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Research Article

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The results of these studies show that an increase in the glucose concentration of the culture medium enhanced the basal as well as the catecholamine-stimulated lipolysis in the short-term incubations. The presence of insulin in the culture medium enhanced the lipolytic process as well. Analogous results were obtained with the cellular rate of glucose conversion to triglycerides in the short-term incubations. The stimulating effects of insulin and glucose were most pronounced in the larger adipose cells possibly due to their enlarged surface areas.

The data suggest that the metabolism of adipose tissue as revealed by short-term studies may be profoundly influenced by the antecedent biochemical environment.

Studies of Human Adipose Tissue in Culture III

INFLUENCE OF INSULIN AND MEDIUM GLUCOSE CONCENTRATION ON CELLULAR METABOLISM

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ABSTRACT Explants of human adipose tissue were maintained in culture for 1 wk in different glucose concentrations with or without the addition of insulin. After this period of time the explants were carefully washed and then subjected to short-term incubations in the same glucose concentration and in the absence of insulin. With this experimental design the influence of long-term exposure to insulin and different glucose concentrations on adipose tissue metabolism could be studied.

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INTRODUCTION

Several recent investigations have shown that adipose tissue metabolism is influenced by the cellular character of the tissue. Thus, glucose metabolism and lipid mobilization are increased in enlarged fat cells (1–6). In contrast, the stimulating effects of insulin on the rates of glucose oxidation and incorporation into the triglycerides are inversely related to the size of the fat cells (7–9).

However, the metabolism of adipose tissue may be influenced by other factors than fat cell size or number. The nutritional status of the donor, for instance, is of importance for the cellular metabolism and sensitivity to hormones. Thus, starvation increases lipid mobilization (10) and decreases both lipid synthesis (9) and the cellular responsiveness to insulin (9, 11). The opposite effects are imposed by refeeding (9–12).

Most studies of adipose tissue metabolism are carried out by incubating the specimens for a few hours in vitro. Under these circumstances factors such as the nutritional status of the donor may profoundly influence the results as outlined above. With the tissue culture technique, however, such an influence may possibly be eliminated. In addition, it offers a unique possibility to alter the biochemical environment in a defined way for prolonged periods of time and the opportunity to study the effect of the manipulation on the cellular metabolism. We have previously described a method to maintain explants of human adipose tissue in vitro for prolonged periods of time with intact morphology (13). Furthermore, the adipocytes are responsive to added insulin and the rates of triglyceride synthesis from glucose and release of glycerol to the incubation medium proceed in a linear fashion for at least 1 wk (14).

In the present study explants from the same individuals were cultured for 1 wk in different glucose concentrations with or without the addition of insulin. After this preincubation period the explants were subjected to short-term incubations analogous to those generally used to study adipose tissue metabolism. With this experimental design, the long-term effects of insulin and glucose on the cellular metabolism in vitro could be studied.

METHODS

Biopsies of subcutaneous adipose tissue were obtained from patients undergoing operations for an isolated abdominal disorder. Clinical data of the patients are shown in Table I.

Part of these data were presented at the 7th Congress of the European Society for Clinical Investigation in Rotterdam, Holland on 26–29 April 1973.

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TABLE I

Clinical Data of Patients in the Metabolic Studies

Patient	Age	Sex	Height	Weight	Diagnosis
	yr		*cm*	*kg*	
1	69	M	179	79.0	Cholecystolithiasis
2	57	F	163	58.0	"
3	43	F	170	59.0	"
4	38	F	164	91.0	Nephrolithiasis
5	78	F	163	65.0	Cholecystolithiasis
6	24	F	152	51.0	"
7	32	M	169	73.0	"
8	50	M	173	85.0	"
9	49	F	173	74.0	Gastric ulcer
10	80	M	175	76.0	Cholecystolithiasis
11	63	F	154	65.0	"
12	28	M	172	72.0	"
13	24	F	167	62.0	Exploratory laparotomy

