Metabolic patterns and insulin responsiveness of enlarging fat cells

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Abstract The rate and pattern of glucose metabolism, basal lipolysis, and intracellular concentration of free fatty acids were determined in isolated epididymal fat cell preparations (mean volume 30–800 pl) from rats on the basis of fat cell number and in relation to the cell volume. The effects of increasing glucose concentrations in the medium and of insulin on the cellular metabolic activities were compared. Expanding fat cell volume correlated positively and significantly ($P < 0.001$) with the synthesis of glyceride glycerol from glucose (correlation coefficient, $r = 0.919$), with rates of basal lipolysis ($r = 0.663$), and with intracellular free fatty acid accumulation ($r = 0.796$); it correlated negatively and significantly with glucose conversion to glyceride fatty acids ($r = -0.814$, $P < 0.01$). The differences in patterns of glucose metabolism and basal lipolysis between small (<100 pl) and large (>400 pl) fat cells were not modified by insulin or by increments in glucose concentration. The results indicate that the reduced capacity of the large fat cells to respond to insulin cannot be attributed solely to a limited capacity of the cells to take up and metabolize increasing amounts of glucose. The acquired unresponsiveness of the large cells to insulin may result from an alteration in the mechanism of action of insulin and may be related to an intracellular metabolic derangement with increased basal lipolysis, free fatty acid accumulation, and accelerated glyceride synthesis resulting from the accumulation of triglyceride.

Supplementary key words glucose metabolism · lipolysis · fat cell size · lipogenesis · glyceride glycerol synthesis · intracellular free fatty acids

Enlarged fat cells from human and other species (1–6) have been shown to be less responsive to the effect of insulin on glucose uptake and metabolism than smaller cells, usually derived from leaner, younger or from weight-reduced subjects (1, 5, 6).

The mechanisms responsible for the progressive decline in insulin responsiveness of the enlarging adipocytes are unclear. One of the difficulties encountered in elucidating this acquired and reversible abnormality (4, 6) of the cell responsiveness to insulin is that the frequently measured cellular responses to the hormonal effect (e.g., enhancement of glucose oxidation, lipogenesis, etc.) are several steps removed from the processes of insulin recognition and binding by the cellular receptors and also from glucose transport across the cell membrane.

In studies carried out with adipose tissue slices (5) from the rat, enlarged fat cells (mean cell volume >400 pl) from older, fatter animals showed, in addition to their limited capacity to respond to insulin, metabolic differences that clearly separated them from the smaller (30–100 pl) fat cells of younger animals. Large fat cells convert greater quantities of glucose to glyceride glycerol and smaller quantities to glyceride fatty acids and have a faster rate of base-line lipolysis and glycerol release per cell (7, 8).

The present studies were undertaken to further characterize in isolated fat cell preparations from the rat the relationship between adipose cell size, metabolic capacities, and the cellular response to the effect of insulin on glucose utilization. In particular, the effects of increasing glucose concentrations in the medium (9) on the rate and pattern of glucose metabolism, on the rate of basal lipolysis, and on the insulin response were compared in selected fat cell preparations of small (<100 pl volume) and large (>400 pl) sizes in order to clarify whether the apparently reduced response to insulin of the large fat cells stems from a rate-limiting capacity to take up and metabolize glucose or from other factors. Preliminary communications of portions of this work have appeared (10, 11).

MATERIALS AND METHODS

Animals, hormone, and reagents

Male Wistar rats (Royalhart Laboratory Animals, New Hampton, N.Y.), fed Purina laboratory chow ad lib. since

Abbreviations: FFA, free fatty acids; KRB, Krebs-Ringer bicarbonate.

[1] Recipient of an Established Investigatorship of the American Heart Association.

weaning, were killed by cervical dislocation between 9 and 11 a.m. The rats were 6 wk to 12 months of age and weighed 150–650 g.

Bovine insulin (lot P.J. 4069, 23.8 U/mg, glucagon content of <0.0003% by weight) was donated by Eli Lilly and Co., Indianapolis, Ind.; bovine serum albumin, fatty acid poor, was from Pentex Inc., Kankakee, Ill.; [U-^{14}C]glucose (5 mCi/mmole) and [U-^{14}C]sucrose (1 mCi/mmole) were from New England Nuclear Corp., Boston, Mass.; and collagenase from *Clostridium histolyticum* (150–175 U/mg) was purchased from Worthington Biochemical Corp., Freehold, N.J.

