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Transport and metabolism of D-glucose in human adipocytes. Studies of the dependence on medium glucose and insulin concentrations

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Uptake and metabolism of the physiologically labelled D-glucose (D-[U-^{14}C]glucose) has been characterized in human adipocytes at several unlabelled D-glucose concentrations in the absence and presence of insulin. Following a 90 min incubation, about 80% of the intracellular radioactivity was incorporated into total lipids at tracer glucose concentration, as well as at higher glucose concentrations in basal and insulin-stimulated cells, whereas 20% was recovered as hydrophilic metabolites. The only ^{14}C-labelled metabolite escaping the cells in detectable amounts was CO_2, which accounted about 4%. At trace glucose concentrations (5 μmol/l), the rate of glucose uptake was linear with time. Comparative studies of initial glucose uptake after 10 s and tracer D-glucose conversion to total lipids after 90 min showed high coefficients of correlation between basal rates ($r = 0.87$), maximal response above basal level to insulin ($r = 0.92$) and insulin sensitivity ($r = 0.78$). Thus, under these conditions glucose transport is rate-limiting for net glucose uptake, and measurements over long time intervals of rates for total cell-associated radioactivity or lipogenesis may serve as reliable estimates of initial glucose influx rates. However, the conversion rate of tracer glucose to metabolites decreased progressively with the glucose concentration and with an apparent K_m of about 0.2 mmol/l. The three metabolic pathways exhibited similar percentage decreases in their activities, suggesting that a common enzymatic step is rate-limiting. In comparison, the K_m for initial D-glucose uptake rate was about 7 mmol/l. Hence, the capacity for total glucose metabolism comprised only a small fraction of the glucose transport capacity at medium glucose concentrations above tracer concentrations. Both basal, half-maximal and maximal insulin-stimulated rates of adipocyte glucose utilization were dependent on the glucose concentration. Thus, comparing lipogenesis at tracer and at 0.5 mmol/l medium glucose concentration, it was shown that the higher medium glucose concentration was associated with a 60% lowering of the basal rate, a 35% reduction in the percentage response above baseline to maximal insulin stimulation and a 4-fold increase in the insulin sensitivity. Obviously, these findings reflect some intracellular step(s) being rate-limiting at medium glucose levels above tracer values.

Introduction

Studies of glucose transport into adipocytes have most often been performed using the non-metabolizable analogue 3-*O*-methylglucose [1,2]. It has been supposed that the rapid metabolization of D-glucose would make direct study of its transport impossible, because radioactive metabolic products would escape from the cells quickly, leading to underestimation of the transport rate [1]. 3-*O*-Methylglucose uptake has to be measured

Abbreviation: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid.

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in very short time intervals (2–5 s) because the waterspace in the adipocytes rapidly equilibrates with the extracellular glucose concentration [1,2] and the use of concentrated cell suspensions is necessary to provide sufficient intracellular waterspace. Recently, we found that a great variability in hexose uptake exists when different batches of 3-*O*-methylglucose are used [3]. This complicates the comparison of transport data based on different batches of 3-*O*-methylglucose as well as the comparison of studies of 3-*O*-methylglucose transport with studies of D-glucose metabolism. It has recently been shown in rat fat cells that D-glucose may be used as tracer in transport studies [4]. Hence, the purpose of the present study was (1) to examine whether the physiologically ^{14}C-labelled D-glucose can be used as tracer in the study of transport in noninsulin-stimulated and insulin-stimulated human fat cells, (2) to study the dependence on glucose and insulin concentrations of glucose transport and further glucose metabolism in the same cells.

Materials and Methods

Chemicals. Collagenase was obtained from Worthington Biochemical (Freehold, NJ). Human albumin from Behringwerke, F.R.G. D-[U-^{14}C]Glucose (specific activity, 300 μCi/μmol) and 3-*O*-[^{14}C]methylglucose (specific activity, 59 μCi/μmol) were from Amersham, U.K. Scintillation fluid (Lipoluma® for lipophilic agents and CO_2 and lumagel® for hydrophilic products) was from Lumac, The Netherlands. Phloretin from ICN Pharmaceuticals, New York, U.S.A. All other chemicals were analytic grade. Fat tissue and isolated fat cells were suspended in a balanced medium containing 10 mmol/l Hepes, as previously described [5]. The pH was adjusted to 7.4 at 37 °C.

Subjects. Subcutaneous fat tissue was obtained from patients undergoing abdominal surgery. The patients were within 20% of ideal body weight. Their age was from 24 to 55 years. None of the donors suffered from endocrine disorders. The patients were fasted about 8 h overnight. General anaesthesia was induced with short-acting barbiturate and maintained with halotane. In the studies of correlations between different transport assays, 30 normal volunteers and 10 noninsulin-dependent diabetic patients were studied. Fat tissue from these subjects was obtained from the gluteral region in local anaesthesia as previously described [5]. Informed consent was given from all subjects according to Helsinki declaration II. Pieces of fat tissue were incubated at 37 °C for 90 min in a 10 mmol/l Hepes buffer containing collagenase (0.5 mg/ml). The cells were then filtrated through a nylon filter (500 μm) and washed twice. Finally, the cells were resuspended in a Hepes buffer without glucose, unless otherwise stated. Cell size was determined as described [5]. Mean cell diameter was 88 ± 8 μm.