The patients were operated upon after an overnight fast. Anesthesia was induced with a short-acting barbiturate and maintained with halothane, nitrous oxide, and oxygen. The biopsies were usually obtained after the skin incision and immediately placed in a sterile vessel containing Parker's medium 199 (for composition see reference 15; Statens Bakteriologiska Laboratorium, Stockholm, Sweden) modified to a glucose concentration of 1.0 mM. The culture procedure has been described in detail previously (13). Briefly, smaller specimens weighing about 5–10 mg each were prepared under sterile conditions, placed between two cover slips and incubated in Leighton tubes (Labora, Stockholm, Sweden). Total tissue weight in each culture tube was about 20 mg. The incubation medium used was medium 199 with a glucose concentration of 1.0, 5.6, or 11.2 mM and with or without the addition of 0.15 IU glucagon-free insulin (recrystallized pork insulin, Vitrum AB, Stockholm, Sweden). No serum was added. All incubations were performed for 1 wk at pH 7.4±0.3 and at 37°C. The gas phase was air.

After 1 wk the explants were removed from the culture tubes, washed carefully with warm medium, and then subjected to short-term incubations as previously described (4). Briefly, after preincubation for 30 min (16), the explants were incubated for 2 h in 2.0 ml medium 199 containing 1.0 mM glucose and with 0.15 μCi [1-^{14}C]glucose (New England Nuclear, Boston, Mass.) or 5×10^{-5} M noradrenaline (Astra AB, Södertälje, Sweden) added. Glycerol was determined on the incubation medium as described by Laurell and Tibbling (17).

Thus, with this design, specimens from the same patients were preincubated for 1 wk under different experimental conditions and then subjected to short-term incubations performed in the same glucose concentration and in the absence of insulin.

After the incubation period the tissue lipids were extracted with chloroform-methanol as described by Folch, Lees, and Sloane Stanley (18). Aliquots of the chloroform phase were evaporated to dryness and 10 ml scintillation fluid (0.4% 2,5-diphenyloxazole and 0.01% 1,4-bis[2-(5-phenyloxazolyl)]benzene dissolved in toluene) added. The radioactivity was determined in a Packard Tri-Carb liquid scintillation spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill.). Quenching was corrected by means of internal standardization.

Aliquots of the chloroform phase were taken for the determination of the glyceride-glycerol (19).

Incorporation of labeled glucose into the triglycerides, and release of glycerol to the incubation medium are expressed on a cellular basis as suggested by several investigators (7, 20). Mean cell diameter of the tissue biopsies was determined on adipocytes isolated from the stroma by treating the tissue with collagenase (Sigma type 1, Sigma Chemical Co., St. Louis, Mo.). With this technique there is no indication of an increased rupture of large adipose cells (21). The mean cell diameters were determined on the freshly excised specimens and in some cases also on explants incubated 1 wk solely for this purpose. The justification for this procedure has been reported previously (14). When the mean cell diameter is known the mean cellular volume can be calculated as suggested by Goldrick (22):

$$v = \frac{\pi}{6}(3\sigma^2 + \bar{x}^2)\bar{x}$$

in which v = volume, σ^2 = variance of the mean cell diameter, and $\bar{x}$ = mean cell diameter.

The mean cellular weight was calculated on the assumption that the density of fat cells is that of triolein (23). The number of fat cells in the specimens was then determined by dividing the triglyceride content of the explants with the mean cellular weight.

It has recently been reported that the uptake of labeled glucose and release of glycerol seem to be better related to cell surface area than to cell diameter (1, 6, 24). The metabolic parameters studied in the present investigation were therefore related to mean cell surface area calculated as suggested by Zinder and Shapiro (24):

$$\text{mean surface area} = \pi(\sigma^2 + \bar{x}^2).$$

Statistical methods. Conventional statistical methods were used. Differences in means were tested with analysis of variance. However, when the variance differed between the groups significance levels were analyzed with the non-parametric sign-test.

