DIRECT MEASUREMENTS OF SUGAR UPTAKE IN SMALL AND LARGE ADIPOCYTES FROM YOUNG AND ADULT RATS

J.N. Livingston and D.H. Lockwood

Departments of Medicine and Pharmacology and Experimental Therapeutics and Division of Animal Medicine
The Johns Hopkins University School of Medicine
Baltimore, Maryland, U.S.A.

Received October 22,1974

SUMMARY. Measurements of basal and insulin-stimulated uptake of D-glucose, 2-deoxy D-glucose and 3-O-methyl-D-glucose were determined in isolated fat cells from young and adult rats by an oil-centrifugation technique. At low sugar concentrations, uptake of D-glucose and 2-deoxy-D-glucose was greater in large cells from older animals than in small cells from young rats while at higher concentrations (3.0 mM-5.0 mM) uptake was similar. Insulin enhanced uptake of both sugars and the amounts accumulated by the two cell types were not significantly different. Also no difference was noted in basal rate of 3-O-methyl-D-glucose uptake or when uptake was accelerated by insulin stimulation. These findings suggest that large adipocytes from adult rats are not as insulin-resistant as previously suggested but, instead, have an efficient D-glucose transport system which is responsive to insulin stimulation.

INTRODUCTION. Several reports have shown that large fat cells obtained from adult rats are less sensitive to insulin stimulation of D-glucose metabolism than small adipocytes from young rats (1-4). The exact nature of the cellular alteration(s) that result in insulin resistance has not been determined. Livingston et al (4) reported that these hormonally resistant cells have the same number of insulin receptors per cell as small adipocytes and that the affinity of the receptor for insulin is also the same. In light of this observation, the alteration in large cells would be expected to be beyond the insulin receptor, quite possibly at the D-glucose transport system. In this report a direct method is described for measuring sugar uptake* by isolated fat cells using a rapid oil-centrifugation technique first reported by Gliemann et al (5). This method has been applied to the study of D-glucose uptake by large fat cells from adult rats and to their response to insulin stimulation.

* Sugar uptake is the amount of sugar and/or metabolic products which are present in the cell following an incubation period of less than one minute for D-glucose and 3-O-methyl-D-glucose and two minutes for 2-deoxy-D-glucose.

MATERIALS AND METHODS. Male Sprague-Dawley rats were maintained on standard Purina Rat Chow and water ad libitum. Rats of 4-5 months of age and weighing 425-500 g were used to obtain large adipocytes. Small fat cells were isolated from rats of 1.5 months of age which weighed 130-160 g. Isolated adipocytes were prepared from epididymal fat pads by the collagenase method of Rodbell (6) and suspended in Krebs-Ringer bicarbonate buffer, pH 7.4, which contained 1.0 g% bovine albumin (fatty acid poor, Pentex). Fat cell number was estimated by determining the DNA content in the preparation as previously described (7). The cellular volume of large cells was approximately three-fold greater than the volume for small cells (4).

D-glucose uptake was determined using a slight modification of the method described by Gliemann et al (5) for rapid separation of isolated fat cells from an incubation medium. The assay was initiated by adding 0.1 ml of fat cell suspension (1.0-3.0 µg DNA) to 0.2 ml of U-[^{14}C]-D-glucose (1.5-3.0 mCi/mM) in a final concentration range of 0.33 mM to 5.0 mM. In assays of sugar uptake by large cells, the amount of DNA in the incubation medium was from one-third to one-half the amount present in assays used to determine sugar uptake by small cells. Sugar uptake was assayed at 24°C unless otherwise indicated. Approximately 15 seconds before termination of the assay, the content of the test tube was transferred to a plastic micro-centrifuge tube (capacity, 0.45 ml) that contained 0.1 ml of silicone oil (Arthur H. Thomas Co.). The incubation was stopped by centrifugation for 15 seconds in a Beckman microfuge. The incubation is considered terminated at the instant when centrifugation begins (5). Following centrifugation, the fat cells were packed in an upper layer over the oil which was interspersed between the cells and the lower aqueous layer. The tubes were quickly cut at the cell-oil interface and the portion containing the fat cell pellet was dissolved in 0.2 ml of Hyamine contained in a glass scintillation vial. After one hour, 10 ml of counting fluid (Instabray, Yorktown Res.) was added to the vial and allowed to set for 12 hours. Radioactivity was then determined using a Beckman Liquid Scintillation counter (counting efficiency of 30%).

