these two metabolic parameters in the presence of insulin (Fig. 3), regardless of the size of the adipose cells. Adipose cell size, on the other hand, appears to correlate directly with both basal glucose metabolism and the response to insulin by this tissue during ingestion of each diet. When studied during ingestion of weight-maintenance, isocaloric diets of comparable composition large adipose cells are less responsive to the stimulatory effect of insulin on glucose oxidation than are smaller cells, and produce $[^{14}\text{C}]$ glyceride-glycerol at a greater rate. These observations are consistent with those reported by Grey, Goldring, and Kipnis (25) and indicate that the antecedent diet may have an important influence on the biologic effectiveness of insulin; they also indicate, however, that the diminished response of enlarged human adipose cells to insulin is demonstrable irrespective of the diet.

Dietary control, therefore, becomes a critical factor in

comparisons of the metabolic character of adipose tissue between and within individuals. Although increasing adipose cell size is associated with a diminishing insulin response when adipose tissue is studied under conditions of weight maintenance and ingestion of diets of similar composition, the response to insulin of large cells obtained from individuals ingesting high carbohydrate diets may be equal to, or even greater than, that of smaller cells obtained during periods of ingestion of isocaloric, low carbohydrate diets. Comparisons of basal glucose metabolism between small and large adipose cells can also be influenced by the nature of the diet being ingested at the time tissue is obtained for study. These observations may explain, then, some of the disparate results of previous studies of basal and insulin-stimulated glucose metabolism in human adipose tissue.

The metabolism of glucose and response to insulin by adipose tissue from spontaneous and experimental human obesity are quite similar when studied during ingestion of diets of similar composition. Slight differences in these metabolic parameters are, however, observed when large adipose cells from spontaneously and experimentally obese are compared under these conditions. Minor differences are also seen when small cells from the reduced-obese and nonobese volunteers are compared. While these observations may reflect primary differences in the metabolism of adipose tissue from nonobese and obese individuals, the analysis of variance (Table II), indicates this to be unlikely. It is more probable that these slight differences reflect the lingering effects of the antecedent gain and loss of weight, factors known to influence the metabolism of this tissue (9, 12, 26). In any case, the similarity of metabolism by adipose tissue from spontaneous and experimental obese subjects is more striking than these small differences.

The finding that the metabolic alterations observed in the enlarged adipose cell from the obese individual can be induced in nonobese volunteers through experimental weight gain and increased cell size, together with the finding that these changes associated with increasing adipose cell size can be reversed through weight loss, suggests that the altered in vitro metabolism of adipose tissue from obese individuals may represent adaptive rather than primary changes in the cell. The decreased effects of insulin reported here coupled with the increased rate of lipolysis noted in other studies (8, 27) suggest, in fact, that the enlarging adipose cell progressively loses its capacity for net glyceride storage. Since these adaptive changes can be overcome, at least in part, either by high carbohydrate or high caloric intake (9, 12), the delivery of excessive lipogenic substrate may be responsible for the maintenance of the enlarged size of the adipose cell in obesity. The source and nature of this substrate(s), whether glucose, lipoproteins, or fatty acids, remains to be more fully examined (28). The failure of the present studies to detect de novo fatty acid synthesis from glucose probably reflects the fact that these tissues were obtained after an overnight fast; Hirsch and Goldrick found 16% of [^{14}C]glucose incorporated into human adipose tissue triglyceride-fatty acids during the fed state, but none after fasting (10, 26).

The present studies do not establish which component of the diet, carbohydrate, or fat, is responsible for the alterations in adipose tissue metabolism associated with changes in dietary composition. Nor do these studies provide insights into the mechanisms by which alterations in adipose cell metabolism are induced by changes in cell size or dietary composition. Freychet et al. have found an impairment of insulin binding to the fat cell membrane in the obese hyperglycemic mouse (29). Kahn, Soll, Neville, and Roth (30) have reported alterations in the binding of insulin to lymphocytes with varying diets, and Grey, Goldring, and Kipnis (25) have demonstrated an effect of glucose on the glucoreceptors and the rate of insulin biosynthesis in isolated pancreatic islets. On the other hand, the metabolic alterations associated with increasing cell size and changes in the composition of the diet may be related to an alteration in some intracellular factor, such as the activity of one or more enzymes or the level of nonesterified fatty acid (27).

Finally, in the present study, the difference in response to insulin between small and large adipose cells is limited to the effects of this hormone on glucose oxidation; alterations in the other insulin-dependent metabolic functions of the cell have not been shown. Until such alterations are demonstrated, conclusions about the insulin "sensitivity" of enlarged adipose cells

should be drawn with more care than heretofore exercised. Furthermore, the present studies indicate that when examined during conditions of similar nutrition and growth, differences in the insulin response of large and small adipose cells remain similar over a wide range of medium glucose and insulin concentration. Until it can be shown that the impaired insulin response of the large adipose cell can be overcome by increasing concentrations of insulin, and/or that the insulin response of large and small cells converge with increasing concentrations of medium glucose and insulin, referring to the diminished in vitro response reported here and in other studies as "insulin resistance" is probably best avoided.

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