Insulin insensitivity and altered glucose utilization in cultured rat adipose tissue

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Abstract Glucose utilization was studied in isolated fat cells prepared from rat adipose tissue which had been cultured for 18 hr in TC 199 medium. When 1% bovine serum albumin (BSA) was in the culture medium, basal rates of $^{14}CO_2$ and [^{14}C]triglyceride production from [1-^{14}C]glucose were markedly depressed and there was no effect of insulin. With 4% BSA, basal $^{14}CO_2$ production was the same as in cells prepared from fresh tissue and basal triglyceride production was greatly increased. Insulin effect on these cells was minimal. One-minute uptake of [^{14}C]2-deoxyglucose was stimulated by 800–1000% in fresh cells and 300–500% in cells cultured with either 1% or 4% BSA. Oxidation of [U-^{14}C]glucose showed a much smaller impairment in cultured cells than for [1-^{14}C]glucose, suggesting that the pentose phosphate shunt was more severely impaired than glycolysis. Glyceride-glycerol production was increased in cultured cells relative to preculture (fresh) cells. There was no effect of insulin in the culture medium in any of these systems. Rates of free fatty acid and glycerol release were markedly increased in cultured cells, especially when insulin was present in the culture medium. The acute antilipolytic effect of insulin was retained, so that insulin in the test incubation decreased lipolysis by 40–80%. Nevertheless, cell-associated fatty acids were increased in cultured cells and FFA/albumin ratios in the medium often reached potentially toxic levels. The reduction in pentose phosphate shunt activity, lipogenesis, and insulin effect resembles other models of insulin insensitivity. The impaired metabolism is probably due to an intracellular defect. A possible toxic role of either intracellular or extracellular fatty acids cannot be excluded. This system should be a useful model in which to study the cellular mechanisms of insulin insensitivity in adipocytes.—**Bernstein, R. S.** Insulin insensitivity and altered glucose utilization in cultured rat adipose tissue. *J. Lipid Res.* 1979. **20:** 848–856.

Supplementary key words 2-deoxyglucose · pentose phosphate shunt · lipogenesis

Adipose tissue from young rats has long been recognized as an insulin-responsive tissue. However many metabolic conditions are known to impair the ability of the fat cell to respond to insulin, including fasting (1, 2), high-fat diets (3–6), glucocorticoids (7–11), and enlargement of fat cells with normal aging (2, 12–15). In all of these conditions there are multiple hormonal changes secondary to the initial alteration, and understanding of the mechanisms of hormone resistance has been hampered as a result. For this reason, our laboratory has turned to an in vitro model of adipose tissue metabolism.

We have worked with the adipose tissue culture system developed by Smith (16, 17) because it has maintained metabolically active human adipose tissue for up to 14 days. He has reported that hormonal sensitivity of the human tissue is maintained during culture in this medium (18). In our laboratory we have studied the regulation of hexokinase-II, an adaptive enzyme in adipose tissue, and have found that it responds appropriately in tissue from fasted rats to sugars, insulin, and glucocorticoids (19, 20). Glucose utilization was present but diminished and insulin responsiveness was absent, as would be expected in fasted tissue. The present study extends these findings to the alterations in glucose utilization and lipolysis in cultured adipose tissue from fed rats, and explores the effects of substrates, insulin, and albumin in the culture medium.

MATERIALS AND METHODS

In all studies, epididymal adipose tissue from chow-fed male Wistar rats weighing 175–200 g was used. Food was available up to the time of killing. The rats were decapitated and fat pads were excised distal to the major blood vessel under sterile conditions. Adipose tissue from 6–10 rats was pooled, cut into pieces of approximately 50 mg, and distributed to 25-ml polycarbonate Ehrlenmeyer flasks for either 18-hr culture or immediate separation into isolated adipocytes. A total of 200–400 mg of tissue was present in each culture flask. The 18-hr culture (20) was performed in 10 ml of TC 199 medium with Hanks salt and

Abbreviations: FFA, free fatty acids; BSA, bovine serum albumin.

without glucose (specially prepared by Gibco, Grand Island, NY) containing 0.3 mg of *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid (HEPES; Sigma, St. Louis, MO), 5000 U of penicillin G, and 50 μg of streptozotocin/ml. Appropriate amounts of bovine serum albumin (BSA) were added prior to adjustment of pH to 7.4 and sterilization through 0.22 μm Millipore filters. Sterile insulin and glucose solutions were added to individual flasks where appropriate to achieve a final concentration of 300 μU/ml and 3 mg/ml, respectively. The flasks were incubated for 18 hr at 37°C in a Dubnoff incubator with air as the gas phase.

