Insulin resistance characterizes glucose uptake in skeletal muscle but not in the heart in NIDDM

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Summary Skeletal muscle insulin resistance and coronary heart disease (CHD) often precede non-insulin-dependent diabetes mellitus (NIDDM). A recent study showed the myocardium of patients with CHD to be insulin resistant, independent of blood flow. We determined whether myocardial insulin resistance is a feature of NIDDM patients with no CHD. Skeletal muscle and myocardial glucose uptake were determined in 10 patients with NIDDM and 9 age- and weight-matched normal men of similar age and body mass index men using [18F]-2-fluoro-2-deoxy-D-glucose and positron emission tomography under normoglycaemic hyperinsulinaemic conditions. Whole body glucose uptake, as determined by the euglycaemic clamp technique, was significantly lower in the patients with NIDDM (35 ± 3 µmol/kg body weight · min) than the normal subjects (45 ± 3 µmol/kg body weight · min, $p < 0.02$). Insulin-stimulated femoral muscle glucose uptake was significantly lower in the patients with NIDDM (71 ± 6 µmol/kg muscle · min) than in the normal subjects (96 ± 5 µmol/kg muscle · min, $p < 0.01$). Whole body glucose uptake was correlated with femoral muscle glucose uptake in the entire group ($r = 0.76$, $p < 0.001$), in patients with NIDDM and in normal subjects. Rates of insulin-stimulated myocardial glucose uptake were comparable between the patients with NIDDM (814 ± 76 µmol/kg muscle · min) and the normal subjects (731 ± 63 µmol/kg muscle · min, $p > 0.4$). Whole body or femoral muscle, and myocardial glucose uptake were not correlated in all subjects, patients with NIDDM or normal subjects. We conclude that insulin resistance of the myocardium is not a feature of uncomplicated NIDDM. [Diabetologia (1998) 41: 555-559]

Keywords Myocardium, insulin resistance, non-insulin-dependent diabetes mellitus, positron emission tomography.

Signs or symptoms of macrovascular disease are frequently found even prior to the development of non-insulin-dependent diabetes mellitus (NIDDM) [1]. This suggests that coronary heart disease (CHD) and NIDDM share a pathophysiologic feature, which predisposes to both diseases. Classic risk factors such as serum cholesterol, age, gender, hypertension and smoking explain only a fraction of the 2–4-fold increase in cardiovascular mortality in patients with NIDDM [1, 2]. Insulin resistance, usually measured by quantitating the rate of insulin-stimulated glucose uptake, qualifies as the underlying pathophysiologic abnormality for both CHD and NIDDM, since it predicts, independent of other risk factors, both disorders [3, 4].

The mechanisms linking insulin resistance to CHD are unclear but could involve lipid abnormalities such as an increase in the concentration of atherogenic small dense LDL particles, which accompanies insu-

lin resistance and hypertriglyceridaemia [5]. On the other hand, insulin resistance has been found to predict CHD even independent of lipid changes [6]. Recently, a defect in the ability of insulin to stimulate myocardial glucose uptake was demonstrated in both non-diabetic [7] and diabetic [8] patients with CHD. This novel defect has been suggested to contribute to, or reflect defects in glycolysis, which characterize heart failure in animal models [9], and might limit utilization of glucose under postprandial and ischaemic conditions [9]. It is, however, unknown, whether insulin resistance in the heart is a cause or a consequence of impaired cardiac function. This question can be answered by quantitating insulin sensitivity simultaneously in both skeletal muscle and the heart in patients who have NIDDM but no CHD. This was the aim of the present study, in which we quantitated heart and skeletal muscle glucose uptake using [18F]-2-fluoro-2-deoxy-D-glucose ([18F]FDG) and positron emission tomography (PET) under normoglycaemic hyperinsulinaemic conditions in patients with NIDDM but no CHD and in non-diabetic subjects.