Conditions of the experiments and analytical techniques

The epididymal adipose pads were removed, weighed, and incubated with collagenase according to the method of Rodbell (12). The incubation medium consisted of Krebs-Ringer bicarbonate (KRB), 4% albumin, at pH 7.4. The washed fat cells were resuspended in medium and added in 1-ml volumes to polyethylene counting vials containing additional medium with 0.5 μCi of [^{14}C]glucose and sufficient glucose to provide 1, 3, 6, or 10 μmoles/ml. Whatman no. 1 paper (2 × 8 cm) for CO_2 collection was added to a hanging center well. After capping the vials with serum stoppers and gassing with 95% O_2–5% CO_2, the incubation of triplicate cell samples for the control and insulin-treated groups was carried out at 37°C in a Dubnoff metabolic shaker at 80 strokes/min. The methodology described by Rodbell (12) was followed to terminate the incubation, to recover the radioactivity in CO_2, total lipid, and saponified lipid fractions, and to calculate the amounts of glucose converted by the fat cells into CO_2, glyceride glycerol, and glyceride fatty acids.[2]

In separate experiments, the rates of glycerol release by duplicate samples of fat cells, prepared in a similar manner, were studied during a 90-min incubation in KRB medium with 4% albumin and no glucose or 3 or 12 mM glucose. Medium glycerol was assayed, prior to the incubation and 90 minutes later, by the method of Eggstein and Kreutz (13), after the cells had been separated from the medium by centrifugation for 30 sec at 300 *g* in ice-cold cellulose nitrate tubes (Beckman) and the medium had been collected by needle-punching of the tube bottom. The zero-time values were subtracted from the 90-min values to assess the "net glycerol" release during the incubation.

The intracellular free fatty acid (FFA) concentration was determined by the method of Angel, Desai, and Halperin (14) in separate experiments in which isolated fat cells differing in mean volume from 30 to 775 pl were incubated for 1 hr in KRB medium containing 4% albumin and either no glucose or 6 mM glucose.

In all experiments, one aliquot of the free fat cell suspension was taken to determine the lipid content by methods previously described (15); another aliquot was stained with methylene blue and the diameters of 300 fat cells were measured by optical sizing to provide a measure of mean cell diameter and volume (15). The number of fat cells contained in the suspension and therefore in the incubation vials was calculated by dividing the lipid content of the cell suspension by the mean fat cell lipid (mean fat cell volume × lipid density) (15). Once the volume and number of the adipocytes were determined, the metabolic activity of the cells was expressed as a function of cell number and of mean fat cell volume.

Statistical evaluation of the data

Means, standard deviations (SD), and standard errors of the mean (SEM) were calculated in the usual way. Relationships between variates were estimated by calculation of the correlation coefficient (r) and regression coefficient (b). Differences between group means, between the correlation coefficient and zero, and between the regression coefficient and zero were tested for significance by Student's t test. Values for P of less than 0.05 were taken to indicate significance.

RESULTS

In a first series of nine experiments, samples of isolated fat cell preparations from epididymal depots of rats fed ad lib., differing in body weight from 150 to 650 g, were incubated in KRB medium containing albumin, [^{14}C]glucose, and 6 mM glucose for 1 hr, in the absence and presence of 1 mU/ml bovine insulin. Detailed results are not shown in tabular form because the data are essentially confirmatory of previous work done with rat adipose tissue slices in our laboratory (5). The results, expressed as μmoles of glucose converted to specified product by 10^7 fat cells in 1 hr, were as follows. Glucose conversion to glyceride fatty acids declined markedly from values ranging between 0.6 and 1.0 μmole observed in fat cells with volume of 30–50 pl to values of 0.3–0.5 μmole for 150–200-pl fat cells and to values of 0.02–0.1 μmole for 500–750-pl fat cells. Glyceride glycerol synthesis from glucose increased markedly from values ranging between 0.5 and 0.6 μmole for the 30–50-pl fat cells to values of 0.8–1.1 μmoles for the 150–200-pl cells and to values of 1.2–2.2 μmoles for the 500–750-pl cells. Glucose oxidation ranged between 1.3 and 1.9 μmoles for the 30–50-pl cells, 1.0–1.2 μmoles for the 150–200-pl cells, and 0.5–1.2 μmoles for the 500–750-pl fat cells. Insulin response (expressed as percent increment in glucose conversion to CO_2 and tri-