The cultured tissue and the fresh (preculture) aliquots were prepared into isolated adipocytes by collagenase digestion (21). Subsequent 2-hr incubations with 3 mM [^{14}C]glucose were performed at 37°C in Krebs-Ringer bicarbonate buffer containing 0 or 250 μU insulin/ml. The BSA concentration was the same as in the culture medium. $^{14}CO_2$ and [^{14}C]-triglycerides were measured by previous methods (10). In some experiments the [^{14}C]triglyceride was hydrolyzed for 1 hr at 80°C in alcoholic KOH. ^{14}C-Labeled fatty acids were extracted with 3 ml of heptane after acidification, evaporated to dryness, dissolved in 10 ml of Bray's solution, and counted as above (10). Hexokinase isoenzymes (22) and adipose cell triglyceride (23) were assayed by previous methods.

Transport of [^{14}C]-2-deoxyglucose was measured by the oil separation technique (24) during 1-min incubations. In these studies, 0.1 ml of a fat cell suspension in Krebs-Ringer phosphate buffer without glucose was preincubated for 45–60 min with 0 or 250 μU insulin/ml at 37°C. Buffer (0.2 ml) containing isotopes was then added, and the suspension was incubated for 1 min at 37°C prior to separation. Each tube contained 0.2 μCi of [^{14}C]-2-deoxyglucose with a final concentration of 0.3 mM. ^{3}H-Labeled inulin (1.2 μCi) was present to correct for trapped extracellular 2-deoxyglucose.

In some experiments lipolysis was studied in preculture and cultured adipocytes, as well as cumulative lipolysis in tissue pieces at the end of culture. For isolated cells, 2 ml of cell suspension containing 40–80 mg of fat-cell triglyceride was incubated for 2 hr in Krebs-Ringer bicarbonate buffer containing 3 mM glucose, appropriate concentrations of BSA and insulin, and 4 μCi of [^{14}C]sucrose as an extracellular marker. At the end of the incubation the cells were separated from the medium by centrifugation, the infranatant medium was aspirated, and the cell float was mixed with 5 ml of isopropyl alcohol–heptane–1 N H_2SO_4 40:10:1 (v/v/v) (Dole's solution) and 1 ml of water and transferred to glass-stoppered centrifuge tubes for extraction. For fat pad pieces, 4 μCi of [^{14}C]sucrose was added to each flask 1 hr before the end of the culture. At the end of the culture, tissue pieces were removed, blotted, and homogenized in 5 ml of Dole's solution and 1 ml of water, and the homogenate was transferred to glass-stoppered centrifuge tubes. After addition of 3 ml each of heptane and water to both preparations, the tubes were centrifuged. The heptane phase was removed, washed with acidified water, and saved for free fatty acid (FFA) determination. One ml of the aqueous phase was mixed with 10 ml of Instagel (Packard Instruments) for determination of [^{14}C]sucrose. Aliquots (0.5 ml) of the incubation and culture media from these experiments were also extracted with Dole's solution for FFA determinations. The remainder of the medium was saved for ^{14}C counting and enzymatic glycerol determination (25). FFA was measured by titration against 0.02 N hyamine using thymol blue as an indicator (26). Cell-associated fatty acids were determined by correcting the FFA measurement in the tissue or cell float for trapped medium, as determined by FFA/^{14}C ratios in the medium (27).

In all cultures, metabolic studies were performed in both preculture and cultured tissue from the same batch. Results of single experiments were compared by Student's *t* test. Results of multiple experiments of identical design were subjected to analysis of variance to eliminate the effects of different batches of animals.

