

Insulin Binding and Hexose Transport in Rat Adipocytes

Relation to Cell Size

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Summary. Insulin binding, initial velocity of [^{14}C]methylglucose transport, uptake of [^{14}C]deoxyglucose and conversion of [$\text{U-}^{14}\text{C}$]glucose to CO_2 , glyceride-glycerol and fatty acids were measured at 37 °C in adipocytes from rats of different weights (135–450 g) and therefore with different mean cell volumes (53–389 pl). Insulin binding per cell increased with increasing cell size and binding was 2.3 times higher in the largest cells than in the smallest cells with tracer alone. The difference was largely accounted for by an increase in the apparent affinity. Influx of methylglucose per cell increased with increasing cell size in the absence of insulin and remained constant as a function of cell size in its presence. The effect of insulin ranged from 11 fold in small cells to 3.5 fold in large cells. The rate of conversion of [$\text{U-}^{14}\text{C}$]glucose to CO_2 and lipids was about half of the rate of methylglucose transport under all conditions. In contrast, the uptake of deoxyglucose in insulin-stimulated cells decreased markedly with increasing cell size. Increasing cell size caused a small decrease in sensitivity which could be explained by a smaller amount of insulin bound per unit surface area. The results show that increasing cell size/animal weight causes changes in insulin binding which may explain changes in sensitivity. In addition, the hexose transport system is modified in a way which is not explained by changes in insulin binding. Finally, changes in deoxyglucose uptake with cell size do not parallel changes in methylglucose transport.

Key words: Insulin receptors, 3-O-methylglucose, glucose, 2-deoxyglucose, hexose transport, hexose metabolism, fat cell size.

insulin binding (receptor number and affinity) and hexose transport rates are key elements in such analyses. However, nearly all possible combinations of results have been reported. Thus, Olefsky [1–3] found that binding of [^{125}I]insulin per cell decreased as rats grew older and fatter, whereas Lockwood and co-workers [4, 5] found no change, and Cushman and Salans [6] an increase. Czech [7] found that transport of tracer hexose (that is in a concentration much lower than K_m), when expressed per cell, was several fold higher in large cells than in small cells both in the absence and the presence of insulin. Livingston and Lockwood [8] reported nearly the same transport rates per cell both with and without insulin. Olefsky [2] and Foley [9] found that transport per cell increased with increasing cell size in the absence of insulin but remained approximately constant in its presence.

We undertook the present study in the attempt to resolve these issues by utilising some recent technological improvements. The major element with regard to binding is the finding that a steady state can be maintained for several hours at 37 °C [10]. In addition, we have recently reported that the binding affinity of monoiodoinsulin iodinated in residue A14 (tyrosine) of the A chain is the same as that of insulin, whereas other iodinated derivatives exhibit reduced affinities [11]. With regard to transport, we have recently described a method to measure the initial rate of uptake of 3-O-methylglucose [12], a sugar analogue which is transported at approximately the same rate as glucose but not phosphorylated. This made it possible to measure unidirectional transport of methylglucose in basal and insulin-stimulated cells over the entire size range. We also measured deoxyglucose uptake under the same conditions as those used for methylglucose, deoxyglucose uptake after incubation for 3 minutes, and finally the conversion

Several authors have investigated the isolated rat fat cell model in the attempt to elucidate the mechanism of the insulin resistance in obesity. Measurement of