[2] Lynn, MacLeod, and Brown (23) have shown that, of the total amount of glucose metabolized by adipose tissue in vitro, 75–80% can be accounted for in the form of CO_2, tissue glyceride glycerol, and glyceride fatty acids.

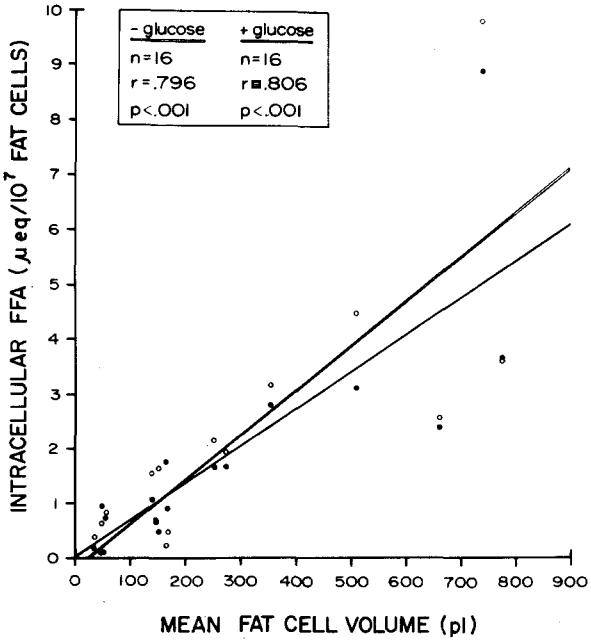

Fig. 1. Relationship between intracellular free fatty acid (FFA) concentration and mean fat cell volume. Isolated fat cells were incubated in Krebs-Ringer bicarbonate medium, containing 4% albumin, in the absence (○) or presence (●) of 6 μmoles of glucose/ml. Each point is the average of duplicate determinations of intracellular FFA at the end of 1 hr of incubation. The lines shown are the calculated regression lines. The correlation coefficient (r) was 0.796 in the absence of glucose and 0.806 in the presence of glucose. The difference between the correlation coefficient and zero was significant, with $P < 0.001$ in both the absence and presence of glucose. The regression coefficient in the absence of glucose was 0.0076 ± 0.0015 (SEM) and in the presence of glucose was 0.0068 ± 0.0013. The difference between the two regression coefficients was not significant ($P > 0.05$).

TABLE 1. Correlation analysis between adipocyte volume[a] and various metabolic parameters

Dependent Variable[b]	Number of Paired Observations	Correlation Coefficient	P
(A) Glucose conversion to glyceride fatty acids	9	−0.814	<0.01
(B) Glucose conversion to glyceride glycerol	9	+0.919	<0.001
(C) Glucose conversion to CO_2	9	−0.623	>0.05
(D) Response to insulin	9	−0.834	<0.01
(E) Glycerol release	35	+0.663	<0.001
(F) Intracellular FFA (no glucose)	16	+0.796	<0.001
(G) Intracellular FFA (with glucose)	16	+0.806	<0.001

[a] Mean fat cell volume, calculated from diameter determination of at least 300 fat cells, was the independent variable.

[b] The relationships between the independent variable (x = adipocyte volume) and each of the dependent variables (y = A to G) presented in Results, were evaluated by correlation analysis. The mathematical model was $y = a + bx$, where x and y are the two variables, a is the intercept, and b is the slope. Mean adipocyte volume was expressed in picoliters (pl); variables A to C were expressed in μmoles of glucose converted by 10^7 fat cells to specified product in 1 hr. The response to insulin (variable D) with enhancement of total glucose conversion to CO_2 + triglyceride was expressed as percent increase of "insulin-treated" values over control values. Glycerol release (variable E) was expressed as μmoles of glycerol released/10^7 fat cells/90 min. Variables F and G were expressed as μeq of FFA/10^7 fat cells at the end of 1 hr of incubation. See text for details.

