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Glucose transport is equally sensitive to insulin stimulation, but basal and insulin-stimulated transport is higher, in human omental compared with subcutaneous adipocytes☆

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Abstract

Excess visceral fat has been found to correlate more closely with morbidity than subcutaneous fat. We found that isolated adipocytes from omental fat of nondiabetic women expressed significantly more of the insulin-regulated glucose transporter glucose transporter 4 protein and exhibited a higher basal and insulin-stimulated rate of glucose transport, at all concentrations of insulin, than subcutaneous adipocytes from the same individuals. In contrast, dose-response relationships for insulin stimulation of glucose transport demonstrated identical sensitivity to insulin in adipocytes from the 2 locations. The results demonstrate that there is no relative insulin resistance to stimulate glucose uptake in visceral compared with subcutaneous fat cells.

1. Introduction

A metabolic difference between lower body and abdominal adipose tissue depots is well known. Excess abdominal adipose tissue has correlated, in several epidemiologic studies, much closer with metabolic diseases such as type 2 diabetes and the so-called metabolic syndrome than has gluteal and femoral fat [1]. Some studies have also indicated differences between subcutaneous and visceral abdominal adipose tissue depots [2]. These differences have been attributed to the particular blood drainage from the visceral omental fat directly to the liver via the portal blood, so that the lipolytically produced fatty acids from the visceral fat may affect liver metabolism. A complementary explanation has been that the fat depots are inherently metabolically different. Examination of gene expression of a range of proteins, including key metabolic enzymes and secreted signaling proteins, however, has indicated that subcutaneous fat and omental fat are quite similar. Exceptions are leptin mRNA that in women exhibits a particularly high copy number in subcutaneous compared with omental fat [3] and glucose transporter (GLUT) 4 mRNA, which has been found to be slightly elevated in omental compared with subcutaneous fat of grossly obese women [4]. It has also been found that omental adipocytes express a higher number of β-adrenergic receptors [5] and show a higher rate of catecholamine-induced lipolysis [6] than subcutaneous adipocytes. Omental adipocytes also appear to be considerably less sensitive to insulin's inhibition of catecholamine-stimulated lipolysis than subcutaneous cells [7]. Consistent with this, it has been reported that the affinity of the insulin receptor for insulin is lower in omental than in subcutaneous fat [8,9]. Furthermore, insulin has been found to be several folds more potent to stimulate tyrosine autophosphorylation of its receptor as well as tyrosine phosphorylation of the downstream IRS1 in subcutaneous as compared with omental adipocytes [7].

In contrast to these marked region-specific effects of insulin on antilipolysis, little has been reported on insulin stimulation of glucose transport in adipocytes from subcutaneous versus omental fat depots, which is a prerequisite for triacylglycerol storage and fat tissue maintenance. It has recently been reported that the basal glucose uptake, but not insulin stimulation of glucose uptake, was increased in omental compared with subcutaneous adipose tissue pieces

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[10], but no investigation of the sensitivity to insulin of isolated cells from the 2 depots has been reported. Also, glucose uptake in deep abdominal properitoneal adipocytes has been shown to respond stronger to insulin than in both subcutaneous and omental adipocytes [4]. We recently described that isolated human fat cells are insulin-resistant because of the surgical cell isolation procedures that are necessary to obtain the cells [11]. The resistance to insulin was manifest downstream of IRS1 and involved a 5-fold reduction in the sensitivity of insulin to enhance glucose transport in the cells. This insulin resistance was, however, in contrast to that of type 2 diabetes, reversible during overnight incubation. Interestingly, the maximal rate of glucose transport was not affected by the overnight incubation, indicating a fundamental difference from rat and mouse cells [11,12].

We now report that insulin's ability to control glucose transport is identical in the subcutaneous and omental adipocytes. The amount of GLUT4 and overall rate of glucose uptake were, however, higher in omental adipocytes, in concord with the demands of enhanced lipolytic activity of the omental fat.

2. Materials and methods

2.1. Subjects

Samples of subcutaneous and omental abdominal fat were obtained from 11 female patients (average 52 [range 32-79] years old with average body mass index of 24 [range 18-27] kg/m^2) during elective abdominal surgery at the University Hospital of Linköping. None of the patients were diagnosed with diabetes. Pieces of adipose tissue were excised at the beginning of the operation from subcutaneous and omental adipose tissue. The study was approved by the local ethics committee, and participants gave their informed approval.

