Improved insulin responsiveness in rat adipose tissue pieces cultured with charcoal-treated albumin and oxygen

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Summary In an effort to simulate the effects of insulin on fat cells prepared from fresh adipose tissue, pieces of epididymal adipose tissue were cultured in a medium containing charcoal-treated bovine serum albumin (BSA) with a gas phase of 100% O_2. Using this system, the insulin effect on [1-^{14}C]glucose oxidation was retained, in contrast to previous results in culture with untreated BSA in room air. Basal [1-^{14}C]glucose oxidation was similar to fresh tissue, and insulin stimulated oxidation by 137%. In contrast to the effects of this culture system on [1-^{14}C]glucose oxidation, tissue cultured with charcoal-treated BSA had lower basal rates of [U-^{14}C]glucose utilization and 2-deoxyglucose uptake than either cells from fresh tissue or from tissue cultured with untreated BSA. The insulin effect on both of these measures was similar for the two culture systems and lower than for fresh tissues. Rates of lipolysis were increased in both types of cultured fat cells. Thus the improvement in [1-^{14}C]glucose oxidation is presumably an effect on the pentose phosphate shunt, does not reflect a change in glucose transport or overall glucose utilization, and is not caused by a reduction in free fatty acid levels.—**Bernstein, R. S.** Improved insulin responsiveness in rat adipose tissue pieces cultured with charcoal-treated albumin and oxygen. *J. Lipid. Res.* 1982. **23:** 360–363.

Supplementary key words 2-deoxyglucose • lipolysis • glucose oxidation • pentose phosphate shunt

Adipose tissue explants, from both man and rats, can be maintained viable for many days in tissue culture medium (1–4). Thus it should be possible to use cultured adipose tissue pieces to study control mechanisms independent of the influences of normal homeostatic mechanisms. However cultured tissue lacks the hormonal responsiveness of fresh tissue, thereby making it difficult to draw conclusions from studies of this model. In particular, stimulation by insulin of [1-^{14}C]glucose oxidation and lipogenesis is absent, and stimulation of [1-^{14}C]glucose oxidation and 2-deoxyglucose uptake is blunted (4). In addition, cells made from cultured adipose tissue pieces show accelerated lipolysis and glyceride-glycerol formation, with unimpaired antilipolytic action of insulin.

The initial studies from this laboratory used 1% or 4% bovine serum albumin (BSA) in the culture medium, and cultures were performed with room air as the gas phase. It seemed possible that tissue hypoxia and/or the presence of fatty acids or other small molecules during culture could have caused the altered metabolic activity of the adipose tissue. Therefore, we used charcoal-treated BSA and a 100% O_2 gas phase for the present studies, and were able to partially restore insulin responsiveness of [1-^{14}C]glucose oxidation in the fat cells.

MATERIALS AND METHODS

In all studies epididymal adipose tissue from chow-fed male Wistar rats weighing 175–200 g was used. Adipose tissue was prepared, cultured, and studied as previously described (4) except for the variations in culture conditions noted in the text. In brief, the distal two-thirds of the epididymal fat pads from 6–8 rats were cut into approximately 50-mg pieces, pooled, and distributed to flasks containing 10 ml of TC-199 medium supplemented with HEPES, 0.3 mg/ml; glucose, 3 mg/ml; penicillin, 5000 U/ml; streptomycin, 50 mg/ml; and 4% BSA (either untreated or charcoal-treated). Tissue pieces were cultured for 18 hr, all pieces from a given culture condition were pooled, and isolated adipocytes were prepared for subsequent studies. [1-^{14}C]Glucose utilization was measured during a 2-hr test incubation with 3 mM glucose and 0 or 250 μU of insulin/ml, and [2-^{14}C]deoxyglucose uptakes were carried out for 1 min by the oil separation method with 0.3 mM 2-deoxyglucose, using [^{3}H]inulin as an extracellular marker. Cell-associated fatty acids and fatty acids in the medium were determined by microtitration, with correction for extracellular space using a [^{14}C]sucrose marker. The BSA and collagenase were from the same lots as used in the previous study. These lots had been selected to give the lowest basal glucose utilization and greatest insulin stimulation of [1-^{14}C]glucose oxidation of all available lots. Charcoal-treated BSA was prepared by the method of Chen (5). This procedure removes fatty acids and other small molecules from the albumin. The BSA was dialyzed against distilled water and lyophilized prior to use. All studies included an incubation of adipocytes prepared from fresh tissue from the same pool of fat pad pieces as the cultured tissue.

Statistical analysis of results was performed on the natural logarithmic transforms of data by analysis of variance of culture condition × insulin in test incubation × replication (6). Geometric means of individual cells were compared by *t*-test against the pooled variance of the second degree interaction term. The transformed data were necessary to provide homogeneity of variance among the various culture and insulin conditions.

