Insulin resistance in patients with cardiac hypertrophy

Giovanni Paternostro\textsuperscript{a}, Domenico Pagano\textsuperscript{b}, Tomaso Gnecci-Ruscone\textsuperscript{a}, Robert S. Bonser\textsuperscript{b}, Paolo G. Camici\textsuperscript{a,\textdagger}

\textsuperscript{a}MRC Cyclotron Unit, Imperial College School of Medicine, Hammersmith Hospital, Du Cane Road, London W12 0NN, UK
\textsuperscript{b}Queen Elizabeth Hospital, Birmingham, UK

Received 28 May 1998; accepted 14 July 1998

Abstract

Objective: Animal studies suggest that left ventricular hypertrophy might be associated with insulin resistance and alterations in glucose transporters. We have previously demonstrated myocardial insulin resistance in patients with post-ischemic heart failure. The aim was to investigate whether myocardial insulin resistance could be demonstrated in human cardiac hypertrophy in the absence of hypertension, diabetes and coronary artery disease.

Methods: Eleven normotensive nondiabetic patients with cardiac hypertrophy due to aortic stenosis and angiographically normal coronary arteries were compared to 11 normal volunteers. Myocardial glucose uptake (MGU) was measured with positron emission tomography and \([\text{F}]_{\text{2}}\)-fluoro-2-deoxy-D-glucose during fasting (low insulinemia) or during euglycemic–hyperinsulinemic clamp (physiologic hyperinsulinemia). Myocardial biopsies were obtained in order to investigate changes in insulin-independent (GLUT-1) and insulin-dependent (GLUT-4) glucose transporters.

Results: During fasting, plasma insulin (7\pm 6 vs. 6\pm 1 mU/l) and MGU (0.12\pm 0.05 vs. 0.11\pm 0.04 \(\mu\)mol/min/g) were comparable in patients and controls. By contrast, during clamp, MGU was markedly reduced in patients (0.48\pm 0.02 vs. 0.70\pm 0.03 \(\mu\)mol/min/g, \(p < 0.01\)) despite similar plasma insulin levels (95\pm 6 vs. 79\pm 6 mU/l). A decreased GLUT-4/GLUT-1 ratio was shown by Western blot analysis in patients.

Conclusions: Insulin resistance seems to be a feature of the hypertrophied heart even in the absence of hypertension, coronary artery disease and diabetes and may be explained, at least in part, by abnormalities in glucose transporters.

Keywords: Glycolysis; Heart failure; Hypertrophy; Membrane transport; Valve (disease); Positron emission tomography

1. Introduction

Whole body insulin resistance, mainly due to a decreased responsiveness to insulin of skeletal muscle [1], has been demonstrated to be associated with systemic conditions such as diabetes (both type 1 and type 2), hypertension and coronary artery disease [1]. More recently, the non-invasive measurement of myocardial uptake of the glucose analogue [\(\text{[F]}\)2-fluoro-2-deoxy-D-glucose (FDG) by positron emission tomography (PET), has made it possible to assess the presence of cardiac insulin resistance in humans. We have previously reported that insulin resistance is present in the non-infarcted myocardium of patients with previous myocardial infarction and heart failure [2]. These myocardial regions, remote from the infarct, are likely to undergo compensatory hypertrophy [3]. On the other hand, in patients with type 1 diabetes, peripheral (skeletal muscle) insulin resistance is not accompanied by a reduced cardiac FDG uptake [4,5]. Likewise, in young subjects with mild hypertension and no cardiac hypertrophy, Nuutila et al. [6] could show skeletal but not cardiac insulin resistance.

Insulin promotes glucose uptake and decreases the utilisation of free fatty acids by the human heart [7–9]. Glucose is a particularly important substrate for the heart. It is more efficient than other substrates in terms of oxygen consumption (more ATP is synthesized per mole of oxygen consumed) and its uptake is increased in myocardial ischemia [10,11]. In addition, glucose seems to be important for the production of ATP for ion channels and pumps [12,13] and has an anaplerotic role, i.e. it provides

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\textsuperscript{\textdagger}Corresponding author. Tel.: +44-181-383-3186; fax: +44-181-383-3742.
\textsuperscript{\textdagger\textdagger}E-mail address: paolo@cu.rpms.ac.uk (P.G. Camici)

\textsuperscript{\textdagger\textdagger}\textsuperscript{\textdagger}See pages 12–14.

