Hexose Transport in Human Adipocytes: Factors Influencing the Response to Insulin and Kinetics of Methylglucose and Glucose Transport

O. Pedersen and J. Gliemann

Division of Endocrinology and Metabolism, Department of Internal Medicine and Department of Clinical Chemistry, County Hospital, Aarhus, and Institute of Physiology, University of Aarhus, Denmark

Summary. Optimal experimental conditions were defined for measuring the initial uptake rate of the non-metabolizable sugar analogue 3-O-methylglucose in non-stimulated and insulin-stimulated human adipocytes. The permeability of the adipocyte plasma membrane for tracer methylglucose (100 $\mu\text{mol/l}$) was $2.9 \times 10^{-7} \text{ cm} \times \text{s}^{-1}$ at 37°C and slightly lower at 20°C . At 37°C and pH 7.4 insulin (5 nmol/l) increased the permeability about twofold (range 1.5 to fivefold) with half maximal effect at about 100 pmol/l. At pH 7.0 the dose response curve for the insulin effect on the uptake rate of methylglucose was shifted about 2.5-fold to the right. The permeability to L-glucose due to simple diffusion was estimated as $3.0 \times 10^{-10} \text{ cm} \times \text{s}^{-1}$ suggesting that uptake of methylglucose occurs almost exclusively by facilitated diffusion. The K_m for methylglucose equilibrium exchange in insulin stimulated cells was about 4.8 mmol/l. The initial uptake of tracer methylglucose in insulin-stimulated cells was inhibited by unlabelled methylglucose and by D-glucose with inhibition constants of about 3.8 and 7.7 mmol/l, respectively. Uptake of tracer 2-deoxyglucose (50 $\mu\text{mol/l}$) in insulin-stimulated adipocytes was linear from 10 s to 5 min whereas the rate of uptake in the presence of 3 mmol/l of D-glucose was markedly decreased suggesting that deoxyglucose uptake after a few minutes is mainly limited by hexokinase in the presence of glucose.

Key words: Methylglucose, glucose, deoxyglucose, insulin, collagenase, agitation

In a preceding paper we have shown that insulin binding to human adipocytes is readily measured at 37°C and that a steady state can be maintained for at

least 2 h [1]. This is an important prerequisite for the study of the relation between insulin binding to adipocytes and its action at physiological temperature. The stimulation of hexose transport is an important and early effect of insulin which has been studied in some detail in rat adipocytes [2]. In the present paper we describe some characteristics of the hexose transport system in human adipocytes both in the absence of insulin and under conditions of steady-state insulin binding.

Materials and Methods

Chemicals, composition of buffer, preparation of adipocytes, and measurement of cell size are described in the preceding paper [1]. Additional chemicals were 3-O- ^{14}C -methyl-D-glucose (59 mCi/mmol), and 2-deoxy-D-1- ^{14}C -glucose (60 mCi/mmol), both obtained from the Radiochemical Centre, Amersham, UK. Phloretin was from ICN Pharmaceuticals, Plainview, New York, USA.

Subjects

Specimens of subcutaneous adipose tissue were obtained from the abdomen of 40 females and 19 males undergoing gynaecological or gastroenterological surgery (sterilisation or duodenal ulcer). The patients were from 18 to 65 years old and their relative body weight was $\pm 15\%$ of ideal weight [3]. None of the donors suffered from endocrine disorders. The subjects were fasted for about 12 h overnight. Preoperative medication was diazepam and atropine. General anaesthesia was induced with a short-acting barbiturate and maintained with halothane, nitrous oxide and oxygen.

Hexose Transport-Studies

Uptake of different hexoses was measured using a modification of the method described by Whitesell and Gliemann [2]. After the last two rinses of cells in glucose-free buffer (10 mmol/l Hepes [1], 50 mg/ml human albumin, pH 7.4) the cell suspension was concentrated to a volume fraction of adipocytes at 0.40 and 40 μl aliquots were carefully placed in round-bottom polypropylene tubes (11 $\times$