The rate of D-glucose uptake was routinely calculated by subtraction of the amount present at 15 seconds from that found at either 30 or 45 seconds depending upon the experimental design. This procedure automatically corrects for the amount of sugar which is trapped extracellularly in the cell pellet. As measured by carboxy-[^{14}C]-inulin, the amount trapped is dependent upon cell size and number and represents a significant portion of the total sugar in the cell pellet. Approximately 50% to 70% of the total sugar in the pellet is trapped following a fifteen second incubation of fat cells in the absence of insulin. This percentage markedly diminishes during longer incubation periods and when sugar uptake is stimulated by insulin.

Measurement of uptake of 2-deoxy-1-[^{14}C]-D-glucose (0.25-1.5 mCi/mM) and 3-O-methyl-[^{14}C]-D-glucose (1.5 mCi/mM) was determined using the same general method described for D-glucose uptake. In studies using 2-deoxy-D-glucose, the rate of uptake was determined by subtracting the amount found at 15 seconds from that present at 2 minutes. A longer period of incubation was possible using this sugar since the initial uptake rate was linear for a period of four minutes.

Fat cells were pre-incubated for 45 minutes in the presence or absence of crystalline bovine insulin (Eli Lilly Co.) before performing the assay. Pre-incubation was carried out under 95% O_2 and 5% CO_2 at the temperature used for the assay.

RESULTS AND DISCUSSION. The rate of D-glucose uptake as determined by the oil-centrifugation method was linearly related to the number of fat cells used in the assay, and to the time of incubation for periods less than one

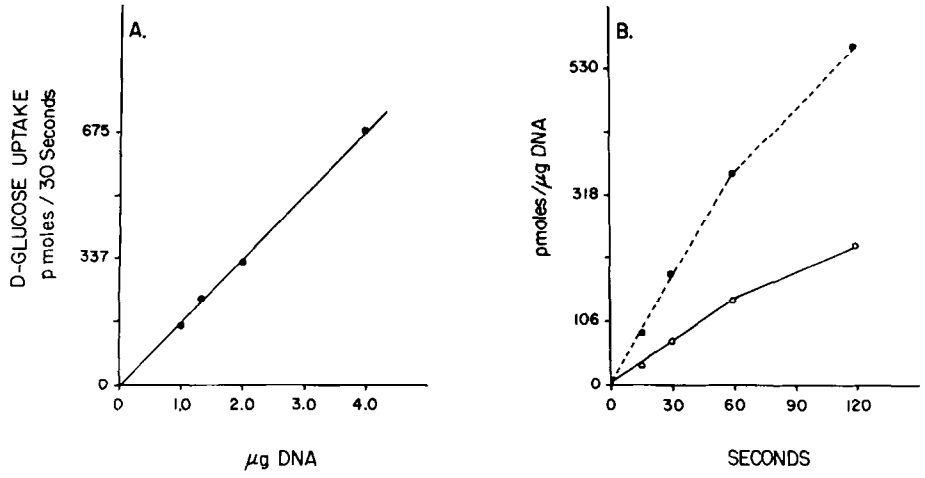

Fig. 1. Relationship of D-glucose uptake to cell number (DNA) and time of incubation of isolated fat cells from 130-160 g rats. A. Fat cells incubated in 1.0 mM D-glucose in the absence of insulin. B. Fat cells incubated in 0.33 mM D-glucose in the presence (●-----●) and absence (0———0) of 1500 μU/ml insulin as described in the Methods.

minute (Fig. 1). Also, as shown in Fig. 1B, pre-incubation of the fat cells with insulin results in an increased rate of D-glucose uptake. Incubation of cells for longer periods resulted in a new rate which was less than the initial rate observed between 15 seconds and 1 minute. The diminished rate found after 1 minute of incubation may represent escape of significant amounts of D-glucose metabolites from the cell.

