Exercise modulates myocardial protein kinase B/Akt in Zucker obese rats

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In type 2 diabetes, heart muscle is insulin resistant. Protein kinase B (PKB/Akt) acts as a mediator of the metabolic effects of insulin, including translocation of the insulin sensitive glucose transporter, GLUT4, in a manner dependent on phosphatidylinositol-3-kinase (PI-3-K). We have previously described dysregulation of PKB/Akt in hearts from a rat model of type 2 diabetes, the Zucker fa/fa rat. Exercise or contraction of heart muscle does not activate PKB/Akt or PI-3-K but can induce GLUT4 translocation and glucose uptake. However, exercise alleviates peripheral insulin resistance at a currently unknown, post-receptor level. To better understand the mechanism whereby this may be accomplished, we investigated whether chronic exercise will induce beneficial changes in the regulation of PKB/Akt that translates into improved myocardial insulin stimulated glucose uptake.

METHODS
To accomplish this, we subjected Zucker fa/fa rats (20 weeks old at the beginning of training) and a control group of age matched Wistar rats, to an exercise training programme, and documented changes in glucose uptake, myocardial GLUT4, and PKB/Akt expression and phosphorylation, in comparison to their sedentary counterparts. Rats were trained by swimming for four weeks, six days per week, on set times each day, starting at five minutes per day, escalating to 90 minutes per day, to avoid stress. The water temperature was 34°C and for half the exercise time, rats were weighted with 2% of their body weight, carried on their tails. Animals were sacrificed by intraperitoneal injection of sodium pentobarbitone (0.1 mg/g) after which ventricular myocytes were harvested via standard methods.

2-Deoxy-D-glucose (2-DG) uptake by cardiomyocytes was measured as previously described while PKB/Akt phosphorylation and GLUT4 expression were determined via standard Western blotting techniques and suitable antibodies. PKB/Akt kinase activity was determined using a commercial non-radioactive assay system (Cell Signaling Technology). Laser scanning densitometry and suitable software (UN-SCAN-IT, Silkscience) were used to analyse the Western blots. Comparisons of datasets were made either by analysis of variance (ANOVA) followed by a Bonferroni correction or a Student’s t test for unpaired data. A probability value of p < 0.05 was considered significant.

RESULTS
Blood glucose determinations (Boehringer Mannheim, Accutrend glucose meter) at the time of euthanasia, demonstrated that Zucker fa/fa rats were diabetic at this age (mean (SEM) 10.3 (0.3) mmol/l (fa/fa) v 7.8 (0.4) mmol/l (Wistar), p < 0.01, n = 6). Training lowered the blood glucose of the fa/fa group to 8.8 (0.6) mmol/l (p < 0.05, n = 7).

2-DG accumulation by cardiomyocytes from sedentary diabetic rats (15.3 (1.4) pmol/mg protein/30 mins) was lower than the sedentary control group (25.5 (1.8) pmol/mg protein/30 mins), while insulin could not elicit a significant response in the former (19.9 (3.6) v 99.6 (8.7)). Exercise had no effect on basal glucose uptake (15.2 (1.7) pmol/mg protein/30 mins), but improved the insulin stimulated response to 38.5 (5.1) pmol/mg protein/30 mins in the diabetic rats. This was, however, still less than controls after exercise. Concurrently, we found a 30% lower GLUT4 content in diabetic versus control hearts. Expression of this protein was upregulated by exercise in the diabetic hearts only, to a level similar to that of control hearts after exercise.

We furthermore documented raised serine-473 phosphorylation of PKB/Akt in diabetic hearts compared to control hearts, under basal conditions. This was coupled to equal expression and kinase activity of the protein. Nevertheless, it was less sensitive to stimulation by insulin. Following training however, insulin stimulated phosphorylation of PKB/Akt was equal to that found in control hearts (fig 1).

DISCUSSION
It is well established that exercise can improve insulin resistance by sensitising muscle to insulin mediated glucose uptake. However, the mechanism by which this occurs is currently unknown, and may include alterations in the regulation of PKB/Akt or PI-3-K expression and activity. Accuracy of this study was further improved by controlling for age and sex differences, which could have influenced the results. Overall, this study provides evidence that exercise training alleviates insulin resistance at a currently unknown, post-receptor level, and that this may be mediated by changes in the regulation of PKB/Akt.

Abbreviations: 2-DG, 2-deoxy-D-glucose; PI-3-K, phosphatidylinositol-3-kinase; PKB, protein kinase B
metabolism, and it has been used to treat or prevent type 2 diabetes in patients successfully. Glucose transport is the rate limiting step in muscle glucose utilisation and the capacity for glucose transport may vary according to the total amount of GLUT4 protein. Exercise raised GLUT4 expression in diabetic hearts; however, despite this upregulation, insulin stimulated glucose uptake was still only 50% of control values, indicating either defective translocation of the transporter or glucose uptake per se.

A high level of serine-473 phosphorylation of PKB/Akt is usually associated with activation of the kinase and should lead to enhanced glucose uptake. However, we found high basal phosphorylation levels associated with low glucose uptake in sedentary diabetic animals. To the best of our knowledge, this is the first demonstration of such dysregulation at the level of PKB/Akt in diabetes. Exercise normalised the phosphorylation pattern of PKB/Akt in diabetic hearts, an effect probably caused by improved insulin signalling to the applicable phosphatases. This would mean that improved PKB/Akt phosphorylation is not the mediator of improved insulin resistance but an effect thereof. Furthermore, despite normal phosphorylation of this kinase in cardiomyocytes from trained diabetic rats and normalisation of GLUT4 expression, insulin stimulated glucose uptake was still curtailed. This result is an important indication that a major alteration in the diabetic heart, leading to faulty glucose metabolism, must be in the functioning or translocation of the GLUT4 protein. These results suggest the possibility that this signalling pathway is a potential target of treatment for type 2 diabetes.

**IMAGES IN CARDIOLOGY**

Value of the unipolar electrogram in the diagnosis of right ventricular perforation following pacemaker implantation

A 76 year old patient with paroxysmal atrial fibrillation and recurrent syncope caused by sinus arrest was admitted for permanent pacemaker implantaion. This was uncomplicated with satisfactory pacing parameters (ventricular lead: threshold 0.7 V at 0.5 ms, R wave 18.1 mV). A routine pacing check the following day revealed complete loss of ventricular pacing and abdominal twitching during unipolar ventricular pacing. The sensed unipolar ventricular electrogram demonstrated a dominant, upright R wave (panel A) and a postero-anterior chest x ray showed the lead tip pointing superiorly, outside the right ventricular boundaries. An epicardial position was suspected and screening in the left anterior oblique projection during subsequent repositioning showed the lead overlying the apical left ventricular free wall (panel B). Repositioning was performed without further complication and a post-procedure unipolar electrogram was obtained (panel C).

Ventricular depolarisation normally occurs from endocardium to epicardium. Endocardial lead positions therefore produce a predominantly negative unipolar electrogram (panel C) and epicardial sites result in a predominantly positive electrogram (panel A). The endocardial electrogram at the repositioned site (panel C) shows a small initial R wave, which is normal for an apical endocardial right ventricular lead position. This is because during normal intrinsic conduction the earliest site of ventricular activation is the septal aspect of the left ventricular endocardium and septal activation then occurs left to right.

Perforation of the right ventricular free wall during pacemaker implantation is often clinically silent. Findings may include chest pain, a pericardial rub, and a right bundle branch block (RBBB) pacing pattern. If hypotension occurs cardiac tamponade must be excluded.

**REFERENCES**