glycerides above basal levels) declined progressively from values ranging between 150 and 250% seen with the 30–50-pl cells to values of 70–140% in the 150–200-pl cells and to values of 20–30% in the 500–750-pl fat cells. The relationships between mean fat cell volume and the above metabolic activities were estimated by regression analysis and are shown in **Table 1.** Statistically significant ($P < 0.01$) negative correlations were found between cell volume and lipogenesis from glucose and between cell volume and the effect of insulin on glucose utilization. There was a highly significant positive correlation (<0.001) between cell volume and glyceride glycerol synthesis from glucose. The modest decline in glucose oxidation with enlargement of the fat cells did not reach statistical significance.

In a second series of 35 experiments we investigated the rate of basal release of glycerol per fat cell in an in vitro incubation of isolated fat cells differing in mean volume from 40 to 600 pl. The incubation was carried out for 90 min in KRB medium at 37°C in the absence of glucose. Net glycerol release, expressed as μmoles/10^7 fat cells/90 min, varied from 0.5–1.5 μmoles for the 40–100-pl cells to 2–5 μmoles for the 200–400-pl cells and to 4–13 μmoles for the 400–600-pl fat cells. A positive correlation between the rate of glycerol release per fat cell and mean fat cell volume was found, with a correlation coefficient of +0.663 and a P value of <0.001 (see Table 1).

A third series of 16 experiments was carried out to determine the intracellular FFA concentration (expressed as μeq of FFA/10^7 fat cells) in fat cell preparations differing in mean volume from 30 to 800 pl, in the absence and presence of 6 mM glucose. **Fig. 1** shows that at the end of 1 hr of incubation at 37°C the intracellular FFA concentration was incremental with increasing mean fat cell volume. Addition of glucose to the medium during the incubation affected the intracellular FFA concentration to only a minimal degree. The correlation coefficients between the adipocyte volume and the intracellular FFA levels are shown in Table 1. A highly significant ($P < 0.001$) positive correlation between these two variates was found in both the absence and presence of glucose in the medium.

Since it has been reported (9) that elevation of the glucose concentration in the medium enhances glucose transport and metabolism in small fat cells from 150–200-g rats, we then proceeded to compare the effect of progressively increasing glucose concentrations in the medium on the rate and pattern of glucose metabolism, on the insulin response, and on the rate of lipolysis in selected prepara-

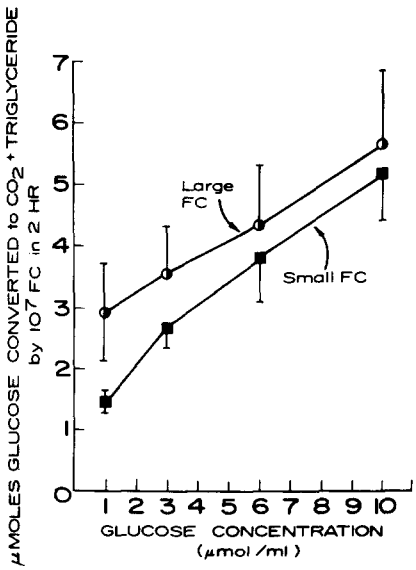

Fig. 2. Effect of increasing glucose concentration in the medium on the amounts of glucose metabolized to CO_2 + triglyceride by small (45 pl volume) and large (650 pl) fat cells (FC) of the rat. Values shown are means ± SEM of triplicate determinations for six experiments with isolated fat cells from epididymal pads of six lean (160 g) and six large (600 g) rats.

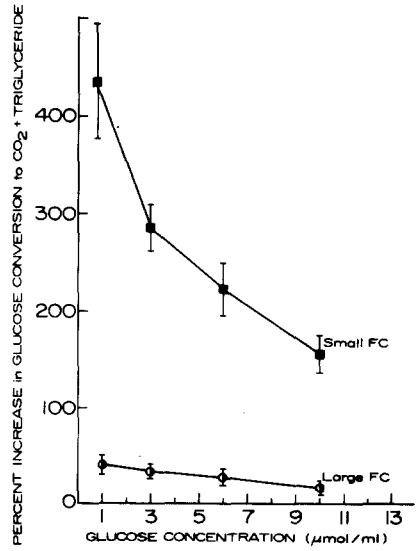

Fig. 3. Effect of insulin on glucose conversion to CO_2 + triglyceride by the fat cells (FC) plotted against glucose concentration in the medium. The effect of insulin is expressed as percent increment in glucose metabolism over base-line control values. Values shown are means ± SEM of triplicate determinations in six experiments with epididymal adipose cells from lean and large rats.

tions of epididymal fat cells of small (<100 pl volume) and large (>400 pl) sizes removed from lean 150–170-g rats and 550–650-g mature obese rats fed ad lib.