3-O-Methyl-D-glucose and phlorizin are reported to inhibit D-glucose transport in adipose tissue (8,9) and as shown in Table 1 both agents markedly diminish D-glucose uptake by isolated fat cells. In agreement with other studies, inhibition was induced with D-mannose (9) while D-fructose did not significantly affect D-glucose uptake (10,11). Stereospecificity of the D-glucose transport system was shown by experiments using L-glucose which did not inhibit D-glucose uptake. This system was also temperature dependent as indicated by a four-fold increase in the rate of uptake when assayed at 37°C (data not shown). These results agree with those found in previous studies using other methods to determine sugar uptake by adipose tissue (9,12-14).

A comparison of the rates of D-glucose uptake by small and large cells

TABLE 1. Effects of various sugars on D-glucose uptake

Addition	(Added Sugar)* / (D-glucose)	Percent Effect
None	---	100
Sucrose	1.0	107
	10.0	98
3-O-methyl-D-glucose	1.0	83
	10.0	45
Phlorizin	1.0	38
	10.0	0
D-Mannose	1.0	99
	10.0	42
D-Fructose	1.0	115
	10.0	95
L-Glucose	1.0	105
	10.0	106

* Millimolar ratio of added sugar to D-glucose. D-glucose concentration in all experiments was 1.0 mM. Data represents mean of two experiments.

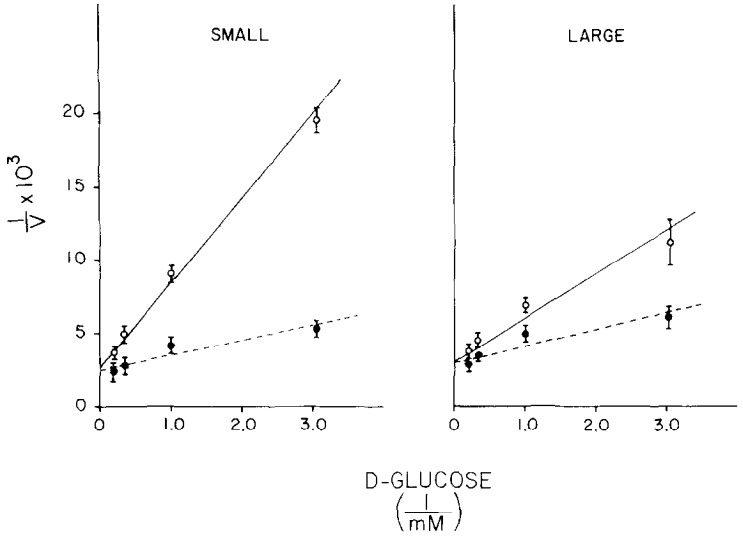

Fig. 2. Double reciprocal analysis of D-glucose uptake by small and large fat cells in the presence (●-----●) and absence (○———○) of insulin (1500 μU/ml). Velocity is expressed as pmoles/μg DNA/15 sec. Results are mean ± S.E.M. of five experiments. Differences between small and large cells were statistically significant ($p<0.05$) in the absence of insulin at D-glucose concentration of 0.33 mM and 1.0 mM by paired t-test.

is shown by double reciprocal plots (Fig. 2). At the higher D-glucose concentrations employed, there were no significant differences between large and small cells in rates of D-glucose uptake in the absence of insulin (basal rates). However, at the lower D-glucose concentrations of 0.33 mM and 1.0 mM, the basal rates for large cells were significantly greater than those found with small cells. As shown in Fig. 2, the "V_{max}" was similar for both cell types, but small cells had an apparent "K_u"* value which was twice the value of 1.0 mM calculated for large cells.