TABLE II

Effect of Insulin and of the Glucose Concentration of the Culture Medium on the Lipolytic Rates in Short-Term Incubations

Culture medium	Glycerol release, *nmol/10⁵ cells*		
1.0 mM glucose (n = 8)	13.5±4.7	<0.05	<0.05
1.0 mM glucose + insulin (n = 7)	23.2±7.9		
5.6 mM glucose (n = 8)	22.0±7.3	NS	<0.05
5.6 mM glucose + insulin (n = 7)	35.3±11.9		
11.2 mM glucose (n = 6)	9.8±2.9		

Explants of human adipose tissue were incubated for 1 wk in medium 199 containing glucose and insulin as indicated. After the culture period the explants were carefully washed, preincubated for 30 min, and then incubated for 2 h in medium 199 with a glucose concentration of 1.0 mM. Results ±SEM. NS = not significant.

RESULTS

Effect of medium glucose concentration and insulin on the lipolysis. The explants maintained in vitro at a glucose concentration of 5.6 mM exhibited a significantly increased lipolysis as compared with those maintained at 1.0 mM ($P < 0.05$, Table II). Increasing the glucose concentration to 11.2 mM did not lead to a further enhancement but rather tended to reduce the lipolysis. Addition of insulin to the culture medium increased the lipolytic rate of the explants in the short-term incubations. This effect of insulin was significant whether the explants were cultured in 5.6 mM glucose or in 1.0 mM ($P < 0.05$, Table II). The lipolytic rates of the explants cultured in 1.0 mM glucose with insulin added were similar to those maintained in 5.6 mM glucose without insulin ($P > 0.1$).

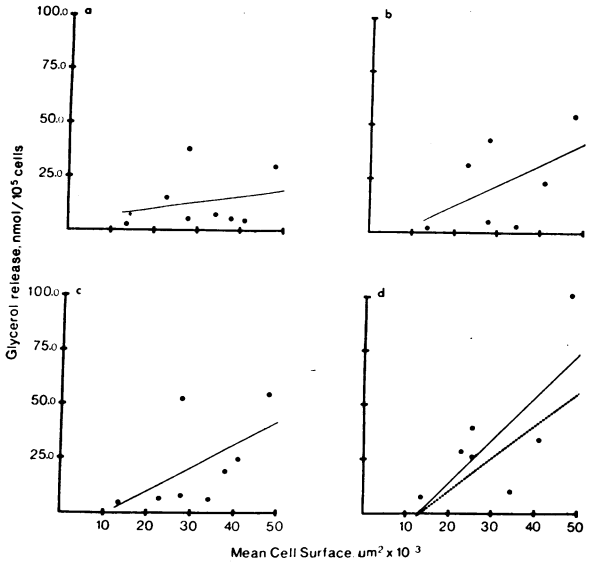

FIGURE 1 Relationship between cell surface area and release of glycerol. The explants were cultured for 1 wk, carefully washed, preincubated for 30 min, and then incubated for 2 h in medium 199 containing 1.0 mM glucose. The culture medium contained 1.0 mM glucose (a) $y = 0.274x + 4.835$, $r = 0.223$, $P > 0.1$); 1.0 mM glucose + 0.15 IU insulin (b) $y = 0.964x - 6.348$, $r = 0.528$, $P > 0.1$); 5.6 mM glucose (c) $y = 1.053x - 11.291$, $r = 0.554$, $P > 0.1$); or 5.6 mM glucose + 0.15 IU insulin (d) $y = 1.998x - 25.971$, $r = 0.728$, $P < 0.05$). The dotted line indicates the regression line previously obtained with freshly excised specimens (4).

Thus, the data show that insulin as well as the glucose concentration of the culture medium influence the lipolytic rates of the explants in the subsequent short-term studies.

Since it has previously been shown that the lipolytic rate is dependent upon the cellular character of the tissue (3–6) the present data were also analyzed in terms of the mean cell surface area (6, 24) of the explants. The results show that the stimulating effects of glucose and of insulin on the lipolysis were most pronounced in the larger cells (Fig. 1). The glycerol release of the explants cultured in the presence of 5.6 mM glucose and with insulin added was significantly correlated with the mean cell surface area ($r = 0.728$; $P < 0.05$). Furthermore, the regression line obtained from the analysis of these data was similar to that previously found (4) in a short-term study with freshly excised specimens (Fig. 1d). Although not significant in any of the other groups the correlation and the regression coefficients between cell size and lipolysis increased as the glucose concentration of the culture medium was raised or when insulin was added.