All reagents were of the highest purity available. The bovine serum albumin (Reheis, Lot #R58706) and collagenase (Sigma Type I, Lot #126C-0435) used were selected from all available lots to give the lowest basal glucose utilization and greatest insulin stimulation. As can be seen from Figs. 2 and 3, there was no difference in glucose utilization in preculture cells between 1% and 4% BSA.

RESULTS

Initial studies of [1-^{14}C]glucose utilization were performed using 1% BSA in the culture and incubation media. In this, as in previous studies, active glucose utilization and stimulation by insulin were seen in fresh ("preculture") adipocytes in Krebs-Ringer bicarbonate buffer with 1% BSA. In contrast, after 18-hr culture of tissue pieces in modified TC 199 medium, regardless of the presence of glucose or insulin, there was markedly diminished basal [^{14}C]glucose metabolism and no stimulation by insulin in subsequent 2-hr incubations of adipocytes made from this tissue (**Fig. 1**). Rates of both $^{14}CO_2$ and [^{14}C]triglyceride formation were higher if glucose was present in the culture medium than in any of the other conditions tested.

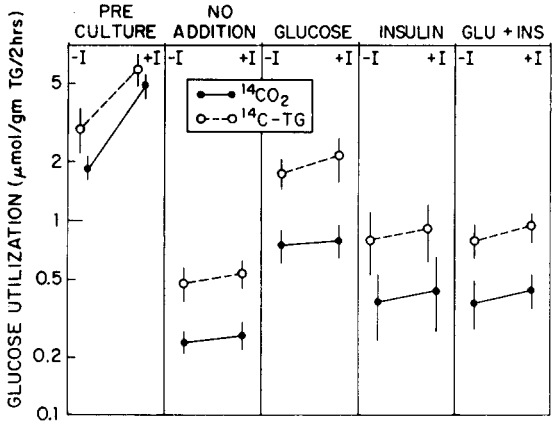

Fig. 1. [1-^{14}C]Glucose utilization in preculture and cultured adipocytes in medium containing 1% BSA. Values are mean ± SEM of five experiments.

The presence of both glucose and insulin or insulin alone did not increase the rate of production of either metabolite above that seen in the absence of glucose and insulin.

Hexokinase activity also decreased after culture in medium containing 1% BSA (**Table 1**). As expected from our previous studies (19, 20), the change in hexokinase-II was greater than that in hexokinase-I. As with glucose utilization, hexokinase-II activity was higher after culture with glucose than with other conditions, but still lower than the preculture enzyme levels. Hexokinase-I activity was significantly reduced from preculture only in tissue incubated without glucose. There was no effect of insulin either with or without glucose.

Because of the possibility that accumulated fatty acids could be responsible for the diminished glucose utilization, these studies were repeated using 4% BSA as a fatty acid acceptor in both the culture and test incubation media. Fresh tissue with 4% BSA behaved similarly to fresh tissue with 1% BSA. Unlike the 1% BSA experiments, the basal rate of glucose oxidation after culture in 4% BSA was the same as before culture, and there was no difference between tissue cultured with or without insulin (**Fig. 2**). However cultured cells had only a 20% increase in glucose oxidation in response to insulin in the test incubation, compared to a 250% increase in the preculture cells. Incorporation of [1-^{14}C]glucose into triglyceride was markedly increased in the cultured cells (**Fig. 3**). Basal rates of glyceride formation in cultured cells were the same as insulin-stimulated rates in the preculture cells. Once again, however, the response to insulin in the cultured cells was only 20%, compared to 440% in the preculture cells. In both 1% and 4% albumin, a much higher proportion of the label went into [^{14}C]-triglyceride relative to $^{14}CO_2$ than in the preculture cells (**Fig. 4**). Unlike the studies with 1% BSA, there was no change in activity of either hexokinase isoenzyme after culture in 4% BSA. (Table 1).