Incubation of the fat cells with insulin enhanced D-glucose uptake particularly at lower D-glucose concentrations. The rate of uptake following stimulation by insulin was not significantly different between the two cell types at any sugar concentration used even though small cells had a tendency to take up slightly more D-glucose. Thus, following insulin stimulation, the "V_{max}" and "K_u" (0.43 mM) are similar for both cell types. If D-glucose uptake is expressed as a percent increase above the basal rate, large cells do not respond as well as small cells to the hormone. This finding results primarily from the elevated basal rates found in large cells and secondarily from the slightly diminished D-glucose uptake following insulin stimulation when compared to small cells.

The D-glucose uptake system was further investigated by the use of 2-deoxy-D-glucose in an attempt to reduce metabolite formation and loss from the cells during the incubation period (8). The rate of 2-deoxy-D-glucose uptake was linear from 15 seconds to 4 minutes, the longest time point tested (data not shown). The difference in results found with D-glucose and 2-deoxy-D-glucose possibly reflects difference in metabolism of the sugars since the deoxysugar cannot be further metabolized beyond phosphorylation (8). As shown in table 2, results were obtained which were qualitatively similar to those found in studies using D-glucose. At the higher concentrations of

---

* "K_u" is the concentration of D-glucose which gives one-half "V_{max}" for D-glucose uptake.

TABLE 2. Uptake of 2-deoxy-D-glucose by small and large fat cells

2-deoxy-D-glucose concentration	Insulin*	2-deoxy-D-glucose uptake at 24°C+	
		Small Cells	Large Cells
0.33 mM	-	150 ± 26	220 ± 9
	+	387 ± 24	366 ± 37
1.00 mM	-	474 ± 49	453 ± 31
	+	786 ± 74	734 ± 69
5.00 mM	-	941 ± 161	1304 ± 204
	+	1704 ± 158	1631 ± 253
		2-deoxy-D-glucose uptake at 37°C‡	
0.33 mM	-	219 ± 4.6	426 ± 76
	+	837 ± 45	751 ± 76
1.00 mM	-	617 ± 74	1024 ± 232
	+	1867 ± 123	1633 ± 193

* Cells incubated in the absence (-) or presence (+) of 1500 uU/ml insulin.

\+ Mean ± S.E.M. of four experiments. Values are expressed as pmoles/ug DNA/10$\bar{5}$ sec.

‡ Mean ± S.E.M. of three experiments.

2-deoxy-D-glucose (1.0 mM and 5.0 mM) or with insulin stimulation at all three concentrations, there was no significant difference in 2-deoxy-D-glucose uptake between large and small cells. However, at the concentration of 0.33 mM, basal uptake by large cells was significantly greater ($p<0.05$) as determined by paired t analysis. The increased basal rate of sugar uptake by large cells is also not an artifact of performing the experiments at 24°C since similar findings are also present at 37°C.

The rate of uptake of a third sugar, 3-O-methyl-D-glucose, was used to study the D-glucose transport systems of large and small cells. 3-O-Methyl-D-glucose uptake is a measure of only the D-glucose transport system since it is transported like D-glucose but not metabolized (12,15). Fig. 3 shows the results of studies in which cells were incubated in 0.5 mM 3-O-methyl-