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PII: S0008-6363(98)00233-8

Time for primary review 19 days.
oxaloacetate, thus replenishing Krebs cycle intermediates [14].

Glucose enters the cell using specific transporters [15]. There are five facilitative glucose transporters, named GLUT-1 to GLUT-5. Of these, only GLUT-1 and GLUT-4 are present in the heart. Immunofluorescence has demonstrated the presence of both on cardiac myocytes [16]. The GLUT-1 isoform, located mainly in the sarcolemmal membrane, is thought to be involved in glucose transport in the basal metabolic state. Under such conditions, less than 1% of GLUT-4 (the insulin-responsive isoform) is located in the sarcolemma [17]. After insulin stimulation, however, glucose transport is accelerated by translocating GLUT-4 from an intracellular pool to the T-tubule and sarcolemmal membrane [17,18]. GLUT-4 levels are decreased in adipose tissue in diabetic patients [19,20], but probably not in skeletal muscle [18]. However, it has been reported that GLUT-4 translocation is diminished in the skeletal muscle of diabetic patients [21]. Much less is known about GLUT-4 in the insulin-resistant heart. We have previously shown that a decreased expression of cardiac GLUT-4 mRNA accompanies insulin resistance in the hypertrophied heart of the spontaneously hypertensive rat [22]. In this model, increased basal deoxyglucose uptake and a reduced response to insulin could be demonstrated [22]. In a different model of cardiac hypertrophy in the rat (aortic banding), a decreased ratio of GLUT-4 to GLUT-1 mRNA expression has been reported [23].

The purpose of the present study was to establish if myocardial insulin resistance is a feature of cardiac hypertrophy in the absence of systemic conditions that are known to be associated with a reduced response to this hormone (diabetes, hypertension, coronary artery disease). Myocardial glucose uptake was estimated using PET in patients with pure aortic stenosis. Furthermore, alterations in the expression of glucose transporters, as a possible molecular basis for insulin resistance, were investigated in cardiac biopsies.

2. Methods

2.1. Study protocol

PET was used to measure myocardial blood flow and FDG uptake at two different levels of plasma insulin: a very low one corresponding to fasting conditions and a level 10 times higher during hyperinsulinemic euglycemic clamp. Five patients and five controls were studied during fasting; six patients and six controls were studied during hyperinsulinemic euglycemic clamp. The expression of the glucose transporters GLUT-1 and GLUT-4 was measured by Western blot analysis in cardiac biopsies from the patients with aortic stenosis. Additional biopsies were obtained from failing and donor hearts at the time of cardiac transplantation. The investigation conforms with the principles outlined in the Declaration of Helsinki.

2.2. Study population

2.2.1. PET

2.2.1.1. Patients The patient population consisted of 11 normotensive, nondiabetic patients (ten men, aged 63 ± 2 years) with pure aortic stenosis and with normal or non-significantly diseased coronary arteries, who were on the waiting list for valve replacement. All patients were in NYHA class II and no attempt was made to standardize medical therapy. Three patients were on ACE inhibitors, one was on digitalis, one was on calcium antagonists and one was on nitrates. Before the PET scan, all patients were studied by echocardiography, according to the guidelines of the American Society of Echocardiography (Esaote Biomedica SIM 7000 CFM echograph). The posterior and septal wall thickness were measured in M-mode in the left parasternal projection (at end diastole). Since these measurements never differed by more than 1 mm, thus excluding asymmetric hypertrophy, only their average thickness is reported in Section 3.

2.2.1.2. Controls The results of the PET studies in patients were compared with those obtained in 11 healthy male volunteers (aged 47 ± 2 years, \( p < 0.05 \) vs. patients) matched for body mass index. All had a negative history for coronary artery disease, normal physical examination, resting 12 lead electrocardiogram and echocardiogram and a treadmill exercise test (Bruce protocol) negative for myocardial ischemia at high workload.