2.2. Materials

2-Deoxy-D-[1-^{3}H]glucose was from Amersham Biotech (Uppsala, Sweden). Rabbit anti-GLUT4 antibodies were from Biogenesis (Poole, UK). Insulin and other chemicals were from Sigma-Aldrich (St Louis, Mo, USA) or as indicated in the text.

2.3. Isolation and incubation of adipocytes

Pieces of tissue from subcutaneous and omental fat were obtained from each patient and were in parallel subjected to identical treatments. Isolated adipocytes were obtained by collagenase (type 1, Worthington, NJ, USA) digestion as described [13]. At a final concentration of 100 μL, packed cell volume per milliliter, cells were incubated overnight at 37°C, 10% CO_2 in Krebs-Ringer solution (0.12 mol/L NaCl, 4.7 mmol/L KCl, 2.5 mmol/L $CaCl_2$, 1.2 mmol/L $MgSO_4$, 1.2 mmol/L KH_2PO_4) containing 20 mmol/L HEPES (4-[2-hydroxyethyl]-1-piperazineethanesulfonic acid, pH 7.4), 1% (wt/vol) fatty acid–free bovine serum albumin, 100 nmol/L phenylisopropyladenosine, 0.5 U/mL adenosine deaminase with 2 mmol/L glucose mixed with an equal volume of Dulbecco's Modified Eagle's Medium (DMEM) containing 7% (wt/vol) albumin, 25 mmol/L glucose, 200 nmol/L phenylisopropyl adenosine, 20 mmol/L HEPES, 50 U/mL penicillin, 50 μg/mL streptomycin (pH 7.4) [11]. Before analysis, cells were washed and transferred to the Krebs-Ringer solution with additions, but without glucose. Average cell diameter was determined from microscopy photo enlargements; approximately 100 cells from each subject were measured using a ruler.

2.4. Determination of glucose transport

Glucose transport was determined as uptake of 2-deoxy-D-[1-^{3}H]glucose [14]. Equal amount of cells as determined by lipocrit (total cell volume) was incubated at 37°C with the indicated concentration of insulin for 10 minutes, when 2-deoxy-D-[1-^{3}H]glucose was added to a final concentration of 50 μmol/L (10 μCi/mL) and the cells were incubated for a further 30 minutes (uptake was linear for at least 30 minutes). Incubations were terminated by separating the cells from the medium during 5-second centrifugation through dinonyl phtalate oil. The cell cake was dissolved in sodium dodecyl sulfate (SDS), and the amount of radioactivity in the cells was determined by scintillation counting. Radioactivity in the medium trapped between cells was corrected for by subtracting radioactivity in incubations that were immediately terminated by addition of cytochalasin B and spinning through the oil. The measurements of glucose uptake were highly reproducible. Reassay the following day of the same cells from subcutaneous fat of 6 subjects gave a correlation coefficient for pairing of 0.99 ($P < .0001$, Student paired t test) for maximal insulin-stimulated glucose uptake. In addition, analysis of cells, from 2 subjects, obtained on 2 separate occasions with 6 months' interval, indicated a very small variation of maximal insulin-stimulated glucose uptake (190 vs 195 and 230 vs 180 cpm, respectively, for the 2 subjects).

2.5. SDS-PAGE and immunoblotting

Cells were separated from medium by centrifugation through dinonyl phtalate. The cells were immediately dissolved in SDS and β-mercaptoethanol with protease and protein phosphatase inhibitors, frozen within 10 seconds, and thawed in boiling water to minimize post-incubation modifications in the cells and protein modifications during immunoprecipitation [13]. Equal amount of cells as determined by lipocrit was subjected to SDS–polyacrylamide gel electrophoresis (PAGE) and immunoblotting. After SDS-PAGE and electrotransfer, membranes were incubated with antibodies against GLUT4 that were detected using ECL+ (Amersham Biosciences) with horseradish peroxidase–conjugated anti-IgG as secondary antibody and evaluated by chemiluminiscence imaging (Las 1000, Image-Gauge, Fuji, Tokyo, Japan).

