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Assay of glucose transport in human fat cells obtained by needle biopsy

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Summary. A method was developed for repeated measurements of glucose transport in human fat cells obtained by needle biopsy aspiration. Assay conditions, reproducibility and normal values of the measurements are described. Transport rates were measured in the absence and presence (25, 50, 100, 200, 800 pmol/l; 8, 80 nmol/l) of insulin using U-(^{14}C)-D-glucose as the tracer. The extracellular glucose concentration was 1.5 μmol/l. The reproducibility of glucose transport measurements was assessed by taking two needle biopsies from opposite sides of the same subject ($n = 11$). The mean coefficient of variation for maximal glucose transport was 11 ± 6%. In 14 subjects, a needle biopsy sample was aspirated immediately prior to surgical removal of fat. The maximal insulin-stimulated glucose transport rates averaged 143 ± 15 and 143 ± 15 fl/cell · s, and the ED50:s 218 ± 124 and 160 ± 28 pmol/l (NS) in fat cells prepared from needle biopsy and surgically removed adipose tissue respectively. The mean coefficient of variation for maximal glucose transport in needle vs. surgical samples was 11 ± 2%. In 6 subjects, a surgical biopsy was taken twice, with a 1-week interval. The coefficient of variation averaged 9 ± 2%. We conclude that measurement of glucose transport rates can be done with similar accuracy using fat cells isolated from needle biopsy aspirates and surgically removed adipose tissue. Use of needle biopsy samples permits, however, study of glucose transport in repeat samples of human fat cells, and may therefore be a useful tool for any perturbation studies.

Key words: Adipocyte, glucose transport, insulin.

Insulin action in cells may be modulated by various mechanisms at either receptor- or postreceptor levels [1]. Especially in human cells, short-term regulatory events are insufficiently characterized due to limited availability of tissues. Acute studies have mostly been confined to measurement of insulin binding to blood cells [2–4]. Changes in the number and affinity of insulin receptors on blood cells correlate, however, poorly with those on other cell types [5, 6] and cannot yield information of insulin action. In adipose tissue both insulin binding and action can be measured, but sampling requires an open surgical biopsy, a procedure which cannot be repeated several times. In the present study we wanted to examine whether glucose transport could be measured from microsamples of adipose tissue obtained by needle biopsy aspiration.

Subjects and methods

Subjects

Adipose tissue was obtained from 29 healthy nonobese (BMI 19 to 24 kg/m^2), nondiabetic (fasting plasma glucose 4.7 to 5.2 mmol/l) volunteers (15 men, 14 women) aged 21 to 35 years. Variation of the needle biopsy method was assessed by comparing results from biopsies taken from the left and right gluteal region within 1 h from the same subject ($n = 11$).

To compare results obtained from fat removed by needle biopsy vs. surgically, needle biopsies were taken immediately prior to surgical biopsy from the lower abdominal region from 14 Pima Indians (8 men, 6 women, aged 18 to 33 years, BMI 26 to 51 kg/m^2, fasting plasma glucose 4.8 to 6.1 mmol/l). To determine the reproducibility of glucose transport measurements from surgical samples, biopsies were taken from 6 Pima Indian men (aged 19 to 32 years, BMI 25 to 67 kg/m^2, fasting plasma glucose 5.0 to 7.2 mmol/l) twice with a 1-week interval. All these subjects were studied in the Phoenix Clinical Research Section after being stabilized for 5 or more days on a weight-maintenance diet (45% carbohydrate, 40% fat, 15% protein). All biopsies were taken between 07.30 and 08.30 h after a 12-h overnight fast.

The nature, purpose and possible risks of the procedures were explained to all subjects before they gave their voluntary consent to participate. All subjects gave informed consent, and the studies were approved by the ethical committees of the Helsinki University Central Hospital, the National Institutes of Health and Indian Health Service, and by the Gila River Indian Community.