Uptake of hexose. Hexose uptake was measured by a modification of our previously described method [2]. All studies were carried out at 37 °C. 40 μl of adipocyte suspension with a volume fraction of 0.4 (unless otherwise stated) were placed in polypropylene tubes and incubated without or with insulin for 45 min. 12 μl (0.24 μCi) of tracer (3-*O*-[^{14}C]methylglucose, final concentration 100 μmol/l, or D-[U-^{14}C]glucose, final concentration 20 μmol/l) were added at time zero and uptake was terminated at the desired time by adding 3 ml of phloretin (0.3 mmol/l in 154 mmol/l saline). 0.8 ml of silicone oil (0.99 g/ml) was layered on the top, and the tubes were spun within 2 min at 2500 × *g*. The cells were collected from the top of the oil and placed in scintillation vials with 5 ml of scintillation fluid. Extracellular trapped radioactivity (blank values) was estimated by adding phloretin before tracer. We found no detectable differences between the two tracers concerning the ability of phloretin to stop further uptake or efflux from the cells or in the amount of extracellular trapped radioactivity, when separation was done within 2 min. Blank values constituted about 30% of noninsulin-stimulated (basal) uptake of D-[U-^{14}C]glucose after 10 s (0.4, v/v) and 3% after 15 min uptake (0.05, v/v) in 15 experiments. For 3-*O*-[^{14}C]methylglucose, blank values made up about 35% of nonstimulated cells after 5 s uptake. Coefficients of variation (S.D./mean) were 0.09 for initial uptake of D-glucose (10 s) and 3-*O*-methylglucose (5 s) and 0.04 for long-term uptake (15 min) of D-glucose under the same conditions. In all experiments with 3-*O*-methylglucose the same batch (No. 17) was used to avoid the dif-

ferences between batches, which would confuse the results.

Estimation of intracellular free glucose concentration. 3-*O*-Methylglucose countertransport at different extracellular D-glucose concentrations (which enables calculation of intracellular versus extracellular glucose concentrations) was measured according to the method of Foley et al. [6]. Triplicates of fat cells (0.4 v/v) were incubated 45 min with 3-*O*-[^{14}C]methylglucose in the absence or presence of D-glucose (0.5, 2 and 5 mmol/l). Cell-associated radioactivity was then estimated as described above. The total coefficient of variation in the absence of D-glucose was 0.05.

Glucose oxidation. The conversion of D-[U-^{14}C]glucose to $^{14}CO_2$ was studied as described earlier [7]. Briefly, isolated adipocytes (0.05, v/v) were preincubated without or with insulin for 45 min, D-[U-^{14}C]glucose (0.4 μCi) was added and the incubation was continued for 90 min. The reaction was stopped by addition of H_2SO_4, and during the subsequent 60 min $^{14}CO_2$ was collected, using phenethylamine as trapping agent. ^{14}C radioactivity was present in average amounts of 20% of nonstimulated CO_2 release in the absence of unlabelled glucose when the incubations were run in the absence of fat cells (blank values). Coefficients of variation averaged 0.10 in noninsulin-stimulated CO_2 production.

Lipogenesis. The experimental procedure for measuring the conversion of D-[U-^{14}C]glucose to ^{14}C-labelled total lipids was the same as described for CO_2 production. After termination of CO_2-trapping, a Dole extraction [8] was performed and a sample for liquid scintillation counting was taken from the upper phase [7]. Blank values (measured as above) constituted about 8% of nonstimulated lipogenesis and the total coefficient of variation of the assay was 0.05.

Determination of hydrophilic ^{14}C-labelled metabolites. The determination of hydrophilic ^{14}C-labelled metabolites inside the cells and of ^{14}C-labelled total lipids in the study with varying medium glucose concentrations was carried out as described for lipogenesis, with the exception that the adipocytes were separated from the medium by centrifugation through silicone oil after incubation with [^{14}C]glucose. H_2SO_4 was then added. After the Dole extraction [8], samples from the upper phase (lipophilic phase) and lower phase (hydrophilic phase) were counted in a liquid scintillation counter for determination of radioactive total lipids and hydrophilic metabolites, respectively. Blank values were measured as hydrophilic metabolite production and total lipid production to time zero and constituted about 6 and 1% of nonstimulated production in the absence of unlabelled glucose, respectively. Total coefficients of variation were 0.08 and 0.04, respectively.

Determination of ^{14}C-labelled metabolites escaping to the medium. Adipocytes (0.2, v/v) were incubated with or without insulin and with or without added unlabelled D-glucose (0.5 mmol/l) for 15 min at 37°C. Then, D-[U-^{14}C]glucose (5 μmol/l) was added and the incubation was continued for 60 min. The incubation was stopped by addition of phloretin and the cells removed by centrifugation through oil. A fraction of the infranatant was layered on an anion-exchange column (Bio-Rad AG 1 × 8), equilibrated with 1 mmol/l D-glucose. The column was then washed with 1 mmol/l D-glucose and the effluents collected for scintillation counting [9].