RESULTS

In order to retain insulin responsiveness of [1-^{14}C]glucose oxidation, it was necessary to treat the BSA subsequently used in the culture with charcoal at pH 3, and to carry out the culture with 100% O_2 as the gas phase. Both of these steps were necessary; culture with untreated BSA or with air as the gas phase did not preserve the insulin effect. Results of all studies are shown in **Table 1.** Basal $^{14}CO_2$ production was similar in the cultured fat cells and fresh cells. Insulin stimulated $^{14}CO_2$ by 275% in fresh cells and by 152% in cultured cells. There was no significant difference in the magnitude of these responses, as demonstrated by a non-significant interaction of insulin vs. culture condition in the analysis of variance. However the combination of the slightly reduced basal activity with the smaller insulin effect caused a significant reduction in $^{14}CO_2$ production in cultured cells compared to fresh cells. The cells cultured with the charcoal-treated BSA contrast with those cultured with untreated BSA (4), in which basal oxidation was identical to fresh cells but there was no response to insulin.

In contrast to oxidation, [^{14}C]triglyceride production in the cells cultured with charcoal BSA showed the same metabolic pattern as with untreated BSA; i.e., markedly increased basal production and little or no insulin response.

The effect of insulin on both [U-^{14}C]glucose oxidation and fatty acid synthesis is similar in cells cultured in either the standard system or with charcoal-treated albumin and O_2. However basal utilization is much more variable, and on the average lower in the latter system. Basal utilization is intermediate in fresh cells, but the insulin effect is greater so that both oxidation and lipogenesis are higher in cells made from fresh tissue than from cultured cells. The majority of the triglyceride production in both types of cultured tissue is glyceride-glycerol synthesis rather than fatty acids. In contrast, fatty acid synthesis predominates in the presence of insulin in fresh cells.

Initial rates of 2-deoxyglucose uptake were measured to determine whether the enhanced [1-^{14}C]glucose oxidation was related to changes in glucose transport. This technique has been shown to reflect transport rate in fresh adipocytes for at least 3 min of incubation (7). As in the previous study (4), basal 2-deoxyglucose uptake was increased in cells cultured with regular BSA compared to fresh cells. In contrast, basal transport was re-

TABLE 1. Metabolism of adipocytes prepared from fresh tissue or tissue cultured with charcoal-treated or untreated BSA

	Fresh Tissue		Charcoal-treated BSA		Untreated BSA	
	−Insulin	+Insulin	−Insulin	+Insulin	−Insulin	+Insulin
[1-^{14}C]Glucose utilization (μmol/g TG/2 hr)						
$^{14}CO_2$	2.16 (1.96–2.39)	8.51^{f} (7.04–10.28)	1.88 (1.61–2.33)	4.45^{a,e} (3.27–6.06)		
[^{14}C]Triglyceride	0.90 (0.74–1.10)	6.42^{f} (5.15–7.99)	3.99^{c} (3.08–5.18)	6.03^{d} (5.13–7.09)		
[U-^{14}C]Glucose utilization (μmol/g TG/2 hr)						
$^{14}CO_2$	2.04 (1.76–2.37)	8.35^{f} (6.21–11.23)	1.14^{a} (0.66–1.94)	1.99^{c,d} (1.24–3.19)	3.44^{a} (3.26–3.63)	5.64^{d} (4.73–6.75)
^{14}C-Labeled glyceride-fatty acid	1.03 (0.80–1.30)	7.26^{f} (5.10–10.33)	0.30^{b} (0.16–0.59)	1.12^{c,f} (0.63–1.99)	1.15 (1.06–1.24)	3.42^{a,e} (2.91–4.01)
^{14}C-Labeled glyceride-glycerol	1.32 (1.15–1.52)	2.59^{f} (2.18–3.07)	1.85^{a} (1.16–2.95)	2.08 (1.39–3.11)	4.37^{c} (4.06–4.72)	4.55^{b} (4.00–5.18)
2-Deoxyglucose uptake (nmol/g TG/min)	4.96 (4.39–5.63)	45.06^{f} (37.36–54.34)	3.17^{b} (1.54–6.52)	9.63^{c,f} (4.81–19.27)	7.50^{c} (6.20–9.05)	24.98^{c,f} (20.07–31.07)
Lipolysis						
Cell-associated FA (μEq/g TG)	0.80 (0.48–1.32)	0.72 (0.35–1.49)	1.16 (0.70–1.92)	1.69 (1.10–2.59)	1.71 (1.27–2.31)	1.16 (0.80–1.68)
Medium free fatty acid (μEq/g TG/2 hr)	2.65 (1.53–4.60)	2.66 (1.66–4.27)	6.70^{b} (3.99–11.26)	5.24^{a} (3.62–7.59)	9.17^{c} (6.00–14.00)	4.57^{a,d} (3.21–6.51)

Values are the antilogs of the means of log-transformed data from five separate incubations. The numbers in parentheses are the antilogs of ±1 SEM for those data. Each type of incubation was performed on separate tissue batches.