Needle biopsy

The skin was anaesthetized with 2 cc of 1% lidocaine without epinephrine, taking care not to infiltrate subcutaneous or adipose tissue. The biopsy needle (9 cm × 2 mm), attached to a 60-cc tight-fitting glass

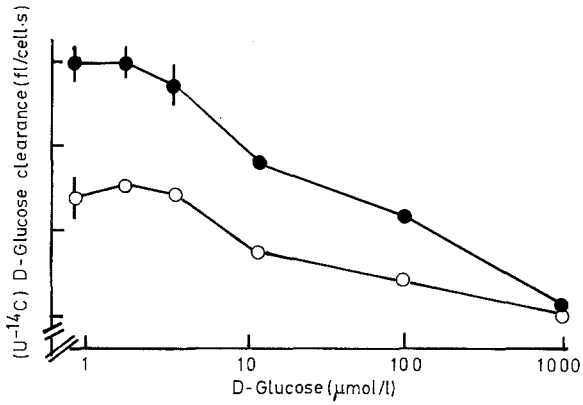

Fig. 1. Effect of extracellular glucose concentration on basal (○) and maximally insulin-stimulated (8 nmol/l insulin, ●), glucose clearance rates

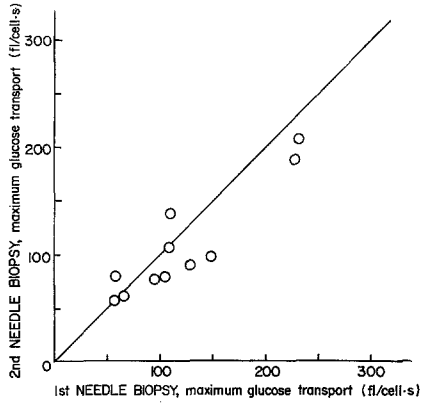

Fig. 2. Reproducibility of measurement of maximally insulin-stimulated (insulin 80 nmol/l) glucose transport rates in two needle biopsy samples taken from the left and right gluteal region from the same subject. The coefficient of variation between the two measurements (method variation) for glucose transport averaged 11 ± 6%

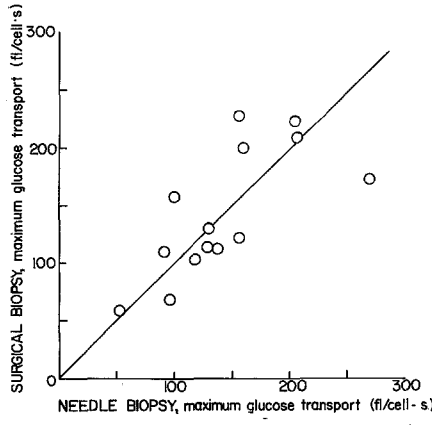

Fig. 3. Comparison of maximal glucose clearance rates in fat cells isolated from needle biopsy samples vs. surgically removed fat. The coefficient of variation averaged 11 ± 2%

syringe was inserted through the infiltrated skin and small samples of adipose tissue were aspirated by vacuum suction outside the infiltrated area [7]. The amount of tissue obtained by two to three aspirations varied from 100 to 700 mg (mean 300 mg). After each aspiration, the syringe was rinsed with Dulbecco's phosphate buffered saline (20–23 °C). The sample was subsequently poured into a plastic strainer, rinsed and transferred to polyethylene tubes containing collagenase buffer for isolation of cells [8].

Surgical biopsy

Subcutaneous adipose tissue (5 g) was removed from the lower abdomen, and the cells were isolated as previously described [8].