Calculations. In the text and in Table I data are given as means ± 1 S.D. In the figures, results are shown as means ± 1 S.E. Linear regression analysis was employed in correlation studies. In some of the figures, data have been expressed as clearance rates. This choice was made because the differences in medium glucose concentrations would make expression as mol substance inappropriate. The ratio between intracellular and extracellular glucose concentrations was calculated from the 3-*O*-methylglucose countertransport data according to the method of Foley et al. [6] from the equation

$$\frac{\text{cell-associated radioactivity in the presence of glucose}}{\text{cell-associated radioactivity in the absence of glucose}} = \frac{1 + (\text{intracellular glucose concn.})/K_{m(\text{D-glucose})}}{1 + (\text{extracellular glucose concn.})/K_{m(\text{D-glucose})}}$$

Results and Discussion

D-glucose transport and metabolism at tracer concentration

Short-term uptake. First, we studied the comparability of short-term uptake of D-glucose and

the conventionally used tracer for measurement of glucose transport, 3-*O*-methylglucose. A strong positive correlation was found between initial uptake rates of these hexoses in noninsulin- as well as maximally insulin-stimulated cells ($r = 0.80$ and 0.84, respectively, $n = 10$ $P < 0.01$). In nonstimulated cells, initial uptake rates (expressed as clearance rates) for 3-*O*-methylglucose and D-glucose were 1.91 ± 0.85 and 1.03 ± 0.43 nl/s per 30 000 cells, respectively. In insulin-stimulated cells the rates were 4.39 ± 2.13 and 2.40 ± 1.23 nl/s per 30 000 cells, respectively. In Fig. 1A, it can be seen that the insulin dose-response curves for 3-*O*-methylglucose and D-glucose exhibit the same characteristics, with the same insulin concentration giving half-maximal response and the same maximal insulin response. These findings suggest that initial uptake rate of tracer amounts of D-glucose is a valid measure of its transport into human adipocytes. The finding of lower transport rates for D-glucose than for 3-*O*-methylglucose is in agreement with the lower affinity for the carrier system of D-glucose (about 7 mmol/l, see below) than of methylglucose (3 mmol/l [1,2]) and not caused by detectable amounts of glucose being converted to metabolic products escaping the cells before counting of radioactivity, as discussed below.

Time course studies. D-Glucose uptake at tracer concentration was then studied at longer time intervals. Fig. 2A depicts the time course for the uptake of tracer amounts of D-glucose (0.4, v/v). The uptake was linear from 2.5 s to about 15 min. At subsequent time points, the uptake rate decreased slightly. This decline may be explained by the fall in medium glucose concentration (about 15% after 30 min), which occurs through the continuous removal and metabolism of glucose in the highly concentrated cell suspension. When the same experiment was performed in a more diluted adipocyte preparation, as in Fig. 2B (0.05, v/v), the rate of D-glucose uptake at tracer concentration (5 μmol/l) is constant for at least 30 min. The linear uptake of tracer D-glucose during the initial 30 min in a diluted cell suspension at a clearance rate that is identical to the initial uptake rate in the more concentrated cell suspension shows that, although glucose in this time interval is phosphorylated and further metabolized, glucose must be the rate-limiting step for glucose