Accumulation of [^{14}C]-2-deoxyglucose during a 1-min incubation was measured to determine whether the impaired glucose utilization was due to changes in glucose transport (**Fig. 5**). This sugar analog is transported and phosphorylated by the same mechanisms as glucose, but subsequently can only be oxidized to the 6-phosphogluconate. Uptake has been shown to be linear for 3 min (24). Chromatography has shown that most of the sugar that enters the cell is rapidly phosphorylated (12), so that early uptake can be used as a probe of the glucose carrier. In preculture cells, insulin stimulated 2-deoxyglucose transport by 800 and 1000%, respectively, in 1% and 4% BSA. 2-Deoxyglucose transport in the absence of insulin was significantly elevated in cells cultured in 4% BSA with glucose alone ($P < 0.001$ by analysis of variance) and was significantly reduced in cells cultured in 1% BSA with

TABLE 1. Hexokinase isoenzymes in adipocytes from preculture and cultured adipose tissue pieces

Condition	1% BSA		4% BSA	
	Hexokinase-I	Hexokinase-II	Hexokinase-I	Hexokinase-II
	mU/g triglyceride			
Preculture	82.0 ± 15.3	300.8 ± 28.3	89.6 ± 30.3	263.4 ± 95.7
Cultured				
No addition	28.8 ± 3.4[b]	87.0 ± 12.1[c]		
Glucose 3 mg/ml	68.2 ± 10.0[e]	187.2 ± 28.6[a,d]	84.6 ± 5.0	276.8 ± 63.6
Insulin 300 μU/ml	33.2 ± 10.6[a]	76.0 ± 27.5[b]		
Gluc. + Ins.	59.4 ± 7.1[e]	131.2 ± 20.7[a]	108.6 ± 21.6	246.2 ± 46.9

[a] $P < 0.05$ vs. preculture.
[b] $P < 0.01$ vs. preculture.
[c] $P < 0.001$ vs. preculture.
[d] $P < 0.05$ vs. no addition.
[e] $P < 0.01$ vs. no addition.
Values are mean ± SEM of five experiments.

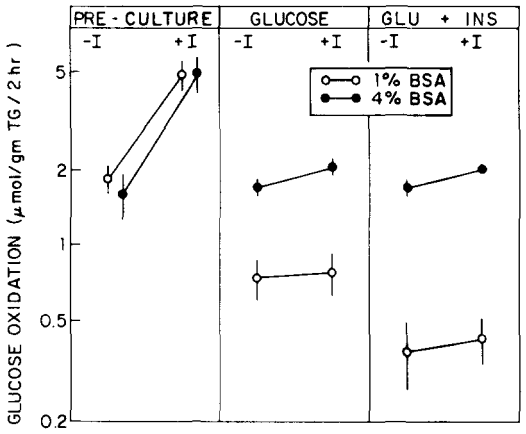

Fig. 2. [1-^{14}C]Glucose oxidation in preculture and cultured adipocytes in media with 1% or 4% BSA. The values for 1% BSA are from the studies in Fig. 1, whereas the values for 4% BSA are the mean ± SEM of five different experiments.

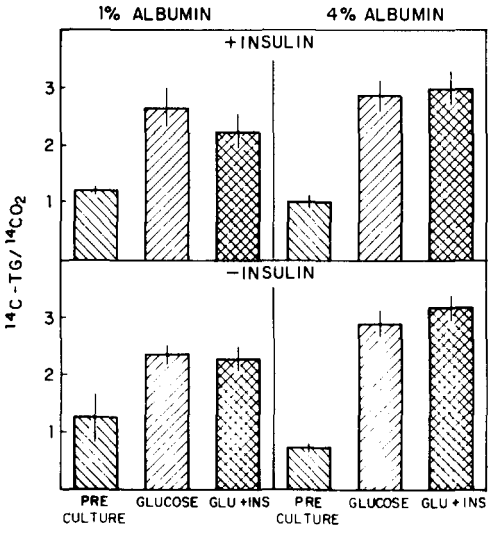

Fig. 4. Ratio of [^{14}C]triglyceride formation to $^{14}CO_2$ formation in the experiments for Figs. 2 and 3.

glucose and insulin, compared to the appropriate preculture cells. Insulin enhanced transport by 300–500% in the various cultured cell preparations. This stimulation was lower than the preculture cells, but much greater than the 20% stimulation of [1-^{14}C]glucose utilization. Cells cultured with glucose alone had higher rates of transport than those cultured with glucose and insulin for both concentrations of BSA, and cells cultured with 4% BSA had slightly higher rates of transport than those cultured with 1% BSA. However the difference between 1% and 4% BSA is not of the order of magnitude of the differences in utilization of [1-^{14}C]glucose.