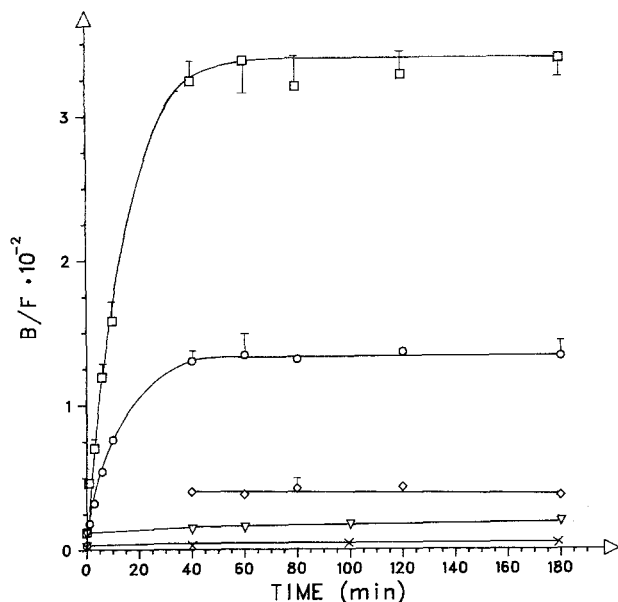


Fig. 1. Time course of binding of [^{125}I]iodoinsulin. Adipocytes from a 140 g (\circ - \circ) and a 430 g (\square - \square) rat were incubated at 37 °C with A14- ^{125}I iodoinsulin 50 pmol/l. Insulin 10 nmol/l (small cells, \diamond - \diamond) or 1 $\mu\text{mol/l}$ (small cells \times - \times , large cells ∇ - ∇) was added to some samples. All incubations were carried out using 24 μl packed cell volume and a total incubation volume of 0.5 ml. The number of cells was calculated as described in Methods. The ordinate (B/F) denotes the ratio: cpm associated with 3×10^5 cells/cpm in the incubation medium. Extracellular radioactivity trapped in the cell pellet was determined using L-glucose and accounted for a B/F of 1.1×10^{-4} . This value, although negligible, has been subtracted from all points. These are mean values of 4 replicates with 1 SD when this exceeds the size of the symbol

of trace glucose to triglycerides and CO_2 after 120 minutes.

The present work represents the first study with concomitant measurements of insulin binding, transport rates of methylglucose and deoxyglucose, and conversion rates of deoxyglucose and glucose at physiological temperature and pH.

Materials and Methods

Adipocytes were obtained from epididymal fat pads of ad libitum fed male Wistar rats in 3 weight groups: 135–145 g, 220–240 g, and 400–450 g. On a given day, experiments were performed on one group of animals using 12, 8, and 3 rats, respectively. HEPES buffer (pH 7.4) with or without albumin was prepared as described previously [10]. Albumin (bovine, fraction V) was obtained from Sigma (St. Louis, USA), collagenase (type I) from Worthington (Freehold, NJ, USA), pig insulin from Nordic Insulin (Gentofte, Denmark), and phloretin from K & K laboratories (Plainview, NY, USA). 3-O- ^{14}C -Methyl-D-Glucose (59 mCi/mmol), 2-deoxy[^{14}C]-D-glucose (54 mCi/mmol), and [^{14}C]-D-glucose (275 mCi/mmol) were from the Radiochemical Centre (Amersham, Great Britain). A14- ^{125}I Moniodoinsulin (about 250 Ci/g) was prepared as described previously [11] by Dr. S. Linde, Hagedorn Research Laboratory. The tracer was 98% precipitable in 12 g/100 ml trichloroacetic acid.

Adipocyte Preparation

Epididymal fat pads were added to 30 ml polystyrene cylinders containing 3 ml of 3.5 g/100 ml albumin-HEPES buffer with collagenase 0.5 mg/ml and minced. A 6 \times 15 mm stir bar was added and the tissue agitated on a magnetic stirrer for 1 h at 37 °C. The cell suspension was passed through a nylon filter (PES 3000 polymer) and washed 5 times. The fraction of the total volume occupied by the cells was measured in duplicate using haematocrit tubes as described previously [13]. The coefficient of variation was 5% ($n = 24$). The cells were then distributed in appropriate amounts to measure binding, transport and metabolism.

Insulin Binding

Packed cells, 24 μl , were incubated in a 14 ml polyethylene tube with [^{125}I]iodoinsulin 50 pmol/l and insulin as indicated. The buffer contained albumin 50 g/l and bacitracin 0.5 g/l. The total volume was 0.5 ml. It has previously been shown that bacitracin markedly inhibits extracellular insulin degradation without interfering with receptor-mediated degradation of insulin or its effect on hexose transport [10, 12]. The cells were incubated for 45 min at 37 °C. The incubation was terminated and the cells recovered as described previously [10].

Transport of Methylglucose and Deoxyglucose

The initial velocity of uptake was measured using 5 s incubations in the absence of insulin and 1 s incubations in cells preincubated for 45 min with 100 nmol/l insulin, a maximally stimulating concentration. The incubations were carried out in a room maintained at 37 °C using 40 μl 40% (v/v) cell suspension, 12 μl isotope solution and timing with a metronome as described by Whitesell and Gliemann [12] with minor modifications as described in detail elsewhere [14]. In brief, transport was stopped with 400 μl 0.1 mmol/l phloretin, and a 400 μl aliquot of the mixture was added to a 550 μl microfuge tube containing 100 μl silicone oil. The tube was spun within 2 min in a Beckman microfuge for 30 s, cut through the oil phase and the cell pellet was added to a 3 ml counting vial with 2.5 ml scintillation fluid. Zero time was determined by adding 400 μl phloretin solution to the isotope solution before adding cells. Under these conditions the extracellular space (zero time space) constituted 6% of the intracellular water space in the cell pellet. The coefficient of variation on 1 s measurements (total counts minus extracellular counts) was 5–10% in small cells and 10–15% in large cells.