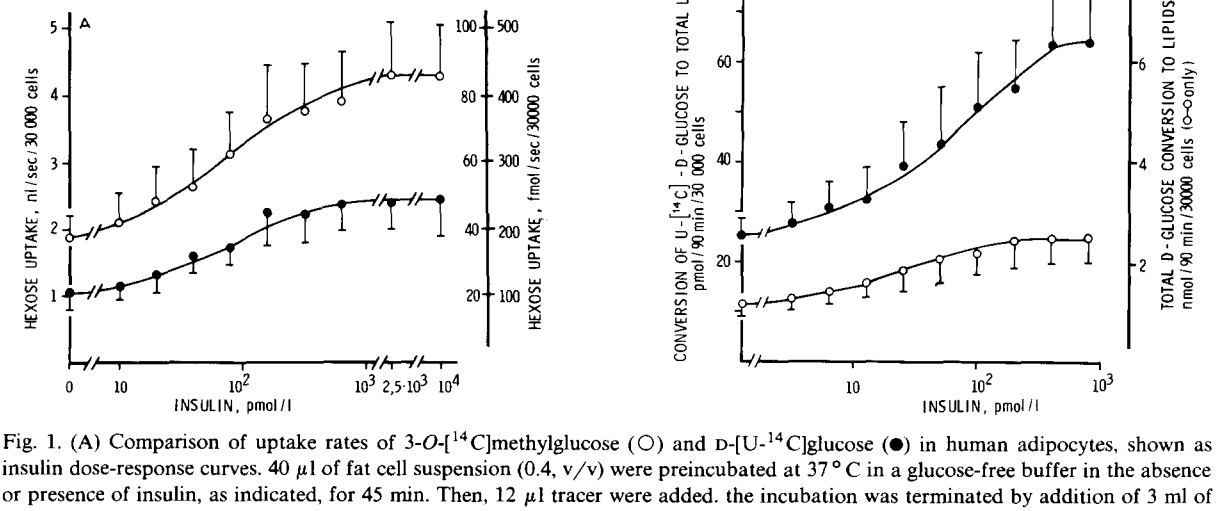

Fig. 1. (A) Comparison of uptake rates of 3-*O*-[^{14}C]methylglucose (○) and D-[U-^{14}C]glucose (●) in human adipocytes, shown as insulin dose-response curves. 40 μl of fat cell suspension (0.4, v/v) were preincubated at 37 °C in a glucose-free buffer in the absence or presence of insulin, as indicated, for 45 min. Then, 12 μl tracer were added. the incubation was terminated by addition of 3 ml of phloretin and centrifuged through oil. Methylglucose uptake was measured over 5 s with a final tracer concentration of 100 μmol/l (right-hand *y*-axis) and D-glucose over 10 s with a tracer concentration of 20 μmol/l (left-hand *y*-axis). Data are mean ± S.E. of nine experiments. (B) Insulin dose-response curves for the conversion rates of D-[U-^{14}C]glucose to total lipids at different medium glucose concentrations. Fat cells were preincubated in the absence (●) or presence (○) of 0.5 mmol/l unlabelled D-glucose with insulin added to final concentrations as indicated. Lipogenesis mas measured as described in Materials and Methods. Incubations were terminated after 90 min. Data are means ± 1 S.E. of ten experiments.

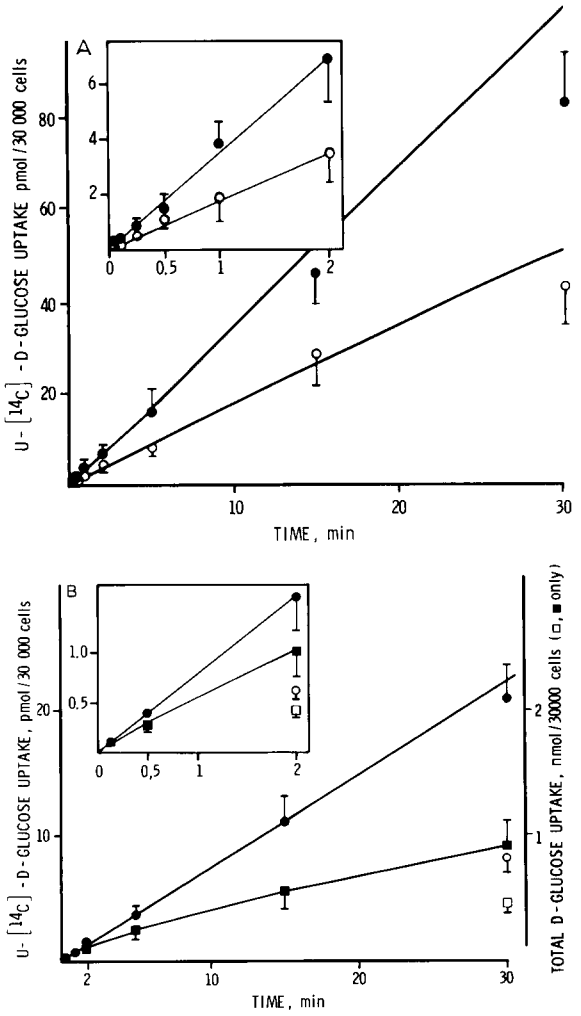

Fig. 2. Time course for uptake of D-[U-^{14}C]glucose in human fat cells. (A) 40 μl of fat cell suspension (0.4, v/v) were preincubated in a glucose-free buffer in the absence (○) or presence (●) of insulin (5 nmol/l) for 45 min. Then, 12 μl labelled glucose were added (final concentration, 20 μmol/l). The incubations were terminated at the indicated times as described in legend to Fig. 1. (B) 200 μl cell suspension (0.05, v/v) were preincubated without (circles) or with (squares) 0.5 mmol/l unlabelled D-glucose in the absence (open symbols) or presence (closed symbols) of 5 nmol/l insulin. Then, 20 μl labelled D-glucose were added (final labelled glucose concentration, 5 μmol/l). The incubations were terminated as described above. Data are means ± 1 S.E. of five experiments.

processing, and escape of metabolites must be of very low importance. Consequently, glucose transport can be measured as the uptake of tracer D-glucose at longer time intervals when diluted cell suspensions are used.

Similar results have been found for rat adipocytes [4]. Kashiwagi et al. [10] have measured glucose transport in human adipocytes using a similar method. With a tracer glucose concentration of 100 nmol/l and an adipocyte suspension at a volume fraction of 0.02, they found a linear uptake of glucose for 1 h. In contrast to our results, they found almost identical transport rates for 3-*O*-methylglucose and D-glucose. This discrepancy may be explained by differences in the affinity for the transport system between the batches of methylglucose tracer used by Kashiwagi et al. and in the present study.