Table 1. Factors influencing the response to insulin

Experimental conditions:	Uptake rate of [^{14}C]-methylglucose at 37°C during the initial 5 s in insulin-stimulated (5 nmol/l) and noninsulin-stimulated (basal) fat cells. Uptake rates are expressed in fractions of equilibrium space for methylglucose.		
	Basal	Insulin	(Insulin-Basal) $\times$ 100 Basal
a Effect of collagenase concentration in buffer.			
0.5 mg/ml	0.10 $\pm$ 0.04	0.21 $\pm$ 0.05	110
1.0 mg/ml	0.14 $\pm$ 0.06	0.26 $\pm$ 0.07	86
2.0 mg/ml	0.17 $\pm$ 0.06	0.26 $\pm$ 0.05	53
3.0 mg/ml	0.18 $\pm$ 0.06	0.26 $\pm$ 0.06	44
b Effect of mechanical agitation			
Control	0.11 $\pm$ 0.04	0.19 $\pm$ 0.05	73
Agitation	0.17 $\pm$ 0.05	0.18 $\pm$ 0.04	6
c Effect of preparation in glucose enriched media			
Effect of preparation in glucose free media	0.10 $\pm$ 0.03	0.25 $\pm$ 0.06	150
	0.11 $\pm$ 0.03	0.21 $\pm$ 0.06	91

Panel a: Adipocytes were prepared with different concentrations of collagenase.

Panel b: Adipocytes were isolated with 0.5 mg/ml collagenase and prepared as described in Methods. Just before measurement of transport activity adipocyte suspensions were shaken (250 cycles/min) for 5 min (agitation).

Panel c: Adipocytes were isolated with 0.5 mg/ml collagenase and prepared in the presence or absence of 5 mmol/l glucose. Hexose transport was measured after two rinses in glucose free buffer. All data are mean values of five individual experiments $\pm$ 1 SD. Statistical analysis was done on the log transformed data

50 mm) in triplicate. Cells were then incubated without shaking for 45 min at 37°C before beginning of experiments. At the indicated times, 12 μl of labelled hexose (0.2 μCi methylglucose/tube or 0.1 μCi deoxyglucose/tube or 0.2 μCi L-glucose/tube) were injected directly into the cell mass with an automatic pipette. The incubation was terminated by the addition of 3 ml of 154 mmol/l saline containing 0.3 mmol/l of phloretin and 0.15% v/v of ethanol. Silicone oil (800 μl , relative density 0.99, viscosity 100 centistokes) was layered on the top and the tubes were centrifuged within 3 min for 1 min at $2500 \times g$. The 16 μl of packed cells on the top of the oil layer was sucked into a plastic disposable pipette tip which was cut off and put into a counting vial with 5 ml scintillation fluid. No variations of quenching were observed. Extracellular trapped radioactivity was measured by adding 1 ml of phloretin solution to cells before injection of isotope. The uptake rates of hexoses were expressed as fractions of the equilibrium value for uptake of the nonmetabolizable sugar analogue methylglucose which was routinely measured after incubation for 30 min. All values were corrected for extracellular trapped radioactivity which for methylglucose averaged 0.036 ± 0.010 ($n = 25$ consecutive experiments) of the equilibrium space for methylglucose. Methylglucose equilibrium space corrected for extracellularly trapped

radioactivity was on several occasions found to be indistinguishable from the intracellular water space ($^3\text{H}_2\text{O}$ space minus L-[^{14}C]-glucose space, data not shown).

Analysis of Data for Hexose Transport

[^{14}C]-methylglucose clearance (i.e. the volume of extracellular medium from which methylglucose is removed completely per unit time by influx into initially sugar free cell water) was determined as counts per minute (cpm) taken up per cell per second/cpm per cm^3 medium. The permeability of the adipocyte plasma membrane for tracer methylglucose was calculated as

$$\frac{\text{clearance (cm}^3 \times \text{s}^{-1})}{\text{mean cell surface area (cm}^2\text{)}} \quad [1]$$

K_m for methylglucose equilibrium exchange (transport of labelled sugar under conditions with equal extracellular and intracellular concentration of unlabelled sugar) was defined as the concentration of methylglucose which gives half maximal exchange of methylglucose and calculated according to

$$t_{1/2} c = \left(1 + \frac{c}{K_m}\right) \times t_{1/2} (\text{tracer}) \quad [2]$$

Where c is the concentration of unlabelled methylglucose in the aqueous phases and $t_{1/2}$ the half time for uptake of labelled methylglucose alone (index tracer) or in the presence of methylglucose at concentration c . K_i was defined as the concentration of unlabelled methylglucose or D-glucose which inhibits the uptake rate of tracer methylglucose 50% in initially sugar free cells.