In the next set of experiments the effect of varying the glucose concentration of the culture medium on the lipolytic response to a maximal dose of noradrenaline was studied. The results show that an increase in the glucose concentration of the culture medium from 1.0 to 5.6 or 11.2 mM significantly enhanced the lipolytic effect

TABLE III

Effect of the Glucose Concentration of the Culture Medium on the Lipolytic Action of Noradrenaline in Short-Term Incubations

Culture medium	Glycerol release, *nmol/10⁵ cells*		
1.0 mM glucose (n = 7)	23.2±5.9	<0.01	<0.01 NS
5.6 mM glucose (n = 7)	44.8±10.8		
11.2 mM glucose (n = 6)	51.6±21.3		

Explants of human adipose tissue were cultured for 1 wk in medium 199 containing glucose as indicated. After the culture period the explants were washed, preincubated for 30 min, and then incubated for 2 h in medium 199 with a glucose concentration of 1.0 mM and with noradrenaline added at a concentration of 5×10^{-5} M. Results ±SEM of the absolute effect of noradrenaline. NS = not significant.

of noradrenaline in the short-term incubations ($P < 0.01$, Table III). Increasing the glucose concentration from 5.6 to 11.2 mM did not significantly further enhance the lipolytic response ($P > 0.1$, Table III). When the data were analyzed in terms of the initial mean cell surface area of the explants the correlation and the regression coefficients between this parameter and the absolute effect of noradrenaline on the explants increased as the glucose concentration of the culture medium increased.

The present study shows that when the explants were preincubated for 1 wk the lipolytic process studied in short-term incubations was influenced by the glucose concentration of the culture medium as well as by the presence of insulin. To study whether a similar effect could be induced during a shorter period of time explants were preincubated for only 3 h. However, neither the presence of insulin nor an increase in the medium glucose concentration during the preincubation period significantly influenced the basal or the noradrenaline-stimulated lipolysis in the short-term incubations.

Effect of medium glucose concentration and insulin on the lipid synthesis from labeled glucose. Analogous to the results obtained with the lipolysis it was found that the preincubation conditions may influence the cellular uptake of labeled glucose in the short-term incubations. Addition of insulin to the culture medium increased the cellular uptake of labeled glucose ($P < 0.05$, Table IV) while an increase in the glucose concentration from 1.0 to 5.6 or 11.2 mM did not significantly enhance the lipid synthesis ($P > 0.1$, Table IV).

TABLE IV

Effect of Insulin and of the Glucose Concentration of the Culture Medium on the Cellular Conversion of [1-¹⁴C] Glucose to Triglycerides in Short-Term Incubations

Culture medium	Incorporation of glucose, *nmol/10⁵ cells*			
1.0 mM glucose (n = 7)	1.7±0.5	NS		<0.05
1.0 mM glucose + insulin (n = 7)	3.4±1.1			
5.6 mM glucose (n = 7)	2.6±1.0		NS	<00.5
5.6 mM glucose + insulin (n = 6)	4.7±1.3			
11.2 mM glucose (n = 6)	1.2±0.3			

The explants were cultured for 1 wk in medium 199 containing glucose and insulin as indicated. After the culture period the explants were carefully washed, preincubated for 30 min, and then incubated for 2 h in medium 199 with a glucose concentration of 1.0 mM and with 0.15 μCi [1-¹⁴C] glucose added. Results ±SEM. NS = not significant.

Analysis of the data in terms of the initial mean cell surface area of the explants showed that the simulating effect of insulin was most pronounced in the larger adipose cells (Fig. 2). The incorporation of label by the explants cultured in the presence of 5.6 mM glucose with insulin added was significantly correlated with the mean cell surface area ($r = 0.876$, $P < 0.01$). Again, the regression line obtained from the analysis of these data was similar to that previously found (4) in a short-term study with freshly excised specimens (Fig. 2*d*). The correlation and the regression coefficients between cell size and the incorporation of label into the triglycerides were not significant in any of the other groups but, again, they were found to increase when the glucose