Since [1-^{14}C]glucose oxidation predominantly reflects pentose phosphate shunt activity, we next studied [U-^{14}C]glucose utilization. Triglycerides were hydrolyzed to see whether the label was in the fatty-acid or glycerol moiety. Patterns of [U-^{14}C]glucose utilization were very similar in both 1% and 4% BSA (**Table 2**). Under both conditions basal oxidation was somewhat elevated relative to preculture cells, but insulin stimulation was suppressed. Basal levels of fatty acid production were similar in all cultured and preculture cells, but the insulin effect was seen only in the preculture tissue. There was also a trend toward an increase in fatty acid synthesis with insulin in the cultured cells, especially in 4% albumin, but the effect was variable and not statistically significant. Glyceride-glycerol production was much higher for the cultured cells than for the preculture. For all metabolites the incorporation in cultured cells was greater in 4% BSA than 1% BSA, but the difference was not as great as for [1-^{14}C]glucose. Insulin effects were greater in 4% BSA.

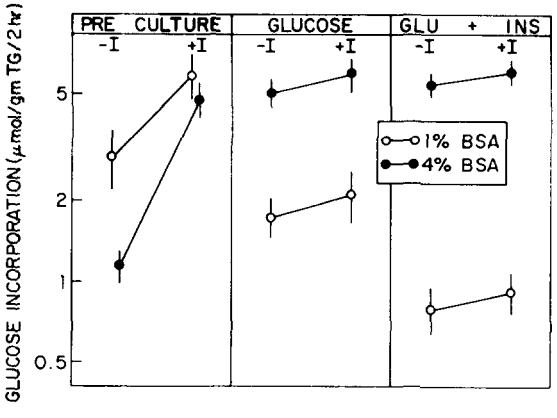

Fig. 3. [1-^{14}C]Glucose incoporation into triglyceride in preculture and cultured adipocytes in media with 1% and 4% BSA. The experiments are the same as in Fig. 2.

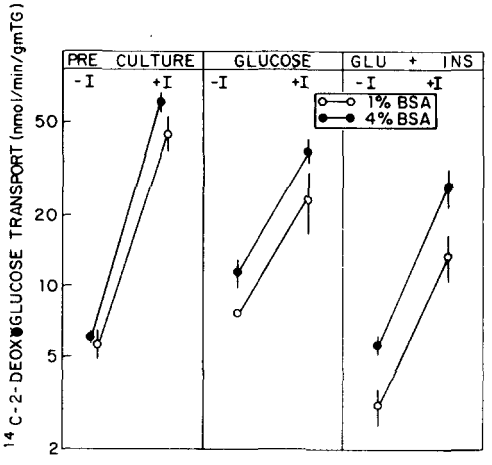

Fig. 5. 2-Deoxyglucose transport in preculture and cultured adipocytes in media with 1% and 4% BSA. Values are mean ± SEM of five experiments, each of which included all conditions shown.

TABLE 2. Effect of 18-hr culture of adipose tissue with glucose (3 mg/ml) on utilization of [U-^{14}C]glucose

	$^{14}CO_2$	Glyceride-FA	Glyceride-Glycerol
		μmol/g triglyceride	
Preculture–1% albumin			
−Insulin	2.90 ± 0.47	2.00 ± 0.25	0.92 ± 0.06
+Insulin	6.91 ± 2.04[a]	7.56 ± 2.52[b]	1.40 ± 0.17
Cultured–1% albumin			
−Insulin	3.73 ± 0.73	2.04 ± 0.63	4.05 ± 0.66[d]
+Insulin	4.61 ± 0.35	2.89 ± 0.81[c]	3.16 ± 0.80[c]
Preculture–4% albumin			
−Insulin	2.29 ± 0.48	1.20 ± 0.24	1.02 ± 0.20
+Insulin	8.63 ± 3.09[a]	7.80 ± 2.67[b]	2.13 ± 0.61
Cultured–4% albumin			
−Insulin	4.64 ± 0.53	1.67 ± 0.17	5.15 ± 0.71[d]
+Insulin	6.77 ± 1.60	3.73 ± 1.04[c]	5.38 ± 0.49[d]

[a] $P < 0.05$ vs. −insulin.
[b] $P < 0.01$ vs. −insulin.
[c] $P < 0.05$ vs. preculture.
[d] $P < 0.01$ vs. preculture.
Values are mean ± SEM of three experiments. P values are from analysis of variance.