Subjects and methods

Subjects. Ten male patients with NIDDM (age 43 ± 2 years, body mass index 27.3 ± 0.7 kg/m^2, blood pressure $126 \pm 4/84 \pm 4$ mmHg, glycosylated hemoglobin A$_{1c}$ 8.1 ± 0.5 %) and 9 normal men (45 ± 3 years, 26.4 ± 0.7 kg/m^2, $127 \pm 4/82 \pm 2$ mmHg, 5.4 ± 0.3 %) volunteered for the studies. The patients did not have clinical or biochemical evidence of diseases other than NIDDM and had no signs of micro- or macrovascular disease. Coronary artery disease was excluded by a normal resting ECG and by a work-conducted maximal bicycle exercise test. Four patients were treated with diet alone while 6 were treated with sulfonylureas and/or metformin. Oral antidiabetic drugs were discontinued for 2 days prior to the studies. The duration of NIDDM averaged 3 ± 1 years. The normal subjects were healthy as judged by history, physical examination and routine laboratory tests, and were not taking any medication. Written informed consent was obtained after the purpose, nature and potential risks were explained to the subjects. The experimental protocol was reviewed and approved by the ethical committee of the Turku University Central Hospital.

Study design. All subjects were studied after a 10–12 h overnight fast. Two catheters were inserted, one in an antecubital vein for infusion of glucose and insulin and injection of [18F]FDG, and another in the opposite radial or brachial artery for blood sampling. The patients with NIDDM were rendered normoglycaemic before the start of the study by using a low-dose insulin infusion according to the algorithm described by Mokan and Gerich [10]. Whole body, skeletal and heart muscle glucose uptake rates were thereafter determined by combining the euglcaemic hyperinsulinaemic clamp technique with [18F]FDG and PET, as described in detail below.

Whole body glucose uptake. Whole body glucose uptake was quantitated using the euglcaemic hyperinsulinaemic clamp technique [11]. Serum insulin was increased for 180 min using a primed-continuous (30 pmol/kg · min) infusion of insulin

(Velosulin; Novo Nordisk A/S, Bagsvaerd, Denmark). Normoglycaemia was maintained using an infusion of 20 % glucose based on frequent arterial plasma glucose measurements. Whole body glucose uptake was calculated from the glucose infusion rate during the period of PET scanning (60–160 min). Serum free insulin concentrations were measured every 30 min by radioimmunoassay [12].

Heart and skeletal muscle glucose uptake. [18F]FDG ($T_{1/2} = 109.8$ min) was synthesized with an automatic apparatus [12]. Approximately 5 mCi (185 MBq) of [18F]FDG was injected intravenously over 2 min. A dynamic scan of the femoral region was performed for 90 min and continued with a dynamic scan of the thoracic region for 18 min. Plasma radioactivity was measured with an automatic gamma counter (Wizard 1480 3"; Wallac, Turku, Finland). An eight-ring ECAT 931/08-tomograph (Siemens/CTI Corp., Knoxville, Tenn., USA) with an axial resolution of 6.7 mm, in plane resolution of 6.5 mm, and final in-plane resolution of 8 mm, was used. Before the emission scan, a transmission scan was performed as previously described [12]. All data were corrected for deadtime, decay and measured photon attenuation. Dynamic FDG scans were reconstructed into a 128×128 matrix using a Hann-filter with a cut-off frequency of 0.5. Regions of interest were drawn within femoral muscles as previously described [12]. For determination of myocardial glucose uptake, large regions of interest were drawn on at least four representative midventricular slices avoiding myocardial borders. All myocardial time-activity curves were corrected for partial volume effect and spillover from cavity [13]. The graphical analysis derived from the three compartment model of [18F]FDG kinetics as described previously was used to quantitate the fractional rate of tracer phosphorylation K_i [12].

Echocardiography. M-mode and two-dimensional echocardiography (Aloca SSD-870; Aloca Co. Ltd., Tokyo, Japan) were performed, and Penn-cube left ventricular mass was calculated as previously described [14, 15].

Statistical analysis. Group comparisons were performed with the unpaired Student's *t*-test for normally distributed data, and correlations were calculated using Pearson's correlation analysis. The results are expressed as mean ± SEM.