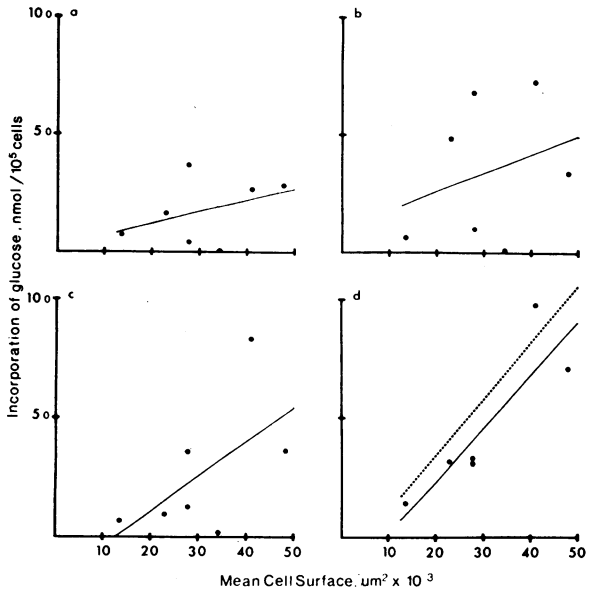

FIGURE 2 Relationship between cell surface area and incorporation of labeled glucose into the triglycerides. The explants were cultured for 1 wk, carefully washed, preincubated for 30 min, and then incubated for 2 h in medium 199 containing 1.0 mM glucose and 0.15 μCi [1-¹⁴C]glucose. The culture medium contained 1.0 mM glucose (*a*) $y = 0.049x + 0.188$, $r = 0.407$, $P > 0.1$), 1.0 mM glucose + 0.15 IU insulin (*b*) $y = 0.077x + 1.087$, $r = 0.295$, $P > 0.1$), 5.6 mM glucose (*c*) $y = 0.145x - 1.815$, $r = 0.585$, $P > 0.1$), or 5.6 mM glucose + 0.15 IU insulin (*d*) $y = 0.222x - 2.032$, $r = 0.876$, $P < 0.01$). The dotted line indicates the regression line previously obtained with freshly excised specimens (4).

TABLE V

Effect of Different Concentrations of Insulin on Basal Lipolysis and Glucose Incorporation into the Triglycerides

Insulin concentration	Glucose incorporation	Glycerol release
μU/ml	*nmol/10⁵ cells*	
—	5.9	25.0
4×10	7.9	34.3
4×10^2	6.3	32.9
4×10^4	10.2	46.0
4×10^5	8.3	40.7

The explants were incubated for 1 wk in medium 199 containing 5.6 mM glucose and insulin as indicated. After the culture period the explants were incubated for 2 h as described in Table IV. The results are the means of two incubations.

concentration of the culture medium was raised from 1.0 to 5.6 mM or when insulin was added (Fig. 2).

The concentration of insulin used in this and in previous studies (13, 14, 25) was supramaximal to avoid significant effects of the insulin-degrading enzymes present in adipose tissue (26). However, subsequent studies indicate that similar results may be obtained with considerably lower insulin concentrations particularly on the side of lipid mobilization (Table V).

Since addition of insulin to the culture medium enhanced the cellular triglyceride synthesis from glucose, control experiments were performed to exclude the possibility that this effect was due to the presence of insulin in the interstitial space in spite of the precautions taken (careful washing and preincubation for 30 min in fresh medium). However, as shown in Table VI, addition of insulin antibodies to the incubation medium did not inhibit the increased rate of lipid synthesis.

TABLE VI

Effect of Insulin Antibodies on the Incorporation of [1-^{14}C]-Glucose into the Triglycerides of Cultured Explants in Short-Term Incubations

Culture medium	Incubation medium	Glucose incorporation	
		Exp. 1	Exp. 2
		nmol/10⁵ cells	
1.0 mM glucose	1.0 mM glucose	3.5±0.3	3.6±0.2
1.0 mM glucose + 0.15 IU insulin	1.0 mM glucose	5.6±1.7	5.7±1.1
	1.0 mM glucose + insulin antibodies	5.8±0.1	8.9±0.4

Specimens of human adipose tissue were cultured for 1 wk with or without the addition of insulin as indicated. After careful washing and preincubation for 30 min the explants were incubated for 2 h in medium 199 with 1.0 mM glucose and with 0.15 μCi [1-^{14}C] glucose added.
Sufficient insulin antibodies to bind 10^3 μU insulin were added. Means ±SEM of duplicate determinations.