Precision of Transport Measurement

In noninsulin-stimulated adipocytes the coefficient of variation was 1) 0.018 for extracellularly trapped radioactivity 2) 0.017 for methylglucose uptake at equilibrium 3) 0.073 for methylglucose uptake rate during the first 5 s ($n = 25$ consecutive experiments). Thus, the total coefficient of variation for measurement of tracer methylglucose uptake rate during 5 s (expressed in fraction of intracellular cell water) was 0.096 in absence of insulin, and slightly lower in its presence.

Evaluation of Experimental Conditions

Unfortunately purified collagenase is unable to liberate adipocytes from fat tissue [4]. The batches of commercial crude collagenase contain variable types and amounts of enzymes with trypsin-like activities [5]. Accordingly, the metabolic activity of isolated cells may depend on the batch and concentration of collagenase used for cell liberation. This study showed a collagenase concentration-dependent increase of basal influx rate of methylglucose (Table 1a). Moreover, we found a twofold shift to the right of the dose-response curve for the insulin effect on the uptake rate of methylglucose when adipocytes isolated with 0.5 mg/ml collagenase were compared with adipocytes isolated with 3.0 mg/ml collagenase (data not shown). Hence, the use of one batch and a constant and low concentration of collagenase is mandatory in comparative studies on isolated adipocytes. Mechanical agitation had an insulin-like effect on hexose transport (Table 1b). Shaking and centrifugation during the preparation procedure should therefore be limited and standardized [6, 7]. Preparation of fat cells in glucose free media caused a reduction of the insulin effect on methylglucose uptake (Table 1c), probably due to energy depletion [7]. The concentration of albumin in final incubation buffer had no consistent influence on hexose transport activity (data not shown). However, as elaborated in the preceding paper [1] a high concen-

Fig. 1. Effect of phloretin on efflux of [^{14}C] methylglucose. Maximally insulin-stimulated (5 nmol/l) adipocytes were incubated with [^{14}C] methylglucose (100 $\mu\text{mol/l}$) for 30 min at 37°C. At time zero, 3 ml of saline containing 0.3 mmol/l of phloretin was added and the tubes were centrifuged at the times indicated. Mean values of three individual experiments $\pm$ 1 SEM

tration of albumin in fat cell buffer is needed to minimize cell traumatization. Bovine serum albumin (fraction V) may contain variable amounts of substances with insulin-like activities [8]. Consequently, highly purified human albumin should be used in studies of human adipocytes.

It is essential that the phloretin stopping solution works efficiently and almost immediately to prevent efflux of cell accumulated hexose. Figure 1 shows that the loss of methylglucose from the intracellular pool of phloretin treated cells was 1.2%/min. Thus, the stopping procedure was adequate since adipocytes were centrifuged within 3 min of stopping the reaction between hexose and cells. The permeability of the phloretin treated cells with respect to tracer methylglucose was about 0.6% of the uninhibited permeability (Fig. 2).

A significant negative correlation was found between fat cell volume and the intracellular water space per volume packed cells ($r = -0.74$, $p < 0.001$, $n = 50$ consecutive experiments). Thus, the difference between the space at time zero and that at any given time will decrease with increasing cell size.

Statistical Methods

In text and table results are presented as mean $\pm$ 1 SD. In the Figures data are given as mean $\pm$ 1 SEM. Student's *t*-test was used to test the significance of paired observations. Linear regression analysis was employed in a correlation study using the least-squares method. The number of samples (*n*) equals the number of persons.

Results

Figure 2 depicts the time course for uptake of methylglucose (100 $\mu\text{mol/l}$) and the effect of insulin. It is seen that the initial velocity can be measured between 5 and 10 s. The permeability at 37°C was calculated from Fig. 2 as $2.5 \times 10^{-7} \text{ cm} \times \text{s}^{-1}$ in the absence of insulin and $5.0 \times 10^{-7} \text{ cm} \times \text{s}^{-1}$ in its

Fig. 2. Time course for uptake of 100 $\mu\text{mol/l}$ [^{14}C] methylglucose. Triangles indicate uptake at 20°C and circles indicate uptake at 37°C. Closed symbols: maximally insulin-stimulated (5 nmol/l) cells and open symbols: basal cells. Cell diameter averaged $76 \pm 3 \mu\text{m}$. Mean values of six individual experiments $\pm$ 1 SEM

Fig. 3. Uptake rate of 100 $\mu\text{mol/l}$ of [^{14}C]-L-glucose. Uptake rate was measured at 37°C in the absence (circles) and in the presence (triangles) of 40 mmol/l of unlabelled methylglucose. Closed symbols: maximally insulin-stimulated (5 nmol/l) cells. Open symbols: basal transport. Mean values of three individual experiments $\pm$ 1 SEM

presence (5 nmol/l). Calculated from the combined data of 30 separate experiments the permeability was $6.1 \pm 2.4 \times 10^{-7} \text{ cm} \times \text{s}^{-1}$ in the presence of insulin (5 nmol/l) and $2.9 \pm 0.4 \times 10^{-7} \text{ cm} \times \text{s}^{-1}$ in its absence. An increase in temperature from 20°C to 37°C nearly doubled the maximal permeability for tracer methylglucose. The insulin effect was slightly greater at 37°C than at room temperature (Fig. 2).