Lipogenesis as a measure of glucose transport. In Table I, left column, the conversion rates for the different pathways for metabolization of D-glucose are given. It is seen that only 4% of the tracer escapes the cells as CO_2, whereas as much as 80% is stored inside the cells as total lipids.

To study whether lipogenesis rates at tracer glucose concentration can serve as a measure of glucose transport, lipogenesis at tracer glucose concentration (method X) as well as initial D-glucose uptake (10 s, method Y) were studied in 30 normal subjects and 10 noninsulin-dependent diabetic patients. In the normal group, high coefficients of correlation were found between basal rates ($r = 0.87$, $P < 0.001$), percentage responsiveness ($r = 0.92$, $P < 0.001$) and insulin sensitivity (ED_{50}, $r = 0.78$, $P < 0.001$). However, absolute rates of basal rates were 77 ± 34 nl/min per 30 000 cells using method X and 61 ± 26 nl/min per 30 000 cells using method Y, thus indicating an apparently higher transport rate in the lipogenesis assay. This apparent paradox was examined in detail. It was found that if the cells were centrifuged through oil before extraction of lipophilic radioactivity, about 20% of the lipophilic radioactivity was lost. The higher levels of lipophilic radioactivity when the cells were not separated from the medium was not caused by 'contamination' with hydrophilic radioactivity from the medium, since washing of the lower phase with new upper phase in a Dole extraction gave radioactivity in the new upper phase identical to blank values. On the other hand, if the separation procedure in method Y was changed, so that the medium and not the cells were centrifuged through oil, an increase in radioactivity of approximately the same

TABLE I

CONVERSION OF D-[U-^{14}C]GLUCOSE TO METABOLITES. DEPENDENCE ON GLUCOSE CONCENTRATION

Adipocytes (0.05, v/v) were preincubated 45 min without (−) or with (+) insulin (5 nmol/l) at the indicated concentrations of unlabelled D-glucose. Then, 0.4 μCi of labelled D-glucose (final concentration, 0.005 mmol/l) was added and the incubation was continued for 90 min. The metabolites were measured as described in Materials and Methods. Total cell-associated radioactivity was estimated by separating the cells from the medium by centrifugation through oil, followed by scintillation counting of the cell pellet. Results are expressed to 30000 cells. Results are means ± 1 S.D. of five experiments.

Conversion of D-[U-^{14}C]glucose to	Insulin	Conversion (pmol/90 min); concn. of unlabelled glucose (mmol/l)			
		0	0.1	0.5	5
Total lipids	−	33.9 ±13.1	20.5 ±6.45	9.30±4.40	1.55±0.40
	+	69.5 ±30.4	34.4 ±11.3	13.95±5.40	1.95±0.45
Water-soluble products	−	6.35±1.95	3.50±0.70	1.80±0.41	0.35±0.15
	+	14.1 ±5.05	6.90±1.95	3.30±0.55	0.50±0.20
CO_2	−	1.43±0.62	0.76±0.46	0.46±0.29	0.07±0.02
	+	2.41±1.56	1.28±0.54	0.70±0.42	0.09±0.02
Total cell-associated radioactivity	−	39.0 ±13.1	20.8 ±4.70	9.75±3.25	1.60±0.30
	+	73.0 ±31.0	35.5 ±8.70	14.8 ±5.00	2.00±0.65

size was observed. This indicates that approx. 20% of the adipocytes are lost by centrifugation through oil, which gives rise to similar underestimation of glucose transport by method Y. The difference between method X and Y was not related to cell size. The insulin responses were identical in the two assays (95 ± 72% in method X and 99 ± 64% in method Y). The insulin sensitivity was 63 ± 51 pmol/l in method X and 92 ± 82 pmol/l in method Y. This discrepancy may be caused by an increased insulin degradation activity in the more concentrated cell suspension in method Y (about 30% of added A_{14}-[^{125}I]monoiodoinsulin (100 pmol/l) was degraded in the medium (trichloroacetic acid-soluble) in an adipocyte suspension with volume fractions of 0.4, versus 2–4% at volume fractions of 0.05). Similar results in all aspects were observed in the group of noninsulin-dependent diabetic patients (data not shown). Hence, conversion of tracer glucose to total lipids is a precise measure of glucose transport.