Another point of importance to consider is the influence of the stromal cells on the results obtained since the incubations were not performed with isolated cells. However, initial experiments were carried out where labeled glucose was added to the incubation medium and the incorporation of label into the intact specimens was compared with that found in isolated cells from the same biopsy. Even in the presence of 5.6 mM glucose and insulin the incorporation of label into the stromal cells could not account for more than 5% of the total incorporation. Thus, even if the metabolism of the stromal cells was influenced by the culture procedure such an effect would not be of importance for the present results.

These studies show that the stimulating effects of insulin and of medium glucose concentration on the cellular rates of lipolysis and lipid synthesis in the short-term incubations are dependent upon the initial mean cell size. Therefore, the mean cell diameters were determined in six incubations on explants maintained at 1.0 and at 5.6 mM glucose with or without insulin added to exclude the possibility that the differences noted between the groups were due to differences in cell size. As shown in Table VII there was a slight decrease in mean cell size during the culture period as also previously found (13, 14, 25). However, there was no significant difference between the groups (P level between the groups $>$ 0.1) and the individual mean cell sizes were similar indicating that the differences noted in the metabolic capacity between the groups were not associated with differences in the cell size during the culture procedure.

DISCUSSION

The tissue culture method used in the present study (13, 14, 25) offers the possibility of varying the biochemical environment for prolonged periods of time and investigating the effect of such manipulations on the metabo-

TABLE VII

Initial Mean Cell Sizes of the Explants Used for the Metabolic Studies and after 1 wk In Vitro

	After 1 wk		
Initial mean	1.0 mM glucose	5.6 mM glucose	5.6 mM glucose + 0.15 IU insulin
μm			
92.2	76.8	89.4	91.8
103.2	104.0	98.5	91.4
64.5	71.2	69.4	70.5
121.2	95.6	103.1	97.3
92.1	83.9	86.3	88.5
84.3	78.9	75.8	78.1
Mean: 93.0±7.7	85.1±5.1	87.1±5.3	86.3±4.1

Mean cell size was determined initially and after 1 wk in culture after treatment of the tissue with collagenase. Results ±SEM.

lism and morphology of the adipose cells. Using this method we have previously shown that the cellular uptake of labeled glucose from the incubation medium as well as the release of glycerol increase in a linear fashion for at least 1 wk (14). Furthermore, the morphology of the adipose cells is maintained during the culture period although large adipocytes tend to decrease in size (13, 14, 25, and Table VII). The incorporation of labeled glucose from the incubation medium is mainly recovered in the glyceride-glycerol moiety. Even when the explants had been maintained in vitro for 2 wk only about 5% of the total radioactivity was found in the fatty acids (14). Thus, the present data on the incorporation of glucose mainly reflect the rate of synthesis of glyceride-glycerol.

In this study, explants from the same individuals were cultured for 1 wk in different glucose concentrations with or without the addition of insulin. After the culture period the cellular metabolism was studied by incubating the explants under identical conditions for 2 h. These studies show that the basal- and noradrenaline-stimulated lipolysis and, to a lesser extent, the uptake of labeled glucose were increased in the specimens cultured in a glucose concentration of 5.6 mM as compared with those maintained at 1.0 mM. A further increase in the glucose concentration to 11.2 mM unexpectedly led to an overall inhibition of the basal- but not the catecholamine-stimulated lipolysis. This inhibitory effect was pronounced in two of the studies but was only slight or not found at all in the other incubations. The reason for the inhibitory effect of an increased glucose concentration in these explants is obscure but has not been found in any study with catecholamines added.