Uptake of Deoxyglucose

Fifty microlitres of 40% (v/v) cell suspension with or without insulin 100 nmol/l was added to vials containing 950 μl buffer with 0.125 μCi 2-[^{14}C]deoxyglucose. Incubation was carried out in a 37 °C water bath for 3 min, and 15 s before the end of the incubation a 400 μl aliquot was added to a microfuge tube containing 100 μl silicone oil and spun for 15 s. Zero time incubations were made by adding cells to vials at 2 °C, immediately followed by centrifugation.

Metabolism of Glucose

Seven microlitres of packed cells were incubated in a 22 ml beta counting vial with 0.2 μCi [^{14}C]glucose and insulin as indicated. The total volume was 1 ml and incubation time 120 min. Incubation was terminated by the addition of H_2SO_4 , and [^{14}C] O_2 was collected as described previously [15]. Labelled glycerides were

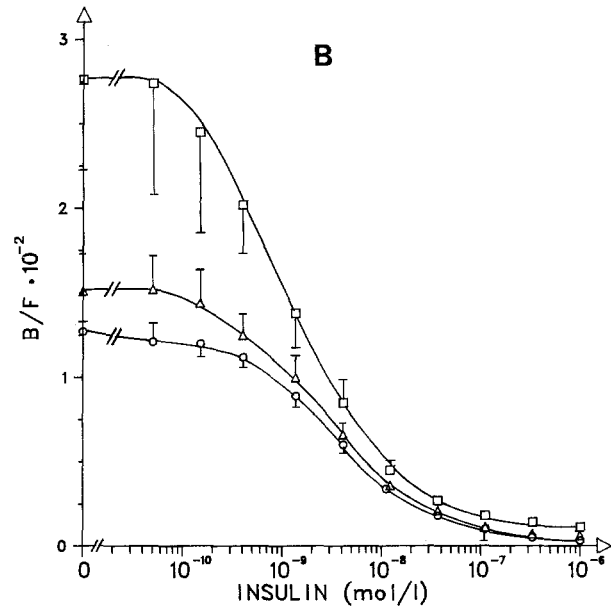
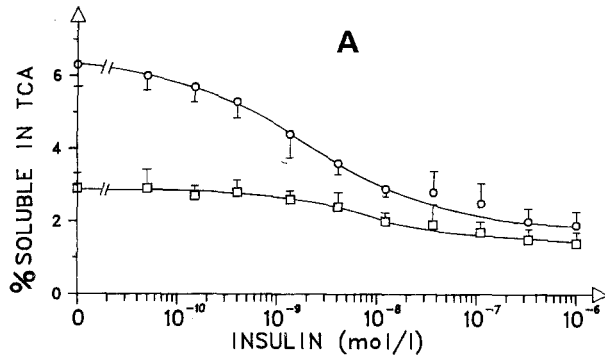
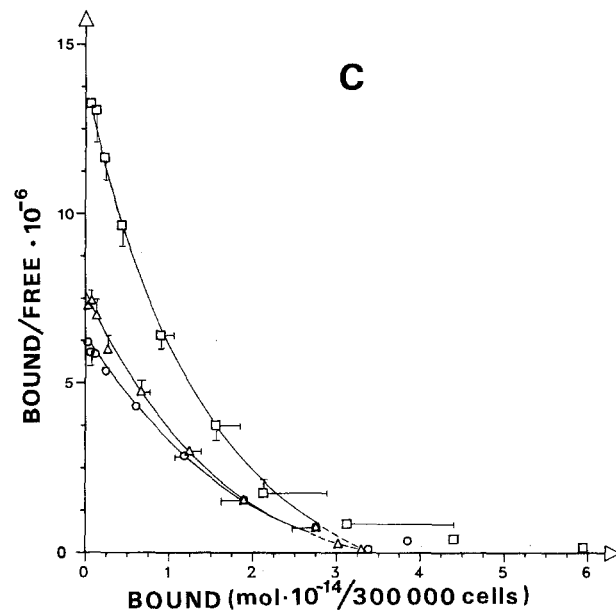