D-Glucose transport and metabolism at increasing glucose concentration

Time course studies. We studied the uptake of tracer glucose when the adipocytes were incubated with 0.5 mmol/l unlabelled D-glucose. Uptake of tracer glucose was initially identical to the uptake rate in the absence of unlabelled glucose (Fig. 2B). However, after 20 s the uptake rate was progressively smaller. After 5 min, the uptake of labelled glucose in the presence of unlabelled glucose was linear, with a slope about one-third of that of the uptake of tracer glucose alone. These findings indicate that glucose transport may no longer be rate-limiting for glucose processing, even at this low glucose concentration. An estimation of the intracellular free glucose concentration would give a more direct answer to the question of whether transport is rate-limiting or not under these conditions. However, direct estimation of free glucose in human fat cells is extremely difficult, due to the small water space [1,2]. On the other hand, free intracellular glucose can be estimated indirectly using glucose-induced steady-state 3-*O*-methylglycose countertransport, as described by Foley et al. [6]. A ratio below 1 of cell-associated radioactivity in the presence versus in the absence of extracellular glucose is indicative of a gradient in glucose concentration over the cell membrane. We found ratios very close to 1 at all the tested extracellular glucose concentrations ($n = 3$, data not shown). This indicates that the intracellular glucose concentration is equal to or very close to the extracellular glucose concentration, thus substantiating our hypothesis that glucose transport is not rate-limit-

ing for glucose uptake in human fat cells at these extracellular glucose concentrations.

Phosphorylation as possible rate-limiting step. Our data do not indicate directly which step in glucose metabolism is now rate-limiting. Phosphorylation by hexokinase may be the rate-limiting step [11], as has been suggested for rat fat cells. This hypothesis may be supported in the following way: The intracellular waterspace in human fat cells is 2–3 pl per cell [2]. From the data in Fig. 1A, the initial clearance rate per cell can be calculated to about 0.8 pl per 10 s in insulin-stimulated cells corresponding to 30% of the water space. Thus, if it is assumed that phosphorylation is the rate-limiting step for glucose processing at 0.5 mmol/l glucose, free, nonphosphorylated glucose would accumulate in the water space at a rate of about 30% per 10 s, minus the rate of phosphorylation. This might be roughly estimated from Fig. 2B to be one-third of the transport rate (differences in slopes estimated from 15 to 30 min). The net accumulation rate of free glucose in the cells would thus be about 20% of the water space per 10 s, resulting in a rapid equilibration with the extracellular glucose concentration. Hence, already within a few seconds, transport is no longer unidirectional and the net influx will progressively decrease until the maximal capacity of phosphorylation is reached. The findings of a deviation from linearity of uptake already after 20 s when the cells are incubated in 0.5 mmol/l glucose is in agreement with this hypothesis. If steps distal to phosphorylation were rate-limiting, a deviation from linearity would be expected at a later time, because the intracellular 'space' for phosphorylated glucose is much larger [11].

It may be argued that escape to the medium of metabolic products may contribute to a major extent to the decrease in intracellular uptake. However, two lines of evidence are against this. Firstly, if fat cells were incubated with labelled D-glucose (5 μmol/l) for 15 min after preincubation for 30 min with increasing concentrations of either 3-*O*-methylglucose (transported, but not metabolized), 2-deoxyglucose (transported and phosphorylated but not further metabolized) or D-glucose, cellular uptake was inhibited with 50% inhibiting concentrations (K_i) of 3, 0.4 and 0.3 mmol/l, respectively ($n = 3$, data not shown). Thus, the inhibition of tracer D-glucose uptake by methylglucose resulted in K_i values close to K_m for methylglucose transport, whereas inhibition by deoxyglucose or unlabelled D-glucose gave almost identical values (K_m for hexokinase for deoxyglucose and D-glucose are similar [12]). Therefore, the decrease in glucose uptake seems to be caused by competition at the level of phosphorylation. Secondly, when we tested the amount of radioactive metabolites escaping to the medium by passing a fraction of the medium through an anion column, it was found that more than 98% of the radioactivity was present as glucose, both at tracer concentration and at higher glucose concentrations. Consequently, escape to the medium of metabolic products cannot explain the decrease in tracer glucose uptake in the presence of unlabelled glucose.

Kinetic studies of glucose transport. Fig. 3A depicts the saturation of labelled D-glucose transport (measured after 10 s where uptake is unidirectional) by increasing concentrations of unlabelled D-glucose basally and with maximum insulin stimulation. The inhibition constant, K_i (estimated as 50% inhibition concentration in individual curves), was found to be the same in basal and in insulin-stimulated cells (7.7 ± 0.6 and 7.3 ± 1.0 mmol/1, respectively), whereas V_{max} (estimated as clearance rate at tracer concentration (20 μmol/l) multiplied by K_m (K_i), [10]) was increased from 70 pmol/10 s per 30 000 cells basally to about 140 pmol/10 s per 30 000 cells in stimulated cells. If data are linearized using Hanes plot (clearance^{-1} versus substrate concentration), approximately the same values are found. The estimated V_{max} values are consistent with the previously reported values for 3-*O*-methylglucose in human fat cells [2], suggesting that a difference in affinity is the main reason for the different tracer uptake rates for the two hexoses. K_m for methylglucose is 3 mmol/l [2].