Because of the possible deleterious effects of free fatty acid accumulation, control of lipolysis was studied in this system (**Table 3**). In both 1% and 4% BSA, there was rapid lipolysis in isolated fat cells prepared from cultured adipose tissue. Unlike the effect on glucose utilization, the antilipolytic effect of insulin is preserved in these cells, both in terms of glycerol and FFA release. In contrast, there was no insulin effect on

TABLE 3. Effect of 18-hr culture of adipose tissue on lipolysis

	Insulin during					
	Culture	Test Incubation	FFA Release	Glycerol Release	Cell-associated Fatty Acid	FFA/Albumin
			μEq/g TG/hr	*μmol/g TG/hr*	*μEq/g TG*	*molar ratio*
1% BSA						
Preculture		0	5.54 ± 1.24	0.95 ± 0.26	2.02 ± 0.19	0.76 ± 0.17
		+	5.61 ± 0.78	1.66 ± 0.07	1.89 ± 0.31	0.77 ± 0.11
During culture	0		0.97 ± 0.11	2.05 ± 0.65	2.25 ± 0.34	1.33 ± 0.05
	+		1.01 ± 0.07	2.11 ± 0.42	2.56 ± 0.32	1.24 ± 0.17
Postculture	0	0	28.32 ± 2.49	16.58 ± 0.56	5.66 ± 0.68	2.27 ± 0.20
	0	+	15.39 ± 2.49	8.99 ± 0.36	4.40 ± 0.56	1.23 ± 0.20
	+	0	49.30 ± 2.66	28.06 ± 2.91	4.91 ± 0.41	7.46 ± 0.40
	+	+	10.34 ± 1.70	11.08 ± 1.32	2.19 ± 0.56	1.56 ± 0.26
4% BSA						
Preculture		0	24.80 ± 3.86	8.87 ± 1.40	1.89 ± 0.31	1.24 ± 0.19
		+	9.36 ± 0.38	8.63 ± 0.51	2.07 ± 0.21	0.46 ± 0.02
During culture	0		0.96 ± 0.31	1.26 ± 0.26	3.23 ± 0.59	0.79 ± 0.04
	+		0.78 ± 0.15	0.77 ± 0.13	3.15 ± 0.63	0.62 ± 0.07
Postculture	0	0	80.13 ± 1.46	25.17 ± 1.02	6.49 ± 0.17	5.95 ± 0.11
	0	+	50.04 ± 0.61	18.54 ± 0.84	3.00 ± 0.63	3.71 ± 0.04
	+	0	161.82 ± 3.26	48.81 ± 0.44	7.13 ± 0.76	5.25 ± 0.10
	+	+	82.80 ± 3.43	27.63 ± 1.14	8.35 ± 1.64	2.69 ± 0.11

Separate experiments were performed with 1% BSA and 4% BSA.
Values are mean ± SEM of three replicates. Preculture and postculture values were derived on adipocytes. During-culture values are on adipose tissue pieces and medium sampled at the end of the 18-hr culture.

TABLE 4. Lipogenesis and lipolysis in cultured adipose tissue pieces in medium containing 4% BSA

	Preculture		Postculture	
	−Insulin	+Insulin	−Insulin	+Insulin
	μmol/g TG			
Incorporation of [U-^{14}C]glucose into				
Glyceride-glycerol	0.54 ± 0.04	1.26 ± 0.11[b]	1.81 ± 0.17[c]	1.61 ± 0.07[c]
Glyceride-fatty acid	0.42 ± 0.06	2.55 ± 0.27[b]	0.18 ± 0.03[d]	0.82 ± 0.16[a,e]
	μEq/g TG			
FFA release	10.73 ± 1.79	8.90 ± 2.12	18.61 ± 2.20[c]	8.75 ± 1.33[a]