Fig. 2 shows the mean amounts of total glucose metabolized in the absence of insulin to CO_2 + triglycerides by equal numbers of small and large fat cells at each of the glucose concentrations employed. Even though no significant difference was found ($P > 0.05$) between analogous group means, the intercept of the regression line for the large fat cells was significantly different from that for the small cells. This would indicate a slightly greater glucose metabolism per cell in the large fat cells. Both small and large fat cells responded to increasing glucose concentration in the medium with increasing rates of glucose metabolism. The rates of the increment in glucose metabolism by each type of cell (small or large), observed with increasing glucose concentrations in the medium, were analyzed by regression analysis and by comparison of the regression coefficients. The increments in glucose metabolism produced by a change in glucose concentration from 3 to 10 μmoles/ml were statistically indistinguishable in small and large fat cells (regression coefficients were, respectively, 0.357 ± 0.011 [SEM] and 0.305 ± 0.021, $P > 0.05$). If the increments in glucose metabolism were compared when glucose concentration changed from 1 to 10 μmoles/ml, a minor divergence of the regression coefficient, of borderline statistical significance, was observed (regression coefficients were, respectively, 0.402 ± 0.035 in small fat cells and 0.303 ± 0.011 for the large cells, $0.05 > P > 0.025$).

The results indicate that large fat cells incubated in the absence of insulin have retained the capacity to take up and metabolize increasing amounts of glucose as the glucose concentration in the medium rises up to 10 mM. However, when the capacity of the large fat cells to respond to insulin with enhancement of glucose utilization was investigated, a marked curtailment of the insulin response was observed. In **Fig. 3** a comparison of the insulin response (expressed as percent increase of "insulin-stimulated" values over base-line control values) is shown for the large and small fat cells.

At each glucose concentration in the medium, the magnitude of the insulin response was significantly smaller ($P < 0.001$) in the large fat cells compared with the small cells. In addition, it was inversely related in both types of cells to the glucose concentration in the medium and to the basal values of glucose uptake and metabolism (see Fig. 2), a finding already reported by Gliemann (3) for small fat cells from 150–200-g rats and now also observed for the large fat cells of larger rats.

The effects of increasing glucose concentrations in the medium and of insulin addition (1 mU/ml) on the patterns of glucose metabolism by both small and large fat cells are shown in **Fig. 4.** The conversion of glucose to CO_2 + glyceride glycerol + glyceride fatty acids was enhanced to a degree in both small and large fat cells by variations in glucose concentration in the medium and/or addition of the hormone. However, the metabolic differences that distinguish glucose metabolism in the large fat cells (namely greater conversion of glucose to glyceride

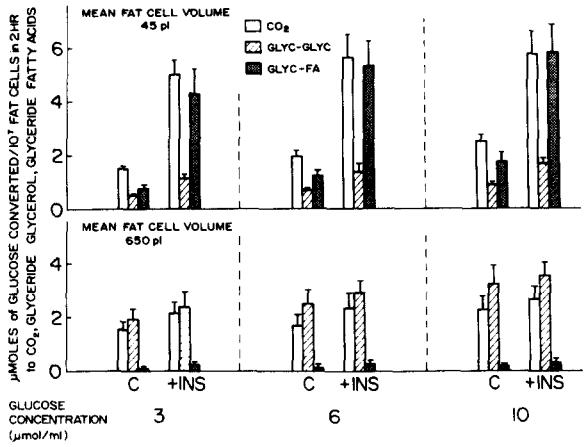

Fig. 4. Effects of increasing glucose concentration in the medium on the rate and pattern of glucose metabolism in the absence (control, C) and presence (+INS) of 1 mU/ml bovine insulin. Glucose conversion to CO_2, glyceride glycerol (GLYC-GLYC), and glyceride fatty acids (GLYC-FA) is indicated for each group of small fat cells (mean volume = 45 pl, upper panels) and of large fat cells (mean volume = 650 pl, lower panels). Values are means ± SEM of triplicate determinations in six animals at each of the glucose concentrations indicated.

glycerol and reduced lipogenesis) from that of the smaller fat cells were not significantly modified by either the increase in glucose concentration or the addition of insulin, or both.