Characteristics of D-glucose metabolism. Glucose can take several pathways in adipose tissue, including oxidation through the citric acid cycle or the hexose monophosphate shunt, conversion to acyl-CoA, and thereby to fatty acids, and formation of alpha-glycerophosphate or glycogen. Our study clearly demonstrates that under the chosen

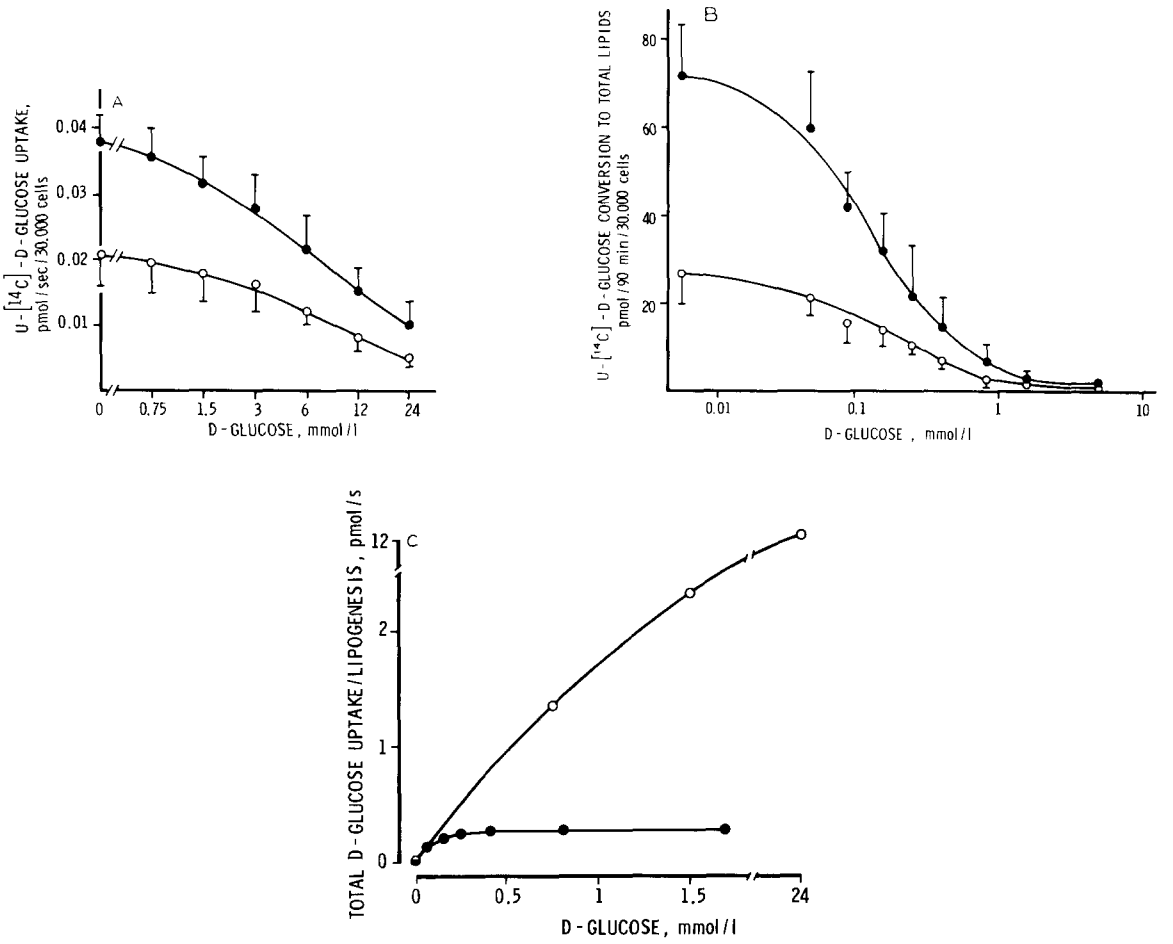

Fig. 3. (A) Initial uptake rates of D-[U-^{14}C]glucose in human fat cells. Dependence on extracellular D-glucose concentrations. 40 μl of adipocyte suspension (0.4, v/v) were preincubated without (○) or with (●) 5 nmol/l insulin. Then, 12 μl labelled D-glucose (final labelled glucose concentration, 20 μmol/l) without or with unlabelled D-glucose to final concentrations as indicated were added. Incubations were terminated after 10 s. Data are means ± 1 S.E. of four experiments. (B) Conversion rates of D-[U-^{14}C]glucose to total lipids. Dependence on extracellular D-glucose concentrations. Experimental conditions were as described in Materials and Methods. Cells preincubated without (○) or with (●) insulin (5 nmol/l). Lipogenesis was measured in the absence or presence of unlabelled D-glucose to final glucose concentrations as indicated. Incubations were terminated after 90 min. Data are means ± 1 S.E. of five experiments. (C) Data from panels A and B expressed as total (labelled + unlabelled) glucose uptake rates (○) and lipogenesis rates (●). Insulin-stimulated cells only.

experimental conditions the synthesis of total lipids is by far the major metabolic pathway. It should be emphasized, however, that the de novo synthesis of fatty acids in human fat cells is very poor [13]. Thus lipogenesis represents first of all glycerol synthesis and esterification of exogenous fatty acids.