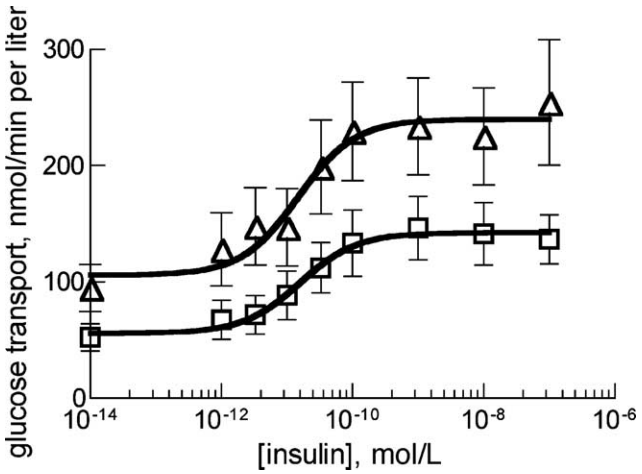

Fig. 1. Insulin stimulation of glucose transport in adipocytes from omental and subcutaneous fat. Adipocytes were incubated at the indicated concentration of insulin for 10 minutes when 2-deoxy-D-[1-^{3}H]glucose was added and cells incubated for another 30 minutes. Glucose transport is expressed as nmol-deoxyglucose/min per liter of packed cell volume. Average of 11 individuals per experiment, each the mean of 2 determinations. Mean ± SE. Squares indicate subcutaneous adipocytes; triangles, omental adipocytes.

2.6. Statistics

Dose-response curves were compared using F test with the sigmoidal curve-fitting algorithm in GraphPad Prism 4 (GraphPad Software, Inc, San Diego, Calif, USA). Student *t* test for paired samples was used to compare differences between the 2 groups of cells.

3. Results

The basal rate of glucose transport was nearly twice as high in the adipocytes from omental as in those from subcutaneous fat (Fig. 1). The same was true for the maximal insulin-stimulated rate of transport and for the extent of stimulation by insulin (Fig. 1). However, the rate of glucose transport varied greatly between individuals in cells from both locations: 33-212 and 59-297 nmol/min per liter of cells in subcutaneous and omental adipocytes, respectively. The mechanisms behind this individual variability are not known but may reflect genetic differences between individuals or lifestyle-related traits acquired over decades [11]. There was no correlation between the degree of obesity (body mass index) of the individual subjects and the ratios of the rate of maximal insulin-stimulated glucose transport in cells from the 2 fat tissues (not shown).

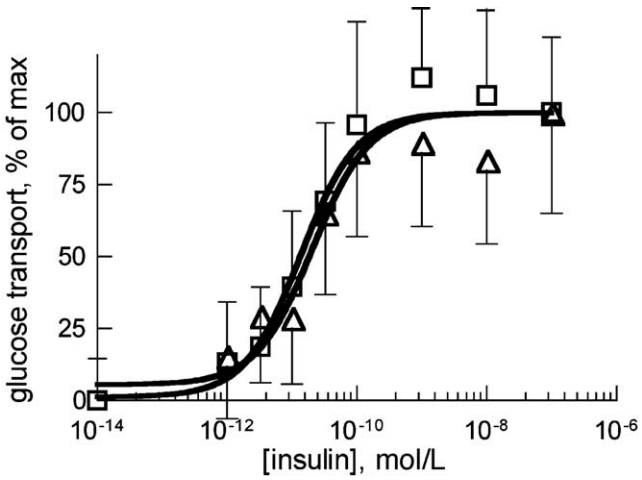

Fig. 2. Sensitivity of glucose transport in adipocytes from omental and subcutaneous fat to insulin stimulation. Data from Fig. 1 were normalized by setting the value with no insulin to 0% and at 100 nmol/L insulin to 100% effect in each experiment. Squares indicate subcutaneous adipocytes; triangles, omental adipocytes.

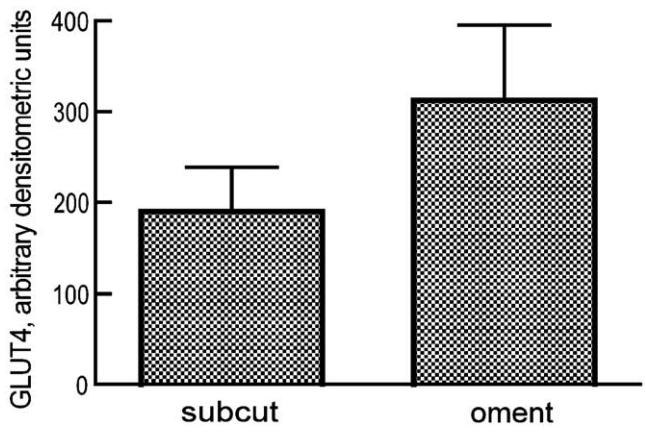

Fig. 3. Amount of GLUT4 protein in adipocytes from omental and subcutaneous fat. Whole cell lysates of equal cell volumes were subjected to SDS-PAGE and immunoblotting with antibodies against GLUT4, in one gel to allow comparison between patients. Mean ± SE (n = 7 patients), $P < .05$ by Student *t* test. Subcut indicate subcutaneous; oment, omental.