The rate of L-glucose uptake (100 $\mu\text{mol/l}$) is shown in Fig. 3. L-glucose was taken up at a much slower rate than D-methylglucose. Insulin stimulated the uptake indicating that carrier-facilitated transport was involved. Moreover, 40 mmol/l of unlabelled methylglucose inhibited L-glucose uptake markedly. The permeability of L-glucose in the presence

Fig. 4. Equilibrium exchange of methylglucose. Uptake rate was measured at 37 °C in maximally insulin-stimulated (5 nmol/l) cells. Uptake of 100 μmol/l [14 C] methylglucose (●—●) and uptake of 10 mmol/l (○—○) or 20 mmol/l (Δ—Δ) methylglucose in cells equilibrated for 1 h with 10 or 20 mmol/l unlabelled methylglucose. The mean half-times for uptake were 45 s for tracer, 140 s for 10 mmol/l methylglucose and 240 s for 20 mmol/l methylglucose. Mean values of six individual experiments $\pm$ 1 SEM

Fig. 6. Steady-state insulin dose-response relationship. Uptake rate of 100 μmol/l [14 C] methylglucose was measured at 37 °C in cells, preincubated at 37 °C for 1 h in buffers at pH 7.4 (○—○) or at 7.0 (●—●) and with insulin in the concentrations indicated on the abscissa. Uptake rate was measured during the initial 5 s. Degradation of labelled insulin in the cell suspension by the time transport was measured, was 2% as estimated by solubility in 12% w/v trichloroacetic acid. Mean values of three individual experiments $\pm$ 1 SEM

of unlabelled methylglucose, which may be considered an estimate of simple diffusion, was 3.0×10^{-10} cm $\times$ s $^{-1}$ or about 1,000 times lower than the permeability of methylglucose in the absence of insulin. The permeability to methylglucose in the presence of

Fig. 5. Inhibition of [14 C] methylglucose uptake rate by methylglucose and glucose. Twelve μl buffer containing [14 C] methylglucose (100 μmol/l) and unlabelled methylglucose (○—○) or unlabelled glucose (●—●) in concentrations three times those indicated on the abscissa were added to 40 μl 40% v/v (with 24 μl of aqueous phase) of maximally insulin-stimulated (5 nmol/l) cells. Uptake rate was measured during the initial 5 s. Mean values of five individual experiments $\pm$ 1 SEM

Fig. 7. Uptake of deoxyglucose. Uptake of 50 μmol/l [14 C]-deoxyglucose was measured at 37 °C in maximally insulin-stimulated (5 nmol/l) cells in the absence (○—○) and in the presence of 3.3 mmol/l of unlabelled glucose (●—●) in the final incubation volume. [14 C]-deoxyglucose and glucose were added to the cells at the same time. Data are expressed as cpm per sample. Mean values of three individual experiments $\pm$ 1 SEM

80 μmol/l cytochalasin B was 5.0×10^{-9} cm $\times$ s $^{-1}$ (data not shown).

Figure 4 shows experiments in which equilibrium exchange rate of methylglucose in insulin stimulated adipocytes was measured both with tracer methylglu-

cose (100 $\mu\text{mol/l}$) alone and with 10 or 20 mmol/l unlabelled methylglucose. K_m (equilibrium exchange) was calculated as 4.7 ± 1.3 mmol/l from the results with 10 mmol/l and as 4.9 ± 0.6 mmol/l from those with 20 mmol/l methylglucose.

The inhibition constant (K_i) of methylglucose on the net uptake (intracellular sugar concentration is initially zero) of tracer methylglucose (Fig. 5) was of the same magnitude (3.8 ± 1.1 mmol/l) as K_m for equilibrium exchange. K_i for net uptake of D-glucose (Fig. 5) was about twice as high as for methylglucose (7.7 ± 1.9 mmol/l).