When the rate of lipolysis, as measured by glycerol release into the medium during the incubation period, was compared in large and small fat cells, it was found that the large fat cells had a three- to fourfold greater rate of base-line lipolysis than the small fat cells ($P < 0.05$) at each of the glucose concentrations employed (**Table 2**). Insulin at 1 mU/ml concentration did not appear to affect the base-line rate of lipolysis to a significant extent. Again, neither addition of glucose to the medium in different concentrations nor addition of insulin was able to modify the metabolic differences in spontaneous rate of base-line lipolysis observed in the large and small fat cells.

TABLE 2. Rate of basal lipolysis during the incubation of small and large epididymal fat cells from 160- and 600-g rats

Group	Glucose Concentration	Net Glycerol Release	
		Control	+ Insulin (1 mU/ml)
	μmoles/ml	*μmoles/10^7 fat cells/90 min*[a]	
Small fat cells,[b]	0	0.73 ± 0.36	0.57 ± 0.58
n = 8	3	0.88 ± 0.43	1.10 ± 0.34
	12	1.39 ± 0.41	1.17 ± 0.28
Large fat cells,[b]			
n = 7	0	3.06 ± 0.71	2.97 ± 0.69
	3	3.90 ± 0.89	4.21 ± 1.69
	12	4.79 ± 1.59	5.53 ± 1.56

[a] Values are means ± SEM of duplicate determinations in number of animals shown for each group.

[b] Isolated fat cells were prepared from the epididymal pads as described in the text. Small fat cells had an average volume of 45 pl (range 36–60), large fat cells an average of 450 pl (range 336–637).

DISCUSSION

The present studies with isolated fat cells of the rat were carried out to investigate the relationship between cell size and metabolic capacities and cellular response to insulin in its effect on glucose utilization in an effort to clarify the mechanisms by which enlarging fat cells become less responsive to this effect of insulin (1–6). The data presented here deal with isolated fat cells of different sizes prepared from the epididymal fat pads of rats fed ad lib. and differing in age from 6 wk to 1 yr and in body weight from 150 to 650 g. We have used mean fat cell volume throughout these experiments to distinguish the cell preparations and to establish certain apparent correlations between metabolic parameters and cell size. However, factors other than adipocyte size, such as age (4), nutritional state of the animals (4, 16), and physical activity may affect glucose metabolism and insulin responsiveness of the fat cells. These factors and the present experimental conditions must be kept in mind for a proper interpretation of the results. Exposure of the cells to collagenase in the preincubation period could also have affected the subsequent metabolic performance of the isolated cells, but in this respect it is comforting to find a marked similarity between results shown here with isolated fat cells and those obtained with rat adipose tissue slices with regard to rate and pattern of glucose metabolism (4, 5) and basal rates of lipolysis (7, 8).

The results shown in Table 1 and Fig. 1 indicate that variations in the rate of basal lipolysis, in the intracellular FFA concentration, and in the rate of glyceride glycerol synthesis from glucose are incremental with increasing cell size; variations in the rate of lipogenesis (fatty acid synthesis) from glucose and in the magnitude of the insulin response are decremental with increasing cell size. In the studies shown in Fig. 2, in which selected populations of small (cell volume <100 pl) and large (>400 pl) fat cells were compared at different glucose concentrations in the medium, the large fat cells presented a rate of total glucose metabolism to CO_2 + triglycerides similar to but not identical with that of smaller cells; both types of cells responded in vitro to increasing glucose concentrations in the medium (from 3 to 10 mM) with progressive and similar increments in glucose metabolism.