Table I depicts the different pathways for metabolism of tracer glucose, namely total lipids, hydrophilic intracellular metabolic products and CO_2 at four medium glucose concentrations in basal and insulin-stimulated cells. For all these metabolic products, we found a decrease in conversion rate of tracer with increasing medium glucose concentration, with apparent K_i of 0.1–0.2 mmol/l. Total lipids accounted for about 80% of glucose taken up by the cell, hydrophilic metabolic products for about 20% and CO_2 production for about 4% at all four medium glucose concentrations. The percentage insulin response was highest at tracer glucose concentration, and a decrease similar for all metabolic pathways was ob-

served at higher glucose concentrations. Fig. 3B shows in more detail the rate of tracer glucose conversion to total lipids, the major pathway for glucose processsing, over a wide range of medium glucose concentrations.

Comparison of glucose transport and metabolism. In Fig. 3C data from panels A and B are shown together, expressed as total (labelled plus unlabelled) glucose transport and conversion to lipids. This visualizes the differences in glucose transport and metabolism capacity directly. It is seen that transport capacity exceeds the metabolism capacity by a factor 3–4 already at subphysiological glucose concentrations. The great difference in K_m is also obvious. These findings also indicate that an intracellular event, and not glucose transport, is rate-limiting for glucose metabolism above tracer glucose concentrations. As discussed above, this step seems to be phosphorylation of D-glucose by hexokinase. The prefix 'apparent' is used for K_m in studies of metabolism, because the conversion of glucose at tracer concentration is limited by transport, thereby underestimating the true phosphorylation rate at these concentrations. The true K_m for this could, therefore, be somewhat lower than the apparent K_m. In rat adipose tissue, hexokinase has an affinity for glucose of approx. 0.07 mmol/l [14].

Fig. 1B shows insulin dose-response curves for lipogenesis, both at tracer glucose concentration and at a medium glucose concentration of 0.5 mmol/l. At tracer concentration, glucose transport is rate-limiting for lipogenesis, and the curve has characteristics almost identical to the D-glucose transport (0–10 s) depicted in Fig. 1A. At a medium glucose concentration of 0.5 mmol/l, a different pattern is observed. Although the total lipogenesis rate is considerably higher, as seen on the *Y*-axis at the right, the uptake of tracer glucose is only one-third of that in cells incubated with tracer alone. This is the same difference as found in Fig. 2B for glucose uptake and is also expected from the findings in Fig. 3B. The percentage insulin response is about two-thirds and the insulin sensitivity 3–4-times higher in adipocytes incubated in the glucose-enriched buffer than in adipocytes incubated with tracer glucose alone (ED_{50}, about 20 versus 70 pmol/l). The different characteristics at a medium glucose concentration of 0.5 mmol/l is obviously caused by some inside-cell step being rate-limiting, and indicates the insulin sensitivity and insulin responsiveness of this step.

Comparison to rat adipocytes

Gliemann et al. [4] have in a recent publication studied the fate of ^{14}C-labelled glucose in rat fat cells exposed to varying glucose concentrations. In maximally insulin-stimulated cells, the results are principally identical to the findings in human fat cells. In particular, it was found that glucose transport is not rate-limiting for glucose utilization, even at subphysiological glucose concentrations. Similar observations have been made by others [6,11]. However, in noninsulin-stimulated rat adipocytes, glucose transport is rate-limiting even at physiological glucose concentrations [6,11]. This fact would give rise to an apparent increase in insulin sensitivity and a decrease in maximal insulin responsiveness in rat fat cells if the insulin effect on glucose metabolism was studied at intermediate glucose concentrations relative to tracer glucose concentrations only. In human fat cells, the changes in insulin sensitivity and responsiveness are real phenomena, because glucose transport is rate-limiting in neither basal nor insulin-stimulated cells. This major difference between rat fat cells and human fat cells is important.

Conclusions

We have studied the characteristics of labelled D-glucose transport and metabolism in human fat cells. We found that in short time uptake studies, D-glucose could replace the more frequently used tracer, 3-*O*-methylglucose. At trace glucose concentrations, transport was rate-limiting for glucose uptake and linear over long time intervals. In the same conditions conversion of glucose to lipids represented more than 80% of glucose uptake. Hence, the glucose transport rate cat be measured as lipogenesis at tracer glucose concentration. In several aspects, this assay was found to be superior to the short-term glucose uptake assay.

At increasing medium glucose concentrations the tracer glucose uptake rate decreased progres-

sively. This was found to be caused by a change in the rate-limiting step, from transport to, most probably, phosphorylation by hexokinase. The major intracellular metabolic pathways for glucose were lipogenesis and water-soluble products, representing about 80 and 20%, respectively, at low as well as high medium glucose concentrations. The only metabolic product escaping the cell in detectable amounts was CO_2, accounting for about 4%. The affinity for glucose of the transport system was about 7 mmol/l, whereas the apparent affinity for glucose conversion to lipids, hydrophilic products and CO_2 was about 0.2 mmol/l. The capacity for glucose conversion to all metabolic products was only a small fraction of that of glucose transport. Hence, even at subphysiological glucose concentrations, glucose transport is not rate-limiting for glucose metabolization in human fat cells. This is the case in both nonstimulated and insulin-stimulated cells.

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