Results

Whole body glucose uptake. Fasting plasma glucose ($8.3 \pm$ vs $5.4 \pm$ mmol/l, NIDDM vs normal subjects, $p < 0.001$) and serum free insulin concentrations (73 ± 24 vs 36 ± 6 pmol/l, respectively, $p < 0.05$), were higher in patients with NIDDM than in normal subjects. During hyperinsulinaemia, serum free insulin concentrations averaged 2434 ± 153 pmol/l in patients with NIDDM and 2666 ± 79 pmol/l in the normal subjects (NS), and plasma glucose concentrations were 5.3 ± 0.1 and 5.4 ± 0.1 mmol/l, respectively (NS). Serum non-esterified fatty acid (NEFA) concentrations were not significantly different between the groups (205 ± 18 vs 155 ± 20 µmol/l, NS). Whole body glucose uptake during hyperinsulinaemia was significantly lower in the patients with NIDDM (35 ± 3 µmol/kg body weight · min) than the normal subjects (45 ± 3 µmol/kg body weight · min, $p < 0.02$).
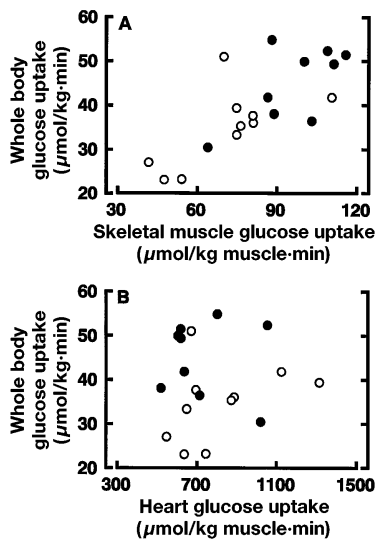
Fig. 1 A, B. Interrelationships between femoral muscle and whole body glucose uptake (**A**), and heart and whole body glucose uptake (**B**) in the patients with NIDDM (○) and in normal subjects (●)

Skeletal muscle and heart glucose uptake. Insulin-stimulated femoral muscle glucose uptake was significantly lower in the patients with NIDDM (71 ± 6 μmol/kg muscle · min) than in the normal subjects (96 ± 5 μmol/kg muscle · min, $p < 0.01$). Whole body glucose uptake was correlated with femoral muscle glucose uptake in the entire group ($r = 0.76$, $p < 0.001$), in patients with NIDDM ($r = 0.66$, $p < 0.05$) and in normal subjects ($r = 0.66$, $p < 0.05$) (Fig. 1).

Left ventricular masses were similar between patients with NIDDM (222 ± 8 g) and the normal subjects (223 ± 11 g, NS). Rates of insulin-stimulated myocardial glucose uptake were comparable between the patients with NIDDM (814 ± 76 μmol/kg muscle · min) and the normal subjects (731 ± 63 μmol/kg muscle · min, $p > 0.4$). Rate-pressure products were comparable during hyperinsulinaemia in patients with NIDDM [9228 ± 684 mmHg · (beats/min)] and the normal subjects (8380 ± 596). There was no significant correlation between whole body and myocardial glucose uptake in all subjects ($r = 0.06$, NS), patients with NIDDM ($r = 0.37$, NS) or normal subjects ($r = -0.08$, NS) (Fig. 1), or between femoral muscle and myocardial glucose uptake (data not shown).

Discussion

In the present study, we quantitated skeletal muscle and heart glucose uptake in patients with NIDDM who had no evidence of CHD clinically or based on a maximal bicycle exercise test. We found insulin resistance at the level of the whole body, the magnitude of which was closely related to that in skeletal muscle. Rates of insulin-stimulated heart glucose uptake, were, however, comparable in patients with NIDDM and normal subjects. These data indicate that patients with NIDDM do not exhibit generalized insulin resistance, and that the insulin resistance which has been found in non-diabetic [7] and NIDDM [8] subjects with CHD is likely to be a consequence of neurohormonal or other alterations secondary to heart failure or CHD and not NIDDM.