2.3. Cardiac biopsies

2.3.1. Patients

Cardiac biopsies were obtained from four of the aortic stenosis patients (all males, age 57 ± 1 years) studied by PET, at the time of aortic valve replacement. In a separate experiment, biopsies were also obtained from five explanted hearts of patients with end stage heart failure (all males, age 45 ± 1 years). Three of these explanted hearts were from patients with non-ischemic cardiomyopathy and two were from patients with severe coronary artery disease.

2.3.2. Controls

Cardiac biopsies were obtained from four donor hearts, at the time of heart transplant. The donors were also all males and aged 32 ± 4 years \( (p < 0.05 \) vs. patients).

2.4. Hyperinsulinemic euglycemic clamp

Before the PET scan, a 20-G polyethylene cannula was inserted in a superficial forearm vein for infusion of
glucose and insulin, as described by DeFronzo et al. [24], in order to obtain a near-steady state at physiological concentration of glucose and at plasma insulin values similar to those of the post-prandial state. A second cannula was threaded retrogradely into a superficial vein of the wrist or hand that had been arterialized using a heating pad set at 50°C. At time zero, a primed-constant insulin infusion (40 mU min⁻¹ m² of body surface area) was started. The body surface area was calculated from the formula of Du Bois and Du Bois [25]. The prime consisted of four times the final constant rate for the first 4 min, followed by two times the constant rate for 3 min. Four min into the insulin infusion, an exogenous D-glucose infusion was started at an initial rate of 1.5 mg min⁻¹ per kg of body weight. The plasma glucose concentration in arterialized blood was measured at baseline and then every 5 min during the clamp. The glucose infusion rate was adjusted according to the change in plasma glucose during the preceding 5 min. Samples for insulin assay were taken from the arterialized vein at 20, 40 and 60 min during the clamp.

Glucose was measured with an automatic analyser (2300Stat, Yellow Spring, USA) which required around 30 s per measurement (this is essential during the clamp because the rate of infusion has to be adjusted as quickly as possible). Insulin was measured in the Endocrinology Laboratory of Hammersmith Hospital using an immunoradiometric assay.

Under the near-steady-state conditions of euglycemic hyperinsulinemia prevailing during the second hour of an insulin clamp, the exogenous glucose infusion rate equals the total amount of glucose metabolized by all tissues and, therefore, is an index of whole-body insulin sensitivity (expressed in μmol min⁻¹ per kg of body weight).

2.5. PET scanning and data analysis

PET scanning was carried out in all subjects, as previously reported [2]. Briefly, the optimal imaging position was determined using a 5-min rectilinear scan and a 20-min transmission scan was then performed. The blood pool was imaged by inhalation of tracer amounts of ¹⁵O-labelled carbon monoxide (¹⁵O₂). Myocardial blood flow (MBF) was measured using inhaled ¹⁵O-labelled carbon dioxide (¹⁵O₂), which is rapidly converted to ¹⁵O-labelled water (H₂¹⁵O) by carbonic anhydrase in the lungs [26]. Myocardial glucose uptake was measured with the glucose analogue FDG. Image manipulation and kinetic analyses were performed using the ANALYZE (Version 3.0, Biodynamics Research Unit, Mayo foundation, Rochester, MN, USA) and the MATLAB (The MathWorks Inc., Natick, MA, USA) software packages, respectively. Cardiac images were resliced in the short axis view. Tissue FDG time-activity curves were analysed by using the linearized approach proposed by Patlak et al. [27] for irreversible processes. Myocardial glucose uptake (MGU) was then obtained by multiplying regional influx rates by the plasma concentration of cold glucose, assuming a lumped constant of one, and by dividing the product by the corresponding tissue fraction. This last step was performed in order to correct for partial volume effect. A conversion for millilitres to grams of perfusable tissue was made by dividing the flow and metabolic data by the tissue density (1.04 g/ml). Thus, MGU is expressed as μmol min⁻¹ per g of water perfusable tissue.