The steady-state insulin dose-response relationship on methylglucose transport at 37°C is depicted in Fig. 6. At pH 7.4 insulin caused a 2.5-fold increase above basal level in initial uptake rate with a maximal effect at an insulin concentration of about 1 nmol/l. Half-maximal stimulation was observed at an insulin concentration of about 0.100 nmol/l. A change of buffer pH from 7.4 to 7.0 caused a 2.5-fold shift of the dose response curve to the right (Fig. 6). In contrast basal and maximally insulin stimulated methylglucose uptake rate was independent of buffer pH in the range 7.0 to 7.6. At pH above 7.6 a decline in insulin enhanced influx occurred (data not shown).

Figure 7 shows the uptake of tracer deoxyglucose (50 $\mu\text{mol/l}$) with time at 37°C in maximally insulin stimulated adipocytes in the absence and presence of 3.3 mmol/l of D-glucose. The uptake of tracer alone appeared linear from 10 s to 5 min whereas the uptake in the presence of D-glucose was progressively non-linear.

Discussion

The half times of uptake of tracer methylglucose in this study were similar to those reported by Ciaraldi et al. [9]. The permeability of the human adipocyte plasma membrane to tracer methylglucose in the absence of insulin ($2.9 \times 10^{-7} \text{ cm} \times \text{s}^{-1}$) was about half of that of the rat adipocyte membrane ($6 \times 10^{-7} \text{ cm} \times \text{s}^{-1}$) [2]. If it is assumed that the turnover is the same on the human and rat carrier, this indicates that the number of functional carriers per unit surface area is roughly half in the human adipocyte as compared to the rat adipocyte. In the presence of insulin the permeability of the human adipocyte ($6.1 \times 10^{-7} \text{ cm} \times \text{s}^{-1}$) was much lower than that of small rat adipocytes ($60 \times 10^{-7} \text{ cm} \times \text{s}^{-1}$) [2]. The effect of insulin may to a large extent be due to recruitment of hexose transport sites from an intracellular pool to the plasma membrane [10, 11]. Therefore, the combined data suggest that the adipocyte of adult man is able to recruit only a limited number of additional

transport sites as a result of binding of insulin to its receptor.

L-glucose was transported very slowly across the cell membrane and partially via the carrier-facilitated and insulin responsive mechanism. Similar findings have been described in rat adipocytes [2]. The permeability of L-glucose accounted for by simple diffusion (i. e. in the presence of a near-saturating concentration of methylglucose) as well as the permeability for methylglucose in the presence of phloretin and cytochalasin B was less than 2% of the carrier-facilitated permeability of tracer methylglucose. Consequently, kinetic data for the rapidly transported sugars, methylglucose and glucose, were not corrected for simple diffusion.

The K_m for equilibrium exchange of methylglucose was about 4.8 mmol/l which is nearly the same as in rat adipocytes [2, 12]. Equilibrium exchange can only be measured for nonmetabolizable sugar analogues and, in order to estimate K_m for transport of D-glucose, net uptake was measured. The initial influx rates tend to be underestimated at high sugar concentrations due to the nonexponential nature of the net flux curve [2]. Thus, K_m for net influx is slightly underestimated and it is likely that the true K_m for net influx of methylglucose (measured value 3.8 mmol/l) is not different from K_m for equilibrium exchange (4.8 mmol/l). This shows that the transport system is not asymmetrical in the same way as that of human erythrocytes in which K_m for equilibrium exchange is more than 10 times higher than that for net influx [13]. The measured K_m for net glucose uptake (7.7 mmol/l) was twice as high as that for methylglucose, which is in agreement with the results of Ciaraldi et al. [9] and Whitesell and Gliemann [2].

The almost identical behaviour of three sugars in human and rat adipocytes suggests that their transport systems are very similar. In this context it should be pointed out that a transport K_m of 1.9 mmol/l has been reported previously for D-glucose in rat adipocytes [14]. This apparent K_m was estimated as the inhibition constant of glucose on the uptake after 3 min of tracer deoxyglucose, a sugar which is phosphorylated by hexokinase but not further metabolized. However, it has been shown in rat adipocytes that glucose not only inhibits transport but also occupies the hexokinase as time progresses and glucose accumulates in the intracellular water [15]. Therefore, tracer deoxyglucose accumulates in the intracellular water due to its diminished rate of phosphorylation and the net uptake rate decreases [15]. The present data on deoxyglucose in combination with glucose (3.3 mmol/l) strongly suggest that the same events occur in human adipocytes. Hence, methylglucose should be used as a probe for meas-

uring hexose transport activity in future clinical studies.