Use of [^{18}F]FDG as a tracer to quantitate glucose uptake assumes that this tracer reflects the behaviour of the tracee. A lumped constant term is used to adjust for differences between the tracer and the tracee. The lumped constant has been found to be unaffected by insulin (5000 mU/l) in the isolated arterially perfused rabbit interventricular septum [16], and by 20-fold differences in glucose metabolic rates in vivo in the heart of anaesthetized dogs [17]. In the isolated perfused working rat heart, however, Hariharan et al. [18], found insulin (1000 mU/l) to increase glucose but not [^{18}F]FDG uptake. The latter result differs from effects of insulin on FDG uptake in the human heart in vivo, in which mean glucose uptake rates, increase approximately 12–34-fold by physiological insulin concentrations [19, 20]. Even at low (NEFA) concentrations, FDG-uptake increases 2-fold by insulin in humans [20]. The latter data are similar to those reported in catheterization studies [21] and support, together with several additional in vivo observations (vide infra) the view that glucose uptake measured with FDG and PET provides information which is fully compatible with physiological knowledge. Similarly, concentrations of plasma NEFA are inversely correlated with rates of FDG uptake [12] and arterial-coronary sinus glucose differences [22]. Several indices of cardiac work are also directly correlated with glucose uptake under normoglycaemic hyperinsulinaemic conditions [12, 14, 23] where glucose is the main fuel for heart energy production.

The present data are consistent with data from previous studies examining insulin sensitivity of heart glucose uptake in various groups differing with respect to their muscle insulin sensitivity. Patients with IDDM [23, 24] with no CHD have insulin resistance in skeletal muscle but no alteration in cardiac work or myocardial glucose uptake under insulin-stimulated conditions. In contrast, in athletes with a decrease in cardiac work per heart mass, insulin-stimulated glucose uptake in the heart is diminished, while peripheral insulin sensitivity is enhanced [14]. In patients with essential hypertension, the opposite picture emerges: heart glucose uptake and cardiac work per unit heart mass are increased in proportion to the elevation of blood pressure, while peripheral insulin sensitivity is decreased [25]. Thus, if changes in insulin sensitivity are associated with alterations in cardiac work (hypertension, physical fitness), heart glucose uptake is altered in proportion to the altera-

tion in cardiac work under insulin-stimulated conditions. If peripheral insulin resistance is not associated with alterations in cardiac work, insulin-stimulated glucose uptake appears normal. The study of Paternostro et al. [7] who found insulin resistance in the myocardium, fits in neither of these categories as 10 of the patients with CHD were classified as NYHA class III and 5 as NYHA class II, and all had symptoms of ischaemic heart disease. The finding of insulin resistance in the myocardium of such patients, even in segments with normal wall motion and blood flow [7], could be due to factors such as increased norepinephrine [26, 27] or NEFA concentrations [27], which accompany heart failure. In contrast to the present data, Voipio-Pulkki et al. [8] found NIDDM to be associated with cardiac insulin resistance in patients with CHD. Although one could argue that NIDDM influences myocardial glucose uptake differently in patients with and without CHD, other explanations are more likely to underlie the discrepant findings. First, in the study of Voipio-Pulkki et al. [8] the patients with NIDDM were studied under hyperglycaemic conditions and the control subjects under normoglycaemic conditions [8]. In the rat heart, the glucose concentration profoundly affects the rate-limiting step for glucose utilization and consequently the lumped constant [28]. Whether this also applies in humans is currently unknown. Another factor to consider is that we used a supramaximal insulin concentration which resulted in comparable suppression of NEFA concentrations in both groups. Since lipolysis is exquisitely sensitive to insulin [29] and since the antilipolytic effect of insulin may be altered by NIDDM or obesity [30], the present data do not exclude the existence of myocardial insulin resistance under conditions where myocardial glucose uptake is dependent upon plasma NEFA availability [12, 31]. Also, the present data do not exclude the possibility that older or more obese patients or patients with longer duration of NIDDM would be characterized by cardiac insulin resistance.

We conclude that insulin resistance in the myocardium is not an inherent feature of uncomplicated NIDDM. Impaired myocardial glucose utilization is therefore unlikely to be a primary defect predisposing non-diabetic individuals or patients with NIDDM for the development of CHD. This does not dispute a role for insulin resistance per se in being a risk factor for cardiovascular disease but suggests that the mechanisms linking the two phenomena are likely to be indirect or could involve some other insulin sensitive process(es) such as resistance to the platelet antiaggregating effect of insulin, which also characterizes patients with NIDDM [32].

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