Addition of insulin to the culture medium containing 1.0 mM glucose enhanced the cellular rates of metabolism to a similar extent as that of increasing the glucose concentration from 1.0 to 5.6 mM. The effect of insulin on the lipid synthesis from glucose could not be attributed to the presence of insulin in the interstitial space since addition of insulin antibodies did not block the effect. Thus, it seems reasonable to assume that the action of insulin on the lipolytic process is associated with its stimulating effect on the cellular uptake and metabolism of glucose. It has previously been found, that an increase in the glucose concentration of the incubation medium may enhance the lipolysis (27–29) and the triglyceride synthesis from glucose (30) in short-term incubations. Furthermore, it is known that glucose may overcome the antilipolytic action of insulin and, in fact, in some cases lead to an enhanced lipolysis (28, 29, 31). These acute effects of glucose on the lipolysis have been suggested to be due to products of the intracellular glucose metabolism which directly stimulate the lipase (21, 32, 33) or influence the lipase-inactivating system (34, 35). The observation of the present study that the increased cellular rates of metabolism are maintained even when the short-term incubations are performed in the same glucose concentration suggest an adaptation of the cellular metabolism possibly due to enzyme induction or equally well, to a change in the membrane permeability. In rodents it seems quite clear that insulin as well as an increase in the glucose concentration may enhance the cellular activity and de novo synthesis of several enzymes in adipocytes as well as other cells (36, 37). In man, however, no clear evidence has yet been obtained for induced enzyme activities in fat cells in starvation-refeeding experiments (38), in response to a glucose load (39) or by a variation in the feeding pattern (40). However, further investigations are required to settle this problem in man and the presently employed tissue culture technique seems well suited for such studies.

Irrespective of the mechanisms involved it seems quite clear from the present data that the acute antilipolytic action of insulin, well-known from short-term incubations, should be separated from the effect on the lipolytic process exerted by chronic exposure to the hormone. It was also found in the present study that the stimulating effect of insulin or of an increase in the glucose concentration of the culture medium was most pronounced in the larger adipocytes. In fact, it was only in the explants cultured in the presence of both insulin and a physiologic concentration of glucose that the relationships between cell size, lipolysis, and uptake of labeled glucose were significant. Using similar conditions for the short-term studies as in the present investigation it has been established that cell size may influence both of these parameters. Thus, the basal and catecholamine-stimulated lipolysis (3–6, 24) as well as the uptake of labeled glucose (1, 2, 8) are increased in enlarged cells. The present study indicates that the cellular metabolism in short-term incubations may be influenced by the antecedent biochemical environment such as the insulin and/or glucose levels. The observation that there is a strong correlation between fat cell size and the insulin level in vivo (41, 42) raises the question to what extent the results of the investigations under discussion are influenced by the hyperinsulinemia associated with large adipocytes. The concept that the cellular rate of metabolism in vitro may be influenced by the antecedent biochemical environment is further supported by our recent observations [1] that there is a significant positive correlation between the insulin level in vivo and the cellular conversion of glucose to lipids in vitro.

The present finding that it was mainly the larger cells that showed increased metabolic rates indicates that the effect may be associated with their increased cell surface

[1] Sjöström, L., U. Smith, and P. Björntorp. In preparation.

area. It has previously been shown in the rat that at the glucose concentrations used in the present study it is the rate of translocation across the cell membrane that is limiting for the cellular rate of glucose metabolism (43). If the same condition applies to human fat cells the increased surface areas associated with large adipocytes may lead to an increased translocation of glucose. Evidence has been presented to show that the cellular rates of glucose metabolism as well as the effect of lipolytic agents may indeed be a function of the surface area (1, 6, 24). The present finding that the effect of insulin was most pronounced in large fat cells seems to be a paradox since the stimulating effect of insulin on glucose oxidation and lipid synthesis is in short-term incubations inversely related to adipose cell size (7–9). It has been suggested (44) that this diminished sensitivity represents an adaptation to the hyperinsulinemia associated with large adipocytes (41, 42). Whether enlarged human fat cells have an increased, unchanged or decreased number of insulin receptors is presently unknown. In rodents, however, genetic obesity (45) but apparently not regulatory obesity (46) is associated with a decreased number of insulin receptors. The present and previous (6) findings make a decreased number of insulin receptors seem unlikely in enlarged human fat cells. However, further studies are needed to elucidate this important question.

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