Fig. 2 A-C. Steady state binding as a function of insulin concentration. Small (○-○), medium (△-△) and large □-□ cells were incubated as described in the legend to Figure 1 for 45 min with concentrations of unlabelled insulin as indicated. **A Degradation.** The ordinate denotes the increase in TCA solubility of [¹²⁵I]iodoinsulin in the incubation medium from 0-45 min. The results are from a representative experiment (mean of 4 replicates) with 1 SD. **B Binding.** The ordinate is the same as that shown in Figure 1. The points on the ordinate indicate binding of the A14-[¹²⁵I]iodoinsulin tracer (50 pmol/l). Each point represents the mean value of 4 independent experiments with 1 SD when this exceeds the size of the symbol. Note that the B/F in the presence of insulin 1 μmol/l, which we define as non-specific binding, is not subtracted. **C Scatchard plots.** The data are the same as those shown in Figure 2B. The abscissa denotes bound [¹²⁵I]iodoinsulin plus unlabelled insulin. The ordinate denotes the ratio: mol total insulin bound specifically per 3×10^5 cells/mol total insulin/l medium. The data of each set of 4 experiments (small, medium or large cells) were normalised in the following way. The ratio of B/F (tracer) in each experiment to the mean B/F (tracer) for the group ($n = 4$) was computed and each point in a given experiment was multiplied by this ratio. The points on the graph represent the mean values of the 4 points generated in this way with one vertical and one horizontal SD when these exceed the size of the symbol. Note that SD is not indicated on points with ordinates of less than 0.4×10^{-6} . The horizontal SD values are very large (more than 2×10^{-14} mol per 3×10^5 cells) due to the impact of non-specific binding on the scatter of specific binding



extracted using Dole's method and in some experiments the glycerides were saponified and the radioactivity in the glycerol and fatty acid moieties determined [16].

Determination of Cell Number

One hundred and fifty microlitres of the cell suspension used for measuring glucose metabolism (0.7% v/v) was added to a 10 ml plastic tube containing 0.6 ml 0.2 g/100 ml osmium tetroxide in 0.154 mol/l NaCl. The cells did not adhere to the sides of the tube and were completely fixed after about 15 min. The diameter distribution was determined [17] from approximately 200 cells

obtained from 3 tubes. The mean diameter was calculated and the mean cell volume determined according to the formula described by Goldrick [18]

$$\bar{V} = \frac{1}{6} \pi (3 \bar{\delta}^2 + \bar{d}^2) \bar{d},$$

where \bar{d} is the mean diameter and $\bar{\delta}$ the variance. $\bar{\delta}$ is a function of the mean cell size and we used previously tabulated values [19] since $\bar{\delta}$ could not be determined with great precision in a sample size of 200. The number of cells was calculated from the total cell volume and the mean cell volume. The coefficient of variation on 12 measurements was 3%.

Calculations

Results are expressed as mean \pm SD. Correlations were calculated using the least square method.

Results

Insulin Binding and Degradation

Figure 1 shows that a constant binding was obtained from 40 min to 180 min, and that non-specific binding, defined as binding of tracer in the presence of insulin 1 $\mu\text{mol/l}$, was only 2% (small cells) to 4% of the total binding. Tracer binding per cell was higher in the large cells from old rats than in the small cells; binding per unit cell volume was, however, considerably smaller in the large cells. This phenomenon was observed in 7 other experiments (data not shown). In these experiments some cells were incubated with tracer alone for 45 min, recovered from the oil layer and reincubated in 1 ml buffer with insulin 1 $\mu\text{mol/l}$ at 37 $^{\circ}\text{C}$ for 60 min. The radioactivity dissociated from the small cells was $49 \pm 8\%$ ($n = 7$) soluble in trichloroacetic acid, indicating that about half of the receptor bound insulin was degraded in small adipocytes. The value for large cells was $41 \pm 16\%$ ($n = 7$). In both groups $95 \pm 3\%$ of the radioactivity was released to the medium by 60 min. Together with the time course shown in Fig. 1, the data demonstrate that the turnover of tracer is nearly the same on receptors from large and small cells.

Figure 2A shows that about 6% of the [^{125}I]iodoinsulin in the medium was degraded during the 45 min incubation of small cells. A large part of the degradation was inhibited half-maximally by insulin 2–3 nmol/l suggesting that this degradation is a consequence of insulin binding to its receptor. The high albumin-bacitracin medium, when preincubated with cells for 45 min, degraded only about 1% of the tracer during a subsequent 45 min incubation (data not shown). The degradation of tracer was consequently smaller in the large cells which contained fewer receptors per unit volume (Fig. 2A).

Figure 2B shows the steady state binding as a function of the insulin concentration for the three groups of rats. The binding per cell was increased in the large cells in the range 50 pmol/l to about 4 nmol/l. Analyses of the combined data from the 12 experiments showed a highly significant correlation between mean cell volume and tracer binding per cell (increase 2.3 fold over the cell size range, $r^2 = 0.68$, $p < 0.001$). The concentration of insulin causing half-maximal displacement of tracer (apparent K_d) decreased with increasing cell size. The apparent K_d