2.6. Western blots

All cardiac biopsies were transmural, taken from the left ventricle in an area with no macroscopic signs of fibrosis. The biopsies from the explanted hearts were cut with a scalpel. The biopsies from the donor hearts and patients undergoing valve replacement were taken using a Tru-cut (Baxter) biopsy needle and were of smaller dimensions (around 30 mg). The tissue was immediately frozen in liquid nitrogen and then stored at −70°C. At the time of analysis, the samples were pulverized on dry ice with a mortar and pestle, then 100 μl of sample buffer were added to the pulverized tissue. The sample buffer contained 62.5 mM Tris, 10% glycerol, 2% sodium dodecyl sulfate (SDS), 2 mM EDTA (all from Sigma) plus the following protease inhibitors: 1 mg/ml AEBSF, 10 mg/ml E64, 1 mg/ml pepstatin (all from Boehringer Mannheim).

After the addition, the tubes were quickly frozen in liquid nitrogen, then boiled and vortex-mixed repeatedly until the tissue appeared to be completely solubilized. After 5 min centrifugation at the maximum speed on an Eppendorf bench top centrifuge, the supernatant was isolated, 10 μl were used for the protein assay and the rest was frozen in liquid nitrogen and kept at −70°C. The protein content was measured using the assay of Lowry et al. [28]. For electrophoresis, β-mercaptoethanol (3% final) was added to a volume of sample equivalent to 30 μg of protein and then volumes were equalized with sample buffer containing bromphenol blue. Western blotting was performed according to standard procedures [29]. The same filter was then probed with two antibodies: anti-GLUT-4 (rabbit polyclonal, a kind gift from Dr Gwyn Gould) [30], anti-GLUT-1 (rabbit polyclonal, from Charles River Laboratories, Wilmington, MA, USA) [31] using dilutions of 1:1300 for GLUT-4 and 1:2000 for GLUT-1. Ponceau S staining of filters was used to assess total protein. Preliminary experiments were performed with control filters in order to show that the expected band (at 50 kilodaltons) was absent if the primary antibody was omitted. Immunodetection was performed with the ECL system (Amersham). After each immunodetection, the filter was stripped using a 30-min incubation in stripping buffer (100 mM β-mercaptoethanol, 2% SDS, 62.5 mM Tris, pH 6.7) and two washing in phosphate-buffered saline (PBS)–Tween at room temperature. The efficiency of stripping was checked by reprobing in the absence of primary antibody. The
intensity of the bands on the autoradiography film were quantified using the image analysis program, ANALYZE.

2.7. Statistical analysis

All results are expressed as mean±standard error of the mean. The Mann-Whitney U test was used to compare mean group values, whereas paired comparisons were performed by the Wilcoxon signed-rank test. Comparisons of more than two groups were performed using the Kruskall-Wallis test. We have used these non-parametric tests because the number of subjects is limited and the assumptions on which parametric tests are based cannot be easily verified [32]. Regression analysis was performed according to standard techniques. A value of \( p<0.05 \) was considered to be statistically significant.

3. Results

3.1. Characteristics of the study population

The body mass index (weight/height [2]) was 25±1 kg/m\(^2\) in controls and 27±1 kg/m\(^2\) in patients (\( p=NS \)). The left ventricular wall thickness was 10±0.3 mm in controls and 16±0.8 mm in patients (\( p<0.01 \)). There was no difference in left ventricular wall thickness between patients studied during fasting and during clamp (16±1 mm in the fasting state and 17±1.4 mm in the clamped state, \( p=NS \)). There were no significant differences between patients and controls in systolic blood pressure (126±3 mmHg in controls vs. 126±4 mmHg in patients), diastolic blood pressure (75±3 mmHg in controls vs. 74±2 mmHg in patients) and heart rate (64±2 bpm in controls vs. 71±4 bpm in patients). The baseline plasma glucose concentration was 5.0±0.2 mM in controls, and 4.6±0.2 mM in patients (\( p=NS \)). During clamp, plasma glucose levels were 6.0±0.4 and 5.5±0.2 mM (\( p=NS \)) in controls and patients, respectively. Plasma insulin was 7±1 and 6±1 mU/l (\( p=NS \)) during fasting and 79±6 and 95±6 mU/l (\( p=NS \)) during clamp, in controls and patients, respectively. The glucose metabolized by the whole body during the last 60 min of the clamp study was 37±4 \( \mu \)mol min\(^{-1} \) kg\(^{-1} \) in controls and 19±2 \( \mu \)mol min\(^{-1} \) kg\(^{-1} \) in patients (\( p<0.01 \)).