Glucose transport

Glucose transport was measured using U-(^{14}C)-D-glucose according to Foley et al. [9]. After isolation, adipocytes were transferred to Krebs-Ringer phosphate buffer (37 °C), pH 7.4, containing 5% bovine serum albumin. The assay was started by adding 50 µl of a 20% cell suspension to tubes containing a trace amount of (U-^{14}C)-D-glucose (0.2 µCi/tube, final concentration 1.5 µmol/l) and various insulin concentrations (0, 25, 50, 100, 200, 800 pmol/l, 8; 80 nmol/l). The incubations were carried out in triplicate at 37 °C for 60 min with continuous shaking (40 cycles/min) at a final volume of 500 µl. The incubation was terminated by centrifuging 350 µl of cell suspension through 100 µl silicone oil [10]. The cell-associated radioactivity and radioactivity remaining in the incubation medium were determined by liquid scintillation counting using ACS as liquid scintillator and Wallac liquid scintillation counter 81000 (LKB Wallac, Turku, Finland). The glucose transport rate was expressed as glucose clearance rate per cell as fl/cell·s or per cell surface as al/µm^2·s.

Determination of cell size

Cell diameters were measured after fixation with osmium tetroxide as previously described [8]. The mean cell volume was converted to micrograms of lipid per cell using the correction factor of 0.91 µg/nl cell volume.

Chemicals. Collagenase (Clostridium histolyticum, no. C-2139, 250 U/mg), was from Sigma (St. Louis, MO, USA), BSA from Finnish Red Cross (Helsinki, Finland) or Armour Pharmaceuticals (Kankakee, IL, USA), silicone oil from Dow Corning (Seneffe, Belgium), insulin from Novo Research Institute (Bagsvaerd, Denmark), and U-(^{14}C)-D-glucose (sp. act. 300 mCi/mmol) from the Radiochemical Centre (Amersham, England).

Statistical analysis

Statistical comparison was done using Student's paired or unpaired t-test. Results are expressed as mean ± SE.

Results

Assay conditions

Glucose transport in adipocytes obtained by needle biopsy was linearly related to fat cell concentration ($r = 0.98$) and time (basal clearance rate 22 ± 1, 42 ± 3, 64 ± 4 and 84 ± 4 fl/cell per s at 15, 30, 45 and 60 min respectively, $n = 3$). The coefficient of variation for triplicate samples was 11 ± 1% for basal transport rates. To confirm that the concentration of glucose (1.5 µmol/l) was not too high to change the rate-limiting step in glucose utilization, basal and maximally stimulated glucose transport rates were measured in the presence of increasing glucose concentrations. Glucose transport

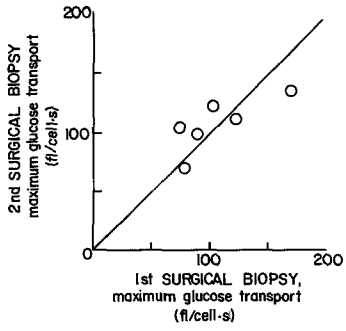

Fig. 4. Comparison of maximal glucose transport rates using fat from two surgical fat biopsies from the same individual. The coefficient of variation was 9 ± 2%

remained constant up to approximately 5 μmol/l and declined thereafter (Fig. 1). Similar data have been reported previously in adipocytes obtained by surgical biopsy [8, 9].

Reproducibility

Needle vs. needle biopsy. In two needle biopsies taken from both sides of the gluteal region, the mean coefficient of variation of maximal clearance rates was 11 ± 6% (Fig. 2). Basal glucose transport averaged 54 ± 6 and 51 ± 6, and maximum transport 122 ± 18 and 108 ± 15 fl/cell · s (NS) in the first and second biopsy respectively.

Needle vs. surgical biopsy. The mean coefficient of variation for maximal glucose transport in needle vs. surgical samples was 11 ± 2% (Fig. 3). The ED50:s were 218 ± 124 and 160 ± 28 pmol/l, basal transport rates 74 ± 8 and 57 ± 6, and maximal transport rates 143 ± 15 and 143 ± 15 fl/cell · s in fat cells prepared from needle and surgical samples respectively (all NS).