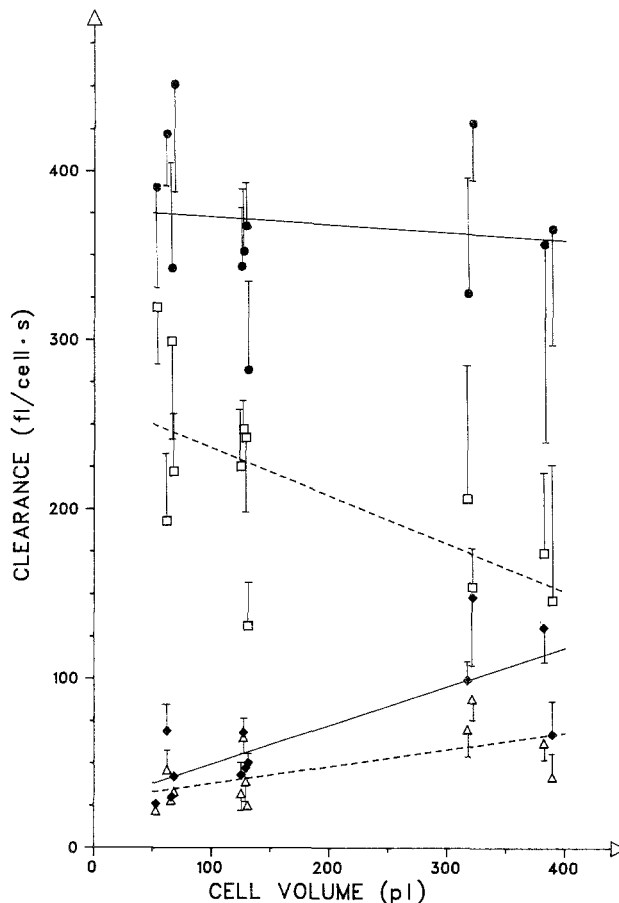


Fig. 3. Initial velocities of methylglucose and deoxyglucose uptake. The results are expressed as clearance rates defined as cpm taken up per cell per s/cpm per fl medium. This expression has the advantage of being independent of the hexose (tracer) concentration when this, as in the present experiments, is much lower than K_m for the process which limits the rate of uptake. Basal cells were incubated with [^{14}C]methylglucose (◆) or [^{14}C]deoxyglucose (△) for 5 s and insulin-stimulated cells were incubated with [^{14}C]methylglucose (●) or [^{14}C]deoxyglucose (□) for 1 s as described in Methods. The points indicate the mean of 4 replicates with 1 SD which includes the variation on the zero time uptake. The following values were obtained with respect to the illustrated regression lines in the presence of insulin: methylglucose clearance (—), $r^2 = 0.02$, NS and deoxyglucose clearance (---), $r^2 = 0.40$, $p < 0.05$. In the absence of insulin: methylglucose clearance, $r^2 = 0.60$, $p < 0.01$, and deoxyglucose clearance, $r^2 = 0.40$, $p < 0.05$. Each transport experiment involved determination of the intracellular equilibrium space for methylglucose (i. e. intracellular waterspace). This ranged from 1.3 to 2.7 pl/cell over the cell size range resulting in an increase of the half times of methylglucose uptake in insulin-stimulated cells from about 1.8 (small cells) to 3.6 s

was 3.6 ± 0.2 nmol/l ($n = 4$) for the smallest cells and 1.2 ± 0.2 nmol/l ($n = 4$) for the largest. Regression analyses of the apparent K_d on mean cell volume ($n = 12$) gave a decrease to 28% in the largest cells, $r^2 = 0.84$, $p < 0.001$.

Figure 2C shows that the Scatchard plots were slightly concave upwards. The initial slopes were well determined by the points corresponding to insulin concentrations of 50 pmol/l – 1.4 nmol/l, and were significantly steeper for the largest cells (calculated K_d 1.0 ± 0.3 nmol/l, $n = 4$) than for the smallest cells (K_d 3.1 ± 0.6 nmol/l, $n = 4$). Regression analyses of K_d on cell mean volume ($n = 12$) gave a decrease to 33%, ($r^2 = 0.74$, $p < 0.001$) in the largest cells. Inspection of the curves suggests that the number of receptors per cell was at least as high in the large cells as in the small cells.

Hexose Transport

Figure 3 shows that the initial velocity of uptake of tracer methylglucose per cell increased with increasing cell size in the absence of insulin (basal conditions) whereas it remained approximately constant in its presence. Thus the insulin effect decreased from about 11 fold in the smallest cells to about 3.5 fold in the largest. Permeabilities were calculated from the data shown in Figure 3 and the mean surface area. In the smallest cells it was $5.79 \times 10^{-7} \pm 1.72 \times 10^{-7} \text{ cm} \cdot \text{s}^{-1}$ ($n = 4$) under basal conditions, and $5.62 \times 10^{-6} \pm 0.74 \times 10^{-6} \text{ cm} \cdot \text{s}^{-1}$ ($n = 4$) in the insulin-stimulated state. Regression analyses of the permeability on cell volume ($n = 12$) showed that the permeability remained constant in basal cells (decrease by 16%, $r^2 = 0.03$, NS) whereas it decreased by 83% in insulin-stimulated cells ($r^2 = 0.71$, $p < 0.001$).