These results and the recent report by Livingston, Cuatrecasas, and Lockwood (17) indicate that large fat cells have preserved the capacity to take up and metabolize glucose in response to elevations of glucose concentration in the medium. Thus, the reduced capacity of the large cells to metabolize more glucose in the presence of insulin cannot be ascribed to an absolute limitation of glucose metabolism but probably reflects an alteration in the cellular response to the action of insulin.

The data in this report do not permit us to define at what site and by what mechanism(s) the response to insulin of the large fat cells is altered. The site of such an alteration is speculative, but it may involve a loss of insulin recognition by a defective insulin receptor in large fat cells, as found in adipocytes of obese hyperglycemic mice (18) but not in adipocytes of obese rats (17); or it may involve a reduced transmission of a "signal" generated from the hormone–receptor interaction; or it may involve an alteration of the insulin-dependent glucose transport. This latter possibility should be tested with studies of glucose transport in isolated fat cells of different sizes, studies that may be difficult because of the small intracellular water space (19).

Of considerable interest are the alterations in the pattern of glucose metabolism, in the basal rate of lipolysis, and in the intracellular FFA levels that appear to be related to the mean fat cell volume. Fig. 4 and Table 2 show that the metabolic differences observed between small and large fat cells (with regard to the rates of glyceride glycerol synthesis and lipogenesis from glucose and to the rates of basal lipolysis) were not readily modified by the addition of either glucose or insulin to the incubation medium. Similarly, glucose did not seem to affect the level of intracellular FFA, which was linearly related to the cell volume (Fig. 1), a relation already observed by Cushman and Salans (20) with a different technique.

These correlations between adipocyte volume, hormonal responsiveness of the cells, and the described metabolic alterations raise the possibility (4, 5) that one or more of these alterations may, in an as yet unidentified way, interfere, as the cells enlarge, with the normal mechanisms of insulin action at one of the sites considered above. In this regard, it is noteworthy that acceleration of lipolysis produced in small fat cells of young rats by lipolytic hormones produces an elevation of intracellular free fatty acids (21), a pattern of glucose metabolism with an increased glyceride glycerol synthesis (22, 23), and an inhibition of the effect of insulin on glucose metabolism and of leucine incorporation into protein (21). The reported findings with small fat cells stimulated by lipolytic hormones are in many respects similar to those observed in the present report with nonstimulated enlarged fat cells and suggest that acceleration of lipolysis and/or elevation of intracellular FFA may play a role in the reduction of the insulin response. The physiological meaning of a mechanism linking cell size to metabolic alterations and to a diminution of the insulin effect on glucose utilization may be visualized as a feedback regulatory attempt in the enlarging fat cells to limit the insulin-stimulated processes of glucose transport, lipid synthesis, and storage, which, if unchecked, could threaten the integrity and survival of the cell itself.

More studies are needed to clarify whether any of these metabolic alterations in the enlarging fat cells are directly responsible for the curtailed insulin response or just coincidental. The results of the present studies raise a hypothesis that needs testing and would provide grounds for future study.

Supported in part by USPHS grant AM 13940 and by a grant-in-aid from the American Heart Association.

Manuscript received 12 March 1973 and in revised form 20 November 1973; accepted 6 March 1974.