Surgical vs. surgical biopsy. The coefficient of variation for maximal glucose transport averaged 9 ± 2% (Fig. 4). Basal glucose transport averaged 40 ± 4 and 45 ± 6, maximal transport 108 ± 9 and 109 ± 13 fl/cell · s and the ED50:s 162 ± 103 and 148 ± 55 pmol/l for the first and the second biopsy (all NS) respectively.

Normal values

Basal glucose transport rates were 1.3 ± 0.2 and 1.1 ± 0.2 al/μm² · s (NS) or 53 ± 7 and 36 ± 6 fl/cell · s ($p < 0.05$) for females and males respectively. Maximal clearance rates were higher in females both when expressed per cell surface (3.4 ± 0.4 and 2.3 ± 0.4 al/μm² · s for females and males, $p < 0.05$), and per cell (136 ± 17 and 75 ± 11 fl/cell · s respectively, $p < 0.01$).

Discussion

In the present study, U-(^{14}C)-D-glucose was chosen as the tracer for measurement of glucose transport since D-glucose uses 25–50% as many cells per sample as 3-O-methylglucose [8, 11]. Furthermore, the coefficient of variation of parallel samples is about 20% for the 3-O-methylglucose assay [8, 11] but was only 5–10% for the D-glucose method of Foley et al. [9] and 11% in this study. Finally, D-glucose is the natural substrate for the glucose transport system.

A large interindividual variation is frequently observed when glucose transport or some other metabolic process is measured in fat cells [5, 8, 9, 12]. This variation could be due to true biological variation between individuals, or it could be caused by methodological variation. To distinguish between these two possibilities and to compare the reliability of needle biopsy samples to that of surgical samples, we studied the reproducibility of the glucose transport measurements using needle and/or surgical samples from the same individual. As judged from the coefficient of variation between two needle biopsies taken from the same subjects within 1 h, the reproducibility was 11%, i.e. small compared to the interindividual coefficient of variation (49% in Figure 2 for the first biopsy). We also studied the reproducibility of the measurements using fat cells from surgical samples. For ethical reasons, the surgical biopsies were taken 1 week apart. Thus, the observed coefficient of variation (9%) may reflect not only method variation but also true intraindividual variation. We would expect the latter component to be small, however, since the subjects were admitted 5 days prior to the first biopsy, and their diet and physical activities habits were strictly controlled throughout the study. Comparison of glucose transport values from fat obtained by needle vs. surgically showed that neither the sensitivity nor the basal or maximal transport values were significantly influenced by the method of sampling. We may thus conclude that method variation is approximately the same for needle biopsy and surgical samples, and that this variation is small compared to the interindividual variation in glucose transport.

Normal values for glucose transport were calculated separately for men and women from the needle biopsy samples. In agreement with the findings of Pedersen et al. [13] in gluteal fat cells and Foley et al. [12] in abdominal fat cells, both basal and maximal glucose transport rates were higher in women than men when expressed per cell. The sex difference in basal glucose transport values disappeared when adjusting for the difference in cell size, whereas the maximally insulin-stimulated glucose transport rate in gluteal fat cells was, in keeping with the results of Foley et al. [12] in abdominal fat cells, higher in women than in men even when expressed per cell surface. Thus, gluteal as well as abdominal fat cells of females are more responsive to insulin than those of males. The finding of enhanced insulin action in females is in keeping with in vivo studies demonstrating higher rates of glucose disposal [14] and better glucose tolerance [15] in women than in men.

In conclusion, the present study indicates that the glucose transport system may be studied using micro-

samples of human adipose tissue. Specifically, the assay described permits determination of the maximal glucose transport capacity and the insulin sensitivity (ED 50) of the fat cells. The reproducibility of the method is good, and therefore it may be used to detect differences both between subjects and changes in one subject in response to a physiological stimulus. The possibility to perform serial studies using insulin target tissue offers a tool to the study of acute changes in insulin action in normal man and in various patient groups.

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