The basal deoxyglucose uptake per cell increased as a function of cell volume although the increase was less than that noted for methylglucose. However, the insulin-stimulated uptake of deoxyglucose per cell, in contrast to that of methylglucose, decreased as a function of cell volume.

Hexose Metabolism

Figure 4 shows that both the basal and the insulin-stimulated rate of glucose metabolism was approximately parallel to the methylglucose transport (Fig. 3) over the entire cell size range. The rate of conversion of glucose to CO_2 and lipids, which accounts for about 80% of the rate of glucose uptake [7, 20] was about half of the methylglucose transport rate. The data show that the rate of conversion of tracer glucose is a reasonable reflection of the changes in methylglucose transport as a function of cell size, whereas the rate of uptake of deoxyglucose is not.

The ratio of CO_2 production to triglyceride synthesis was found to decrease as a function of cell size

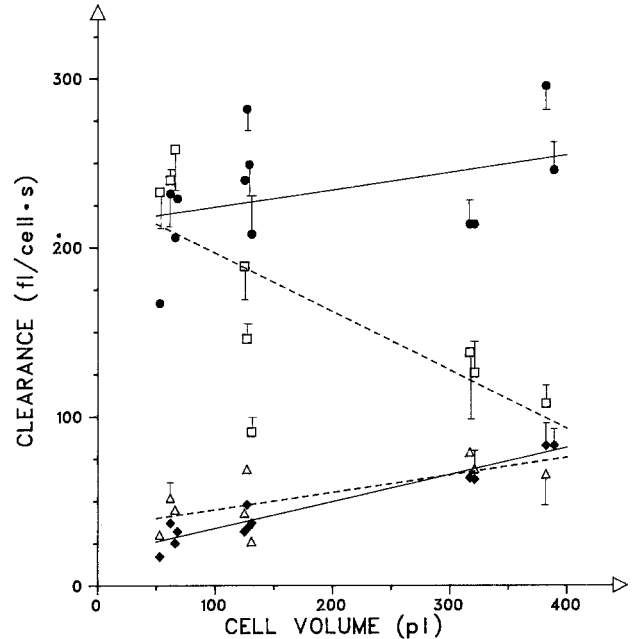


Fig. 4. Rate of conversion of deoxyglucose and glucose. Cells were incubated with [^{14}C]deoxyglucose for 3 min in the absence (Δ) or presence (\square) of insulin or with [^3H]glucose for 120 min in the absence (\blacklozenge) or presence (\bullet) of insulin as described in Methods. The points represent the mean values of 4 replicates with 1 SD. Regression lines in the presence of insulin: glucose clearance (—) on cell volume, $r^2 = 0.15$, NS and deoxyglucose clearance (---), $r^2 = 0.50$, $p < 0.01$. In the absence of insulin: glucose clearance, $r^2 = 0.92$, $p < 0.001$, and deoxyglucose clearance, $r^2 = 0.50$, $p < 0.01$.

in insulin-stimulated cells. In addition, fatty acid synthesis in the presence of insulin 0–200 pmol/l was virtually absent in the largest cells (data not shown) as described previously by others [7, 21, 22].

Insulin Binding and Biological Sensitivity

Figure 5A shows that the sensitivity with respect to glucose metabolism decreased (ED_{50} increased) as a function of cell size. Insulin binding was nearly proportional to the insulin concentration in the biological dose response range (10–150 pmol/l) and binding per cell was higher in the large cells (Fig. 5B). However, the binding per unit surface area decreased slightly as a function of cell size. The mean decrease in binding per μm^2 in the concentration range 10–150 pmol/l was 36% from the smallest to the largest cells. Regression analyses of binding per μm^2 as a function of cell volume gave $r^2 = 0.45$, $p < 0.02$, $n = 12$. It seemed possible, therefore, that the sensitivity was determined by the number of occupied receptors per unit surface area. Figure 5B shows that the dose-response curves after correction for binding per unit surface area (cf. legend) are superimposable.