P values from analysis of variance: ^{a}, $P < 0.05$ vs. fresh tissue; ^{b}, $P < 0.01$ vs. fresh tissue; ^{c}, $P < 0.001$ vs. fresh tissue; ^{d}, $P < 0.05$ vs. −insulin; ^{e}, $P < 0.01$ vs. −insulin; ^{f}, $P < 0.001$ vs. −insulin.

duced to about 60% of fresh cells in cells prepared after culture with charcoal BSA and O_2. The insulin responsiveness in cultured cells was approximately 2-fold, compared with an 8-fold increase in fresh cells.

Rates of lipolysis were measured in order to determine whether the improved insulin effect on [1-^{14}C]glucose oxidation after culture with charcoal-treated BSA and O_2 is mediated by a reduction in the high levels of cell-associated and extracellular free fatty acids previously observed in cultured cells (4). Although basal fatty acid release was slightly lower after culture with charcoal-treated BSA than with untreated BSA, the difference was not significant, and both were higher than for fresh tissue. Cell-associated fatty acids also tended to be higher in cultured tissue than in fresh tissue, but results were variable. Insulin consistently had an antilipolytic effect only in tissue cultured with untreated BSA. Thus the changes in fatty acid release can not explain the improved [1-^{14}C]glucose oxidation.

DISCUSSION

Culture of adipose tissue pieces in the presence of 4% charcoal-treated BSA with a 100% O_2 atmosphere has preserved the insulin effect on [1-^{14}C]glucose oxidation. In contrast, other measures of adipocyte glucose utilization, including triglyceride formation from [1-^{14}C]glucose, 2-deoxyglucose uptake, lipolysis, and all pathways of [U-^{14}C]glucose utilization are similar to or lower than the previous system, and the absolute rates show greater variability after culture with the current system. It is likely that the improvement in cell metabolism is limited to enhancement of the pentose phosphate shunt.

The mechanism of these changes is not clear. It would appear that relief of hypoxia is a major effect, and that the variable response might be caused by inconsistent O_2 tension depending on the size of the adipose tissue pieces. However a similar enhancement of both [1-^{14}C]glucose oxidation and fatty acid synthesis can be seen after culture with 20% human serum despite an atmosphere of room air (8). Other explanations could include effects of charcoal treatment on insulin-like growth factors (9) that might stimulate basal glucose utilization, or on adenosine deaminase (10, 11) that might inhibit the enhancement of insulin effect by adenosine. The lipolysis experiments demonstrate that removal of fatty acyl CoA is not likely to explain the effects of charcoal on glucose utilization.

The findings in this study are somewhat at variance with other studies of adipose tissue in culture. Vernon (3) has shown that the presence of albumin or serum diminishes [U-^{14}C]glucose oxidation relative to culture in the absence of either. All cultures had reduced oxidation relative to fresh tissue, and insulin responsiveness was not determined in Vernon's studies. The acute incubations in that study were performed on tissue slices, not cells. We have found that increasing BSA concentrations from 1% to 4% increases the rate of [1-^{14}C]glucose oxidation (4). Some of the discrepancy between these two studies may be due to the presence of inhibitors or stimulators in the lot of BSA used, the use of tissue slices, and the location of the glucose label. [1-^{14}C]Glucose emphasizes the pentose shunt, which would be sensitive to fatty acyl CoA accumulated in the absence of a receptor for fatty acids in the medium (12). The improvement in oxidation in Vernon's study (3) with insulin in the culture medium contrasts with our findings, and may be due to an antilipolytic effect of insulin in the tissue cultured without albumin or serum. Livingston, Purvis, and Lockwood (13, 14), using a medium with 0.2% albumin and 95% O_2:5% CO_2 gas phase, showed that insulin in the culture medium diminishes insulin sensitivity, but does not alter the maximum insulin responsiveness of 2-deoxyglucose transport. Smith (1, 2) has shown very little insulin effect in his cultures of human adipose tissue, but no loss of responsiveness during culture. Of course, human tissue is less responsive than rat tissue under all circumstances. No albumin is used in culture in Smith's studies.

There is a striking similarity between the direction and magnitude of 2-deoxyglucose transport in these studies and the magnitude of all measures of basal glucose utilization. In addition, the degree of insulin stimulation of [U-^{14}C]glucose oxidation and fatty acid synthesis is similar to that of 2-deoxyglucose transport. These results suggest, but certainly do not prove, that for these pathways transport is rate-limiting. This contrasts with the effects in fat cells from normal rats, rats with large fat cells (15, 16), fasting rats (17, 18), diabetic rats (19, 20), or high fat-fed rats (21), in which steps subsequent to transport appear to regulate glucose utilization in the cell. It is possible that the limited transport in the cultured cells does not saturate subsequent metabolic steps, and thus prevents these steps from limiting utilization. In the case of [1-^{14}C]glucose oxidation however, it is clear that the effects of culture with O_2 and charcoal-treated BSA are in the opposite direction from changes caused by transport alone. The system described in this report preserves insulin effect better than the previous culture system. However it is clear that further adaptations are necessary to achieve a culture system that replicates in vivo adipose tissue control.