Relationships Between Insulin Receptor Binding and Hexose Transport

About 1 nmol/l insulin is required to obtain maximal stimulation of hexose transport (Fig. 6). Thus, about 25% of the insulin receptors are needed to obtain a maximal biological effect of insulin (cf. Fig. 4 of [1]). In adipocytes from young rats a maximal insulin effect is obtained with an occupancy of about 4% [16]. Hence, the phenomenon of "spare" receptors appears less marked in adipocytes from adult man than in those from growing rats.

Insulin binding is considerably lower at pH 7.0 when compared to pH 7.4 [1]. The functional consequence of a decrease of insulin binding is an impaired insulin sensitivity. Accordingly, a change of buffer pH from 7.4 to 7.0 caused a rightward shift of the dose response curve for the insulin effect on hexose transport.

Acknowledgements. For supply of fat biopsies we are indebted to the staff of the Departments of Surgery, Aarhus Amtssygehus and Aarhus Kommunehospital, and the Department of Obstetrics and Gynecology, Aarhus Kommunehospital. T. Skrumsager, L. Busch, J. Jørgensen and L. Blak are thanked for skillful technical assistance. The study was supported by grants from Danish Medical Research Council, Landsforeningen for Sukkersyges Fond, Aarhus Universitets Forskningsfond, Novo Fond and Nordisk Insulin Fond.

References

1. Pedersen O, Hjøllund E, Beck-Nielsen H, Lindskov HO, Sonne O, Gliemann J (1981) Insulin receptor binding and receptor mediated insulin degradation in human adipocytes. *Diabetologia* 20: 636–641
2. Whitesell RR, Gliemann J (1979) Kinetic parameters of transport of 3-O-methylglucose and glucose in adipocytes. *J Biol Chem* 254: 5276–5283
3. Geigy Scientific Tables (1970) 7th edn
4. Kono T (1969) Roles of collagenases and other proteolytic enzymes in the dispersal on animal tissues. *Biochim Biophys Acta* 178: 397–400
5. Fain JN (1975) Isolation of free brown and white fat cells. *Methods Enzymol* 35: 555–561
6. Gliemann J (1967) Assay of insulin-like activity by the isolated fat cell method. I. Factors influencing the response to crystalline insulin. *Diabetologia* 3: 382–388
7. Vega FV, Kono T (1979) Sugar transport in fat cells: Effects of mechanical agitation, cell-bound insulin and temperature. *Arch Biochem Biophys* 192: 120–127
8. Lawrence JC Jr, Guinovart JJ, Lerner J (1977) Activation of rat adipocyte glycogen synthase by insulin. *J Biol Chem* 252: 444–450
9. Ciaraldi TP, Kolterman OG, Siegel JA, Olefsky JM (1979) Insulin-stimulated glucose transport in human adipocytes. *Am J Physiol* 236: 621–625
10. Cushman SW, Wardzala LJ (1980) Potential mechanism of insulin action on glucose transport in the isolated rat adipose cell. *J Biol Chem* 255: 4758–4762
11. Suzuki K, Kono T (1980) Evidence that insulin causes translocation of glucose transport activity to the plasma membrane from an intracellular storage site. *Proc Natl Acad Sci USA* 77: 2542–2545
12. Vinten J, Gliemann J, Österlind LK (1976) Exchange of 3-O-methylglucose in isolated fat cells. Concentration dependence and effect of insulin. *J Biol Chem* 251: 794–800
13. Regen MD, Tarpley HL (1974) Anomalous transport kinetics and the glucose carrier hypotheses. *Biochim Biophys Acta* 339: 218–233
14. Olefsky JM (1978) Mechanisms of the ability of insulin to activate the glucose transport system in rat adipocytes. *Biochem J* 172: 137–145
15. Foley JE, Foley R, Gliemann J (1980) Rate limiting steps of 2-deoxyglucose uptake in rat adipocytes. *Biochim Biophys Acta* 599: 689–698
16. Gammeltoft S, Gliemann J (1973) Binding and degradation of ¹²⁵I-labelled insulin by isolated rat fat cells. *Biochim Biophys Acta* 320: 16–32

Received: 15 September 1980
and in revised form: 13 October 1980

Dr. Oluf Pedersen
Medical Department III
County Hospital
Tage Hansensgade
DK-8000 Aarhus C
Denmark