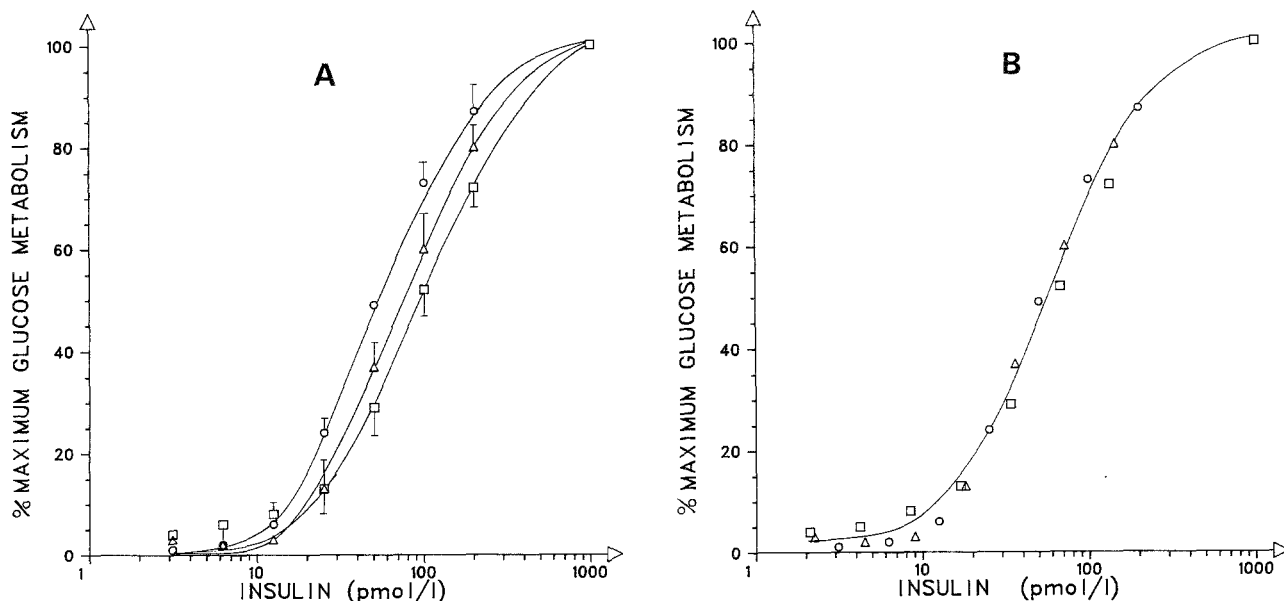


Fig. 5 A and B. Insulin dose-response relationship. Small (O-O), medium (Δ - Δ) and large (\square - \square) cells were incubated for 120 min with [14 C]glucose and insulin as indicated. The ordinates show the rate of conversion of glucose to CO_2 plus triglycerides in per cent of the maximal conversion rate. The points represent the mean values of 4 independent experiments with 1 SD. The abscissa in panel **A** shows the insulin concentrations in the medium. The results in panel **B** are identical with respect to the small cells whereas the insulin concentrations for the medium size and large cells are those which would give the same number of occupied receptors per unit surface area as in small cells. This normalization was carried out by multiplying the insulin concentrations of the incubation medium of large (or medium size) cells in panel **A** with the ratio: tracer bound per unit surface area of large cells/tracer bound per same unit surface area of small cells, where the binding per unit surface area was calculated from the mean cell surfaces (cf. mean cell volumes in Fig. 3) and the binding of tracer per cell (cf. Fig. 2B). This calculation is legitimate because binding was nearly proportional to the insulin concentration in the range considered (Fig. 2B)

Discussion

Binding

The present results are different from those reported by Lockwood and co-workers [4, 5], who found no change, and are in marked contrast to those reported by Olefsky [1-3] who found a 50 per cent decrease in binding with increasing cell size over approximately the same size range as that studied in the present work. We do not know with certainty the reasons for the differences. We have previously shown that a steady state of binding can be maintained for a prolonged period of time in small cells [10], but this phenomenon has to our knowledge not been reported before in large cells, which are known to be more fragile. It seems possible, therefore, that steady state kinetics have previously been applied in situations where a steady state was not prevailing. The present finding of an increased binding per cell as a function of cell size is in agreement with the results of Cushman et al. [6]. In a preliminary report from this group [23] it was also stated that this increase was mainly due to an increase in affinity. This is again in agreement with our results, but in contrast to those of

Olefsky [1-3] who found no change in the slopes when the data were plotted according to Scatchard.