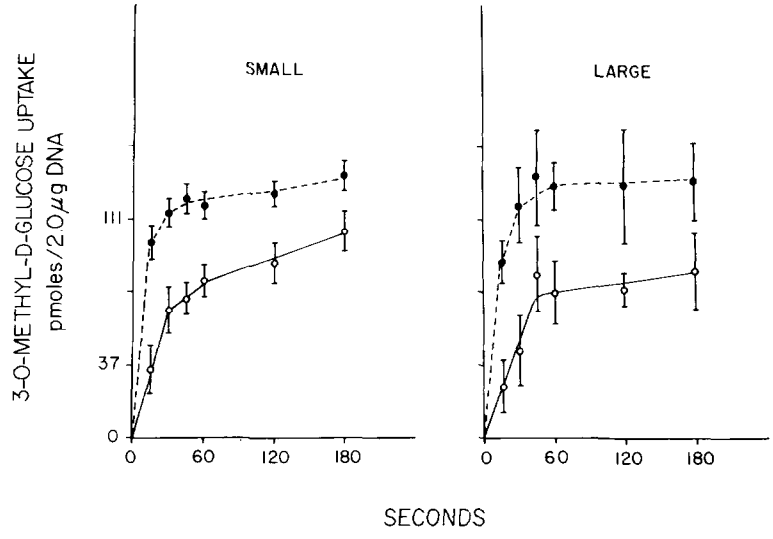

Fig. 3. Uptake of 3-O-methyl-D-glucose by small and large cells in the presence (●-----●) and absence (O———O) of insulin (1500 μU/ml). Fat cells were incubated in 0.50 mM 3-O-methyl-D-glucose for the periods indicated. Results are the mean ± S.E.M. of three experiments.

D-glucose in the presence or absence of insulin. Unfortunately, influx of 3-O-methyl-D-glucose was very rapid with equilibrium between intracellular and extracellular sugar concentrations being approached by approximately one minute. This restricts the accuracy of values for initial rates of uptake. However, as shown, no differences were observed between large and small cells for 3-O-methyl-D-glucose uptake either in the presence or absence of insulin stimulation. This finding suggests that the translocation of sugar itself is not involved in the difference found in basal uptake of D-glucose between the two cell types.

Results of this study indicate that large fat cells may not be as insulin-resistant as previously reported by our laboratory (4) and others (1-3) which indirectly estimated D-glucose transport by the formation of CO_2 and/or lipids. It also appears that the D-glucose transport system is not the site of the alteration responsible for the markedly diminished production of glucose metabolites following insulin stimulation and suggests that other intracellular processes may be involved.

ACKNOWLEDGEMENTS. We are indebted to Drs. Oscar B. Crofford, Mario DiGirolamo, and Pedro Cuatrecasas for helpful discussions during the course of this work. These studies were supported by grant AM-13562 from NIAMD, a grant from the American Diabetes Association, Inc. and a grant from the Weight Watchers Foundation, Inc. D.H.L. is a recipient of PHS Career Development Award, AM-42579.

REFERENCES.

1. DiGirolamo, M., and Rudman, D. Endocrinology (1968) 82, 1133-1141.
2. Gries, F.A., and Steinke, J. J. Clin. Invest. (1967) 46, 1413-1421.
3. Salans, L.B., and Dougherty, J.S. J. Clin. Invest. (1971) 50, 1399-1410.
4. Livingston, J.N., Cuatrecasas, P., and Lockwood, D.H. Science (1972) 177, 626-628.
5. Gliemann, J., Østerlind, K., Vinten, J., and Gammeltoft, S. Biochim. Biophys. Acta (1972) 286, 1-9.
6. Rodbell, M. J. Biol. Chem. (1964) 239, 375-380.
7. Livingston, J.N., Cuatrecasas, P., and Lockwood, D.H. J. Lipid Res. (1974) 15, 26-32.
8. Czech, M.P., Lynn, D.G., and Lynn, W.S. J. Biol. Chem. (1973) 248, 3636-3641.
9. Crofford, O.B., and Renold, A.E. J. Biol. Chem. (1965) 240, 3237-3244.
10. Froesch, E.R., and Ginsberg, J.L. J. Biol. Chem. (1962) 237, 3317-3324.
11. Fain, J.N. J. Biol. Chem. (1964) 239, 958-962.
12. Rodbell, M. J. Biol. Chem. (1966) 241, 130-139.
13. Letarte, J., and Renold, A.E. Biochim. Biophys. Acta (1969) 183, 350-356.
14. Gliemann, J. Acta Physiol. Scand. (1968) 72, 481-491.
15. Clausen, T. Biochim. Biophys. Acta (1969) 183, 625-634.