3.2. Myocardial blood flow and glucose uptake (Table 1)

There was no difference amongst the four groups in terms of myocardial blood flow rates. There was also no difference in glucose uptake between patients and controls during fasting. By contrast, myocardial glucose uptake during clamp was 31% lower in patients. Thus, a 13-fold increase in plasma insulin could increase glucose uptake by 6.4-fold in controls but only four-fold in patients (Fig. 1). During clamp, cardiac glucose uptake was linearly related to whole-body glucose utilization (\( r=0.70, \ p<0.01 \)).

3.3. Glucose transporters

In the patients with cardiac hypertrophy and aortic stenosis, the GLUT-4/GLUT-1 ratio was decreased compared to controls (1.2±0.2 vs. 2.3±0.2, \( p=0.009 \)). The GLUT-4/GLUT-1 ratio was equally decreased in the explanted hearts of the patients with heart failure (1.2±0.1) compared to controls (2.6±0.3; \( p=0.002 \)) (Fig. 2). When GLUT-1 and GLUT-4 were expressed as a ratio to total protein (in arbitrary density units), the changes were not significant. In patients with aortic stenosis, GLUT-4 was 1.6±0.3 vs. 2.3±0.7 in controls and GLUT-1 was 1.3±0.3 vs. 1.0±0.4 in controls. In the explanted hearts of patients with heart failure, GLUT-4 was 1.9±0.3 vs. 2.6±0.4 in controls and GLUT-1 was 1.5±0.4 vs. 1.0±0.4 in controls. The cause of heart failure did not seem to influence the GLUT-4/GLUT-1 ratio, which was 1.3±0.1 in the three patients with heart failure due to non-ischemic cardiomyopathy and 1±0.1 in the two patients with heart failure due to coronary heart disease.

4. Discussion

The results obtained in patients with aortic stenosis are similar to those we have previously reported in the non-infarcted myocardium of patients with post-ischemic heart failure [2] and suggest that insulin resistance accompanies cardiac hypertrophy per se, even in the absence of coronary artery disease, diabetes and/or hypertension.
4.1. Glucose transporters

We found a reduced GLUT-4/GLUT-1 protein ratio in hearts from patients with end-stage heart failure and in those with aortic stenosis and we hypothesize that this is a possible molecular basis for the cardiac insulin resistance shown by PET. This is also consistent with previous reports of lowered GLUT-4/GLUT-1 mRNA ratio in animal models of cardiac hypertrophy [22,23]. Interestingly, transgenic mice lacking GLUT-4 expression have cardiac hypertrophy and a decreased life span [33], raising the possibility that alterations of glucose metabolism might contribute to and not simply be a consequence of cardiac disease. Furthermore, Sun et al. [34] have shown that myocardial ischemia causes translocation of GLUT-4 to the plasma membrane of cardiac myocytes, leading to increased glucose uptake. GLUT-4 depletion could therefore limit glucose availability under conditions of hypoxia and contribute to myocardial dysfunction. On the other hand, it is well known that cardiac hypertrophy is associated with the reappearance of a pattern of gene expression that is present in the fetal and newborn heart [35]. The fetal rat heart has less GLUT-4 and more GLUT-1 than the adult [36,37] and our results are in line with the concept of re-expression of fetal genes in cardiac hypertrophy. Our finding that comparable reductions in the GLUT-4/GLUT-1 ratio could be demonstrated in biopsies from patients with cardiac diseases of different severity and etiology suggests that this might be part of a general response of the myocardium to insult. Similar cardiac isoform switches have been reported for several other proteins, including myosin heavy chain [38], Na–K-ATPase [39] and creatine kinase [40].