[a] $P < 0.01$ vs. −insulin.
[b] $P < 0.001$ vs. −insulin.
[c] $P < 0.05$ vs. preculture.
[d] $P < 0.01$ vs. preculture.
[e] $P < 0.001$ vs. preculture.
Values are mean ± SEM of six replicates.

the low rate of lipolysis in the preculture cells in 1% BSA. The apparent antilipolytic effect seen in FFA release in preculture cells tested in 4% albumin is probably due to reesterification, since glycerol release is not inhibited. Cell-associated fatty acids tended to rise during culture, but there was no accumulation in the culture medium. In contrast, both cell-associated fatty acids and FFA/albumin ratios were very high during the incubation of cultured cells. These were elevated in both albumin concentrations, and thus cannot be the cause of the differential effects of the two culture conditions. However the high levels of cell-associated fatty acids might be responsible for increased re-esterification with consequent glucose incorporation into glyceride-glycerol. As in human adipose tissue (28), the presence of insulin in the culture medium enhanced subsequent lipolysis.

The increased lipolytic rates were found after isolation of cells and washing. In order to determine the effect of this procedure, rates of [U-^{14}C]glucose incorporation into lipids and of FFA release were determined during incubations of fat pad pieces in culture medium for the initial and final 2 hr of culture (**Table 4**). It is clear that the metabolic alterations had already begun prior to collagenase treatment. Glyceride-fatty acid synthesis was reduced, glyceride-glycerol synthesis was increased, and the insulin effect was reduced. In contrast, basal lipolysis was enhanced and the insulin effect was intact in the cultured tissue. Thus the changes were due to culture, not to isolation of cells.

DISCUSSION

Studies of human adipose tissue by Smith and his collaborators have shown that the culture medium used in those experiments preserves viability and that, if insulin is omitted from the culture medium, antilipolytic and lipogenic effects of the hormone on the tissue are preserved (17, 18, 28). No comparison was made between the responses of fresh and cultured human tissue, but the magnitude of the response was similar to that seen by other investigators in fresh human tissue. In contrast, the studies reported here on rat tissue demonstrate a loss of insulin responsiveness in some pathways, and a marked change in the metabolism of the fat cell. The reason for these discrepancies is not clear, but it may be that the human adipose tissue begins with impaired glucose utilization relative to the rat (29), and is unable to further reduce its response.

Changes in the cultured rat tissue include: *a*) decreased $^{14}CO_2$ production from [1-^{14}C]-labeled glucose, indicating reduced pentose phosphate shunt activity; *b*) decreased fatty acid synthesis from glucose; *c*) increased rates of glyceride-glycerol formation; *d)* reduced effectiveness of insulin on all pathways of glucose utilization; and *e*) increased lipolysis with preservation or enhancement of the antilipolytic effects of insulin. These changes are qualitatively similar to those seen in rats on high-fat diets (3, 4) and in old rats with enlarged adipocytes (2, 30), and it is tempting to postulate a common cellular mechanism for the alterations in these three models. In this case, study of the factors that influence the cultured rat tissue may shed light on the causes of the more physiological alterations described previously.

The metabolic changes in the cultured tissue are probably the result of one or more intracellular alterations. It is unlikely that there were large changes in insulin receptors, since the effects of the hormone on 2-deoxyglucose uptake and lipolysis were largely intact. In particular, the 3- to 5-fold stimulation of transport should have been sufficient to supply sub-

strate for the pentose phosphate shunt. Furthermore, although reduced hexokinase-II (10, 20, 22) might play a role in the cultures with 1% BSA, the enzyme was unchanged from preculture levels in 4% BSA. Thus the reduced pentose phosphate shunt activity was most likely due to loss of some component of the rate-limiting glucose-6-phosphate dehydrogenase reaction, i.e., either loss or inhibition of the enzyme, reduction in NADP levels, or removal of glucose-6-phosphate into a more favorable pathway. The formation of other metabolic products, such as glyceride-glycerol or CO_2 from pyruvate oxidation, is increased under basal conditions. As with 2-deoxyglucose transport, the insulin response was reduced but, once again, oxidation of [U-^{14}C]glucose was not impaired to the same extent that of [1-^{14}C]glucose. The reduced insulin effect on fatty acid synthesis is consistent with other models, but the rates of lipogenesis seen were greater than we see in high-fat diets using the same methodology (6), probably because of specific inhibition of pyruvate dehydrogenase and enzymes of lipogenesis (4, 31–33) in the latter model.