REFERENCES

1. Salans, L. B., J. L. Knittle, and J. Hirsch. 1968. The role of adipose cell size and adipose tissue insulin sensitivity in the carbohydrate intolerance of human obesity. *J. Clin. Invest.* **47:** 153–165.
2. Gries, F. A., and J. Steinke. 1967. Comparative effects of insulin on adipose tissue segments and isolated fat cells of rat and man. *J. Clin. Invest.* **46:** 1413–1421.
3. Gliemann, J. 1965. Insulin-like activity of dilute human serum assayed by an isolated adipose cell method. *Diabetes.* **14:** 643–649.
4. DiGirolamo, M., and D. Rudman. 1968. Variations in glucose metabolism and sensitivity to insulin of the rat's adipose tissue, in relation to age and body weight. *Endocrinology.* **82:** 1133–1141.
5. DiGirolamo, M., and S. Mendlinger. 1972. Glucose metabolism and responsiveness to bovine insulin by adipose tissue from three mammalian species. *Diabetes.* **21:** 1151–1161.
6. Horton, E. S., E. Danforth, Jr., E. A. H. Sims, and L. B. Salans. 1972. Correlation of forearm muscle and adipose tissue metabolism in obesity before and after weight loss. *Clin. Res.* **20:** 548. (Abstr.)
7. DiGirolamo, M., and S. Mendlinger. 1969. Unstimulated rate of lipolysis and FFA reesterification in lean and obese rat fat cells. *Clin. Res.* **17:** 43. (Abstr.)
8. Hartman, A. D., A. I. Cohen, C. J. Richane, and T. Hsu. 1971. Lipolytic response and adenyl cyclase activity of rat adipocytes as related to cell size. *J. Lipid Res.* **12:** 498–505.
9. Jeanrenaud, B., and A. E. Renold. 1959. Studies on rat adipose tissue in vitro. IV. Metabolic patterns produced in rat adipose tissue by varying insulin and glucose concentrations independently from each other. *J. Biol. Chem.* **234:** 3082–3087.
10. DiGirolamo, M. 1972. Fat cell size, metabolic capacities and hormonal responsiveness of adipose tissue in spontaneous obesity in the rat. *Israel J. Med. Sci.* **8:** 807.
11. DiGirolamo, M., M. D. Howe, J. Esposito, and L. Thurman. 1972. Variations in patterns of glucose metabolism, basal rate of lipolysis and unresponsiveness to insulin of enlarged adipocytes. Program, 4th International Congress of Endocrinology, abstr. no. 414. Excerpta Med. Found. Int. Congr. Ser. no. 256.
12. Rodbell, M. 1964. Metabolism of isolated fat cells. I. Effects of hormones on glucose metabolism and lipolysis. *J. Biol. Chem.* **239:** 375–380.
13. Eggstein, M., and F. H. Kreutz. 1966. Eine neue Bestimmung der Neutralfette im Blutserum and Gewebe. Prinzip, Durchführung und Besprechung der Methode. *Klin. Wochenschr.* **44:** 262–267.

14. Angel, A., K. S. Desai, and M. L. Halperin. 1971. Intracellular accumulation of free fatty acids in isolated white adipose cells. *J. Lipid Res.* **12:** 104–111.
15. DiGirolamo, M., S. Mendlinger, and J. W. Fertig. 1971. A simple method to determine fat cell size and number in four mammalian species. *Amer. J. Physiol.* **221:** 850–858.
16. Salans, L. B., and J. W. Dougherty. 1971. The effect of insulin upon glucose metabolism by adipose cells of different size. Influence of cell lipid and protein content, age, and nutritional state. *J. Clin. Invest.* **50:** 1399–1410.
17. Livingston, J. N., P. Cuatrecasas, and D. H. Lockwood. 1972. Insulin insensitivity of large fat cells. *Science.* **177:** 626–628.
18. Freychet, P., M. H. Laudat, P. Laudat, G. Rosselin, C. R. Kahn, P. Gorden, and J. Roth. 1972. Impairment of insulin binding to the fat cell plasma membrane in the obese hyperglycemic mouse. *FEBS Lett.* **25:** 339–342.
19. Crofford, O. B., W. Stauffacher, B. Jeanrenaud, and A. E. Renold. 1966. Glucose transport in isolated fat cells. Procedures for measurement of the intracellular water space. *Helv. Physiol. Pharmacol. Acta.* **24:** 45–57.
20. Cushman, S. W., and L. B. Salans. 1973. Cell associated fatty acids: a role in the mediation of cell size effects on adipose cell function. *Clin. Res.* **21:** 620. (Abstr.)
21. Fain, J. N., and L. Rosenberg. 1972. Antilipolytic action of insulin on fat cells. *Diabetes.* **21(Suppl. 2):** 414–425.
22. Cahill, G. F., Jr., B. Leboeuf, and R. B. Flinn. 1960. Studies on rat adipose tissue in vitro. VI. Effect of epinephrine on glucose metabolism. *J. Biol. Chem.* **235:** 1246–1250.
23. Lynn, W. S., R. M. MacLeod, and R. H. Brown. 1960. Effects of epinephrine, insulin, and corticotrophin on the metabolism of rat adipose tissue. *J. Biol. Chem.* **235:** 1904–1911.