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REFERENCES

1. Smith, U. 1972. Studies of human adipose tissue in culture. I. Incorporation of glucose and release of glycerol. *Anat. Rec.* **172:** 597–602.
2. Smith, U. 1974. Studies of human adipose tissue in culture. III. Influence of insulin and medium glucose concentration on cellular metabolism. *J. Clin. Invest.* **53:** 91–98.
3. Vernon, R. G. 1978. Maintenance of rat adipose tissue in tissue culture. *Int. J. Biochem.* **9:** 505–512.
4. Bernstein, R. S. 1979. Insulin insensitivity and altered glucose utilization in cultured rat adipose tissue. *J. Lipid Res.* **20:** 848–856.
5. Chen, R. F. 1967. Removal of fatty acids from serum albumin by charcoal treatment. *J. Biol. Chem.* **242:** 173–181.
6. Cochran, W. G., and G. M. Cox. 1957. Experimental Designs. 2nd edition. John Wiley & Sons, New York.
7. Olefsky, J. M. 1978. Mechanisms of insulin's ability to activate the glucose transport system in rat adipocytes. *Biochem. J.* **172:** 137–145.
8. Bernstein, R. S. 1980. Preservation of adipocyte glucose utilization after culture with human serum. *Aliment. Nutr. Metab.* **1:** 230.
9. Froesch, E. R., H. Burgi, W. A. Muller, R. E. Humbel, A. Jakob, and A. Labhart. 1967. Nonsuppressible insulin-like activity of human serum: purification physicochemical and biological properties and its relation to total serum ILA. *Recent Prog. Horm. Res.* **23:** 565–616.
10. Fain, J. N., and P. B. Weiser. 1975. Effects of adenosine deaminase on cyclic adenosine monophosphate accumulation, lipolysis and glucose metabolism of fat cells. *J. Biol. Chem.* **250:** 1027–1034.
11. Schwabe, U., P. S. Schonhofer, and R. Ebert. 1974. Facilitation by adenosine of the action of insulin on the accumulation of adenosine 3′,5′-monophosphate, lipolysis, and glucose oxidation in isolated fat cells. *Eur. J. Biochem.* **46:** 537–545.
12. Eger-Neufeldt, I., A. Teinger, L. Weiss, et al. 1965. Inhibition of glucose-6-phosphate dehydrogenase by long chain acyl coenzyme A. *Biochem. Biophys. Res. Commun.* **19:** 43–49.
13. Livingston, J. N., B. J. Purvis, and D. H. Lockwood. 1978. Insulin-dependent regulation of the insulin-sensitivity of adipocytes. *Nature.* **272:** 394–396.
14. Livingston, J. N., B. J. Purvis, and D. H. Lockwood. 1978. Insulin-induced changes in insulin binding and insulin sensitivity of adipocytes. *Metabolism.* **27:** 2009–2014.
15. Olefsky, J. M. 1977. Mechanism of decreased insulin responsiveness of large adipocytes. *Endrocrinology.* **100:** 1169–1177.
16. Czech, M. P. 1976. Cellular basis of insulin insensitivity in large rat adipocytes. *J. Clin. Invest.* **57:** 1523–1532.
17. Olefsky, J. M. 1976. Effects of fasting on insulin binding, glucose transport and glucose oxidation in isolated rat adipocytes. *J. Clin. Invest.* **58:** 1450–1458.
18. Kasuga, M., Y. Akanuma, Y. Iwamoto, and K. Kosaka. 1977. Effects of fasting and refeeding on insulin receptors and glucose metabolism in rat adipocytes. *Endrocrinology.* **100:** 1384–1390.
19. Kasuga, M., Y. Akanuma, Y. Iwamoto, and K. Kosaka. 1978. Insulin binding and glucose metabolism in adipocytes of streptozotocin-diabetic rats. *Am. J. Physiol.* **232:** E175–E182.
20. Kobayashi, M., and J. M. Olefsky. 1979. Effect of streptozotocin-induced diabetes on insulin binding, glucose transport and intracellular metabolism in isolated rat adipocytes. *Diabetes.* **28:** 87–95.
21. Lavau, M., S. K. Fried, C. Susini, and P. Freychet. 1979. Mechanism of insulin resistance in adipocytes of rats fed a high-fat diet. *J. Lipid Res.* **20:** 8–16.