The absence of an insulin effect on glucose oxidation is at variance with the study of Livingston, Purvis, and Lockwood (34) using a similar culture system. In part, this might be due to different culture conditions, such as the use of 95% O_2–5% CO_2 in the latter study. However a more likely explanation is that the test incubations in the study of Livingston et al. were performed with 0.1 mM glucose, whereas this study was conducted with 3 mM glucose. The similar curves for transport and oxidation suggest that transport may be rate limiting in the presence of low glucose concentrations but that an intracellular step becomes saturated when medium glucose is in the physiological range. Preservation of insulin response at low glucose concentrations is also seen in large fat cells from old rats (12).

The reason for the effect of BSA concentration is not clear. Most of the increased triglyceride production is due to glyceride-glycerol formation. Since glycerol phosphate produced in the pentose phosphate shunt from [1-^{14}C]-labeled glucose is not labeled, this must be due to increased flux through the glycolytic pathway as far as the triose phosphates. This might be from a direct stimulation of re-esterification by elevated levels of intracellular fatty acids. However the improved [1-^{14}C]glucose oxidation, 2-deoxyglucose transport, and hexokinase activity with 4% BSA imply a more general effect. It is possible that this is due to a contaminating smaller molecule rather than to the albumin itself. In either case, the stimulator must be different from insulin or nonsuppressible insulin-like activity (35), since there is no difference in basal glucose utilization between 1% and 4% BSA.

The relation of increased lipolysis to the impaired glucose utilization is intriguing. Glucose metabolism might be reduced by a detergent effect of extracellular fatty acids on the cell membrane or by increased intracellular accumulation of the fatty acids or some derivative. Certainly the levels of fatty acids achieved in the medium during the test incubation of cultured cells could be toxic. However the extracellular fatty acids are not increased during culture, whereas glucose utilization is impaired. In contrast, cell-associated fatty acids begin to rise during the culture. High levels of fatty acyl CoA have been shown to impair glucose-6-phosphate dehydrogenase (36) and acetyl CoA carboxylase (37), although this may not be valid under physiological conditions. It will be of interest to see whether antilipolytic agents such as nicotinic acid can restore glucose utilization to normal.

The long-term effects of insulin in vitro deserve comment. As in human adipose tissue (18), rat adipose tissue cultured with insulin displays increased rates of lipolysis. In contrast, exposure to insulin causes no alteration in glucose utilization. The cultures were performed with 300 μU insulin/ml because this concentration of hormone in culture was shown to cause maximal increase in hexokinase-II activity (19). Insulin was not measured in the medium at the end of culture, but would be likely to be lower because of proteolytic activity in the fat cells. Indeed, a reduction of 60–90% was seen by Livingston et al. (34). Thus it is possible that a higher insulin concentration in the culture medium would have altered glucose utilization. However the physiological significance of exposure to such high amounts of insulin is questionable.

Unlike the human tissue (18), the antilipolytic effect of insulin is not reduced by prior exposure to the hormone. It has been shown that culture of IM-9 lymphocytes in the presence of insulin reduces the number of insulin receptors (38). Other, less direct, evidence also suggests a "down regulation" of insulin receptors in vivo by insulin (39–41). Reduction in the number of insulin receptors would not necessarily reduce the maximum insulin response. Because occupancy of only 10% of the receptors is necessary for full action (11, 42), reduced receptor number might merely shift the insulin dose-response curve to the right. This has recently been demonstrated in a similar culture system (43). The insulin dose in this study (300 μU/ml) is well below the amount necessary to reduce high-affinity insulin receptors (34, 43) in fat cells.

Current work in this laboratory is devoted to improv-

ing the insulin responsiveness of cultured adipose tissue. If this can be accomplished, the cellular alterations will provide a model for the regulation of adipocyte glucose metabolism in insulin insensitivity.

Supported by NIH grant AM-17200. This work was performed during Dr. Bernstein's tenure as Otto G. Storm Investigator of the American Heart Association.

Manuscript received 5 February 1979; accepted 4 May 1979.

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