Transport and Metabolism

The increase in the basal rate of methylglucose transport per cell as a function of cell size and the lack of change in insulin-stimulated cells was also observed by Foley [9] using L-arabinose at 37 °C. In contrast, Czech [7] reported that the initial velocity of methylglucose transport per cell at 37 °C was increased by the same factor in basal and insulin-stimulated large cells. However, Czech probably underestimated the transport rates in the presence of insulin (discussed in [12]) since these were less than 10 per cent of the rates of conversion of tracer glucose, which is inconsistent with the affinities of the two sugars for the transport system [12]. Furthermore, this underestimation may have been less marked in the large cells since the half time of methylglucose transport is longer in these cells than in small cells, cf. text to Figure 3. Livingston and Lockwood [8] reported the same uptake of methylglucose per cell both in the absence and presence of insulin. However, these results are difficult to inter-

pret since no attempt was made to measure transport rates.

The present study shows that deoxyglucose uptake rates do not parallel methylglucose transport rates using incubations for either 1 s or 3 min at 37 °C. We have recently demonstrated that in small cells tracer deoxyglucose is transported at a slower rate than tracer methylglucose even though the inhibition constants of the two sugars on the initial velocity of [¹⁴C]methylglucose uptake are the same [14]. We now show that the difference between insulin-stimulated deoxyglucose and methylglucose uptake rates increases as the cell enlarges. This result is in contrast to that of Olefsky [2] who found the same uptake of deoxyglucose (3 min) per cell in small and large adipocytes. Olefsky used incubations at 24 °C and this is perhaps the reason for the difference.

The rate of conversion of tracer glucose (0.7 μmol/l) was proportional to methylglucose transport under all conditions, indicating that transport was rate determining for the metabolism of glucose at this very low concentration. This comparison has not been carried out before but it has been noted by others that transport of glucose in high concentrations is not rate limiting in small or large cells in the presence of insulin [7, 20], and that metabolism becomes rate limiting at lower glucose concentrations in larger cells [4, 23]. The very small effect of insulin in large cells on the rate of conversion of glucose at physiological concentrations is therefore to a considerable extent caused by their low capacity to metabolise glucose [7, 20–23].

Sensitivity

We observed a small decrease in sensitivity as a function of cell size which disappeared when corrected for the small decrease in binding per unit surface area. Foley [9] using L-arabinose transport and Czech [7] using metabolism of glucose (0.2 mmol/l) found no detectable changes in sensitivity. However, the changes observed in the present study are so small that the three studies are probably in broad agreement with each other. In contrast, Olefsky [2, 3] found a large decrease in the sensitivity with respect to deoxyglucose uptake.

General Implications

When the rat grows older and fatter the adipocytes enlarge and both the insulin binding system and the hexose transport system undergo changes. Until now we have discussed changes in binding and transport per cell because previous authors have chosen this way of expression. However, it may be physiolog-

ically more meaningful to consider the cell surface as the functional unit.

The number of receptors per μm² appears to be roughly inversely proportional to the mean surface area, but at the same time the affinity increases with increasing cell size. As a result, the binding of insulin in low concentrations, including those which cause near-maximal effects on glucose transport [12] and metabolism, is only slightly decreased per μm². Thus, the increase in affinity can be looked upon as a compensatory process. It is unknown how this change is brought about. However, it seems possible that embedding of receptors in a membrane which has undergone more general changes may cause a shift in their affinity. In this connection it is worth noting that receptors of adipocytes from adult man exhibit an even higher affinity than that of the largest rat adipocytes [24].

In previous work [25] we found no evidence for receptor heterogeneity. However, the non-specific binding was for technical reasons much higher than in the present work and the scatter on specific binding became therefore very large at high insulin concentrations (Fig. 2C). The curvilinearity of the Scatchard plots observed in the present work is slight in the sense that K_d calculated from the initial slope is only about 20 per cent lower than the concentration of insulin causing half-maximal displacement (Fig. 2B and C). The upward concave Scatchard plots are qualitatively in agreement with the finding of others [26]; however, it should also be noted that marked non-linearity can be generated using less suitable experimental designs [10].

Previous work [12, 27] has shown that insulin increases V_{max} of hexose transport without changing the affinity, and recent work by Wardzala et al. [28] suggests that the effect is largely caused by an increase in the number of transport sites. The present data show that transport of [¹⁴C]methylglucose remains constant per cell (Fig. 3). This implies that the permeability (clearance per unit surface area) in insulin-stimulated cells decreases in inverse proportion to the surface area. The permeability in the absence of insulin remains constant suggesting that the large cells incorporate a higher fraction of the total number of transport sites in the pool available even in the absence of insulin. Consequently, the large cells would be able to recruit less additional sites in the presence of insulin at a maximally stimulating concentration. Insulin at lower concentrations would then recruit a fraction of the additional sites according to the number of occupied receptors per unit surface area. In addition, the finding that the permeabil-

ity to deoxyglucose in the presence of insulin decreases more than the permeability to methylglucose and glucose as a function of cell size (Fig. 3) suggests that the properties of the transport system change with increasing cell size in another as yet undefined manner.

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