To examine if the cells exhibited different sensitivities to insulin stimulation of glucose transport, the data in Fig. 1 were normalized to maximal insulin-stimulated transport. As seen from Fig. 2, the adipocytes from subcutaneous and from omental adipose tissue exhibited identical or very similar sensitivities to control by insulin.

To gain insight in the difference in overall glucose transport rates, we next determined the total amount of GLUT4 protein in cells from the 2 depots. By SDS-PAGE of total cell lysates and immunoblotting with antibodies against GLUT4, the amount of GLUT4 was nearly twice as high in the same volume of cells from the omental compared with subcutaneous fat (Fig. 3). This compares well with the increased rate of glucose transport in the omental adipocytes (Fig. 1). The average cell size was slightly larger in the subcutaneous compared with the omental cells (101 ± 22 and 93 ± 24 μm diameter, respectively, n = 6 subjects, $P < .01$), with large intraindividual variations.

4. Discussion

Our findings clearly demonstrate that in human beings, there is a difference between abdominal subcutaneous and omental adipocytes in terms of glucose uptake; basal glucose transport and insulin-stimulated glucose transport

were approximately doubled in the omental adipocytes compared with subcutaneous cells. Insulin-stimulated glucose uptake in both lean and obese subjects has indeed been reported to be significantly higher per volume of tissue in visceral compared with subcutaneous fat in whole-body examinations by positron emission tomography during euglycemic hyperinsulinemic clamp [15]. The small difference in cell size that we found involves such a small difference in total cell surface area that this cannot explain the difference in transport rates. We found that the total amount of GLUT4 protein was significantly higher in cells from the omental adipose tissue. It is therefore possible that the increased insulin-stimulated glucose uptake by the omental adipocytes is related to a higher expression of GLUT4 in these cells compared with the subcutaneous cells. Part of the increased basal rate of glucose uptake by the omental adipocytes could, however, also be caused by different expression levels of GLUT1. Further analysis is required to unequivocally establish the relative contributions of GLUT1 and GLUT4. Omental adipocytes are lipolytically more active than the subcutaneous cells [6,16]. This is compatible with, and indeed requires, a higher overall glucose uptake in the omental adipocytes to provide glycerol 3-phosphate for the increased rate of triacylglycerol synthesis, required to maintain unchanged levels of triacylglycerol stores in a tissue with a high rate of lipolysis.

The near-identical dose-response curves for insulin stimulation of glucose transport in the 2 cell populations, on the other hand, unequivocally demonstrate that there is no difference in insulin sensitivity between subcutaneous and omental adipocytes in nonobese individuals. This implies that insulin signal transduction from the insulin receptor and downstream to relocation of GLUT4 is equally sensitive to insulin stimulation in subcutaneous and omental adipocytes. However, these findings do not preclude such differences in insulin sensitivity in adipocytes from obese or diabetic subjects.

Omental adipocytes have been reported to express higher levels of β-adrenergic receptors [5] and to be lipolytically more responsive to catecholamines than are subcutaneous adipocytes [6], which likely explains the higher concentrations of insulin needed to inhibit catecholamine-stimulated lipolysis, that is, relative insulin resistance, in omental compared with subcutaneous adipocytes [7]. This, however, does not explain the reported relative resistance in omental versus subcutaneous adipocytes to insulin stimulation of insulin receptor autophosphorylation, IRS1 tyrosine phosphorylation, or phosphatidylinositol 3-kinase activation [7-9], which are signaling intermediates likely to be common to both stimulation of glucose transport and inhibition of lipolysis. We have previously demonstrated that directly after surgical excision of subcutaneous adipose tissue, the adipocytes are strongly insulin-resistant and that this insulin resistance is reversed by overnight incubation of the cells; maximal insulin responses were not affected, but the sensitivity to insulin increased [11]. Herein, cells were incubated overnight to avoid interference from the insulin resistance after the surgical procedures. It is possible that omental fat is more prone than subcutaneous fat to become insulin-resistant in response to the stress during surgical procedures, which may explain the above discrepancy. It has, for example, been described that there is separate autonomous innervation of these 2 adipose depots [17].

In conclusion, we have shown that adipocytes from subcutaneous and visceral (omental) fat have the same sensitivity to insulin. The visceral adipocytes, however, exhibited elevated levels of GLUT4 and glucose uptake, presumably to provide the cells with the increased amount of glucose needed for triacylglycerol synthesis to balance the increased lipolysis in the visceral fat tissue.

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