4.2. Myocardial blood flow and glucose uptake

Changes in blood flow could affect glucose uptake [41], but our PET measurements do not show any significant effect of insulin on myocardial blood flow during hyperinsulinemic euglycemic clamp. While there is growing evidence that insulin can act as a vasodilator in human skeletal muscle [42], several studies have shown that hyperinsulinemia has no effect on coronary blood flow in
The FDG–PET results obtained during fasting, with low plasma insulin, are different from those reported by several in vitro animal studies, both by us and by others (e.g. [22,45]), where deoxyglucose uptake in the absence of insulin was shown to be higher in hypertrophied hearts compared to controls. No other human studies of myocardial glucose uptake in global cardiac hypertrophy have been published. An in vivo NMR animal study [46] has also reported an increase in deoxyglucose-6-phosphate accumulation in hypertrophied hearts, in the presence of low circulating insulin values. A dog model of hypertrophy secondary to aortic constriction was employed. A possible reason for the different results obtained in man is the presence of generalized metabolic alterations that occur in patients with heart failure [47], especially an increase in circulating free fatty acids, since substrate competition regulates cardiac glucose uptake [48,49]. Alterations in fatty acid levels were however not present in the experiment of Zhang et al. [46] and this might explain why their results differed from those we report in man.

The results of our study are consistent with those of Maki et al. [50] in patients with coronary artery disease and demonstrate that, in the human heart in vivo, the increase of glucose uptake in response to insulin is much higher than that in isolated perfused hearts [45,51–53]. A possible explanation for this difference could be that in vivo insulin-stimulated myocardial glucose uptake is not only due to stimulation of GLUT-4 translocation, but also to a decrease in circulating fatty acids [8,54]. Furthermore, the possible limitations [55] inherent with the use of FDG for the quantitation of myocardial glucose uptake, which are discussed below, are more serious when different metabolic states are compared.

Several medications are known to affect whole body insulin resistance. ACE-inhibitors are known to improve insulin sensitivity, while other drugs (e.g. diuretics) might worsen it [56]. It might be an objective for future studies to elucidate the effects of ACE-inhibitors on cardiac insulin resistance.

### 4.3. Possible limitations

Heart failure and hypertrophy are accompanied by an increase in extracellular collagen deposition [57,58]. Therefore, normalizing the Western blot results by total protein might be inappropriate. The choice of an internal control is also difficult, since it implies that the control protein is known not to be altered. Although the GLUT-4/GLUT-1 ratio provides a relative measurement, this has a clear physiological meaning and has the additional advantage of correcting for possible variations in the handling of the samples or in the efficiency of protein transfer during the blotting procedure. Any change in this ratio will influence glucose responsiveness to insulin (that is, the difference in glucose uptake between high and low insulin concentrations), but could be due to a decrease in GLUT4, an increase in GLUT1 or to both. Changes in GLUT1 and GLUT4 expressed as a ratio to total protein were not significant, but, as mentioned above, these measurements can be considered less reliable than the ratio of the two transporters. In fact, Western blots from cardiac biopsies are often quantified as a ratio of two proteins [59–62].

For ethical reasons, we could obtain only very limited amounts of tissue from donor hearts and from the hearts of patients undergoing valve replacement, therefore, we were unable to investigate this point further. However, several authors have quantified their glucose transporter measurements as a ratio of the two transporters [23,63,64].

PET allows for attenuation correction of annihilation photons, thus enabling the accurate measurement of absolute radioactivity concentration in tissues [66]. In the case of FDG measurements, however, there are some assumptions that we have discussed in detail previously [2]. Briefly, in order to account for differences in affinity between FDG and glucose, both for glucose transporters and hexokinase, the estimate of glucose uptake obtained from FDG–PET is normally divided by the so-called lumped constant [67–69]. However, studies in isolated rat hearts have shown that the lumped constant might vary under different experimental conditions [55]. Therefore, it has been recommended that PET studies of FDG uptake in the heart should be performed under controlled metabolic conditions [55,70], as those employed in the present study.

It is worth noting that the difference in myocardial glucose uptake between fasting and euglycemic clamp estimated with FDG–PET is very similar to that measured in humans during simultaneous arterial and great cardiac vein catheterization [8].

### 5. Conclusions

The response of glucose uptake to insulin in the hypertrophied human heart is reduced and this reduction is independent of myocardial blood flow and predisposing conditions (hypertension, coronary artery disease, diabetes). A lower GLUT-4/GLUT-1 ratio is a possible molecular basis for this decreased responsiveness. It remains to be seen if interventions that are known to improve whole body insulin resistance might have an effect on cardiac insulin resistance and the progression to heart failure.
References


