Exercice training attenuated the PKB and GSK-3 dephosphorylation in the myocardium of ZDF rats

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Exercise training attenuated the PKB and GSK-3 dephosphorylation in the myocardium of ZDF rats. In conclusion, exercise training may ameliorate the regulation of PKB/GSK-3 phosphorylation. These anomalies in the ZDF diabetic heart remain partially normalized with swimming exercise. These data support the premise that exercise training may protect the heart against the deleterious consequences of diabetes.

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TYPE 2 DIABETES IS A PREVALENT CAUSE of morbidity and mortality, mainly due to cardiovascular complications (14, 29, 53). The underlying events remain unclear, but a decrease of myocardial glucose oxidation represents an early pathophysiological event and occurs before both contractile and pathological changes (1). The serine (Ser)/threonine (Thr) PKB is an intracellular signaling enzyme activated after insulin binding to its cognate receptor and implicated in glucose homeostasis (28). The latter was confirmed in transgenic studies, as mice lacking PKB-β expression displayed many features of Type 2 diabetes observed in humans, including hyperglycemia, insulinemia, and hepatic insulin resistance (7). PKB is biologically active after the dual phosphorylation of the Thr308 residue in the kinase domain and the Ser473 residue in the hydrophobic C-terminal regulatory domain (2). The mechanisms behind this dual phosphorylation remain unclear. Phosphorylation can be induced by IGF-I or insulin, through activation of the phosphatidylinositol 3-kinase (PI3K) pathway (3). Phosphorylation of Thr308 can then be mediated by 3-phosphoinositide-dependent kinase-1. Regulation of the phosphorylation of the Ser473 residue is less understood. It could involve various signaling events, including the putative kinase phosphoinositide-dependent kinase-2, that could be modulated by exercise or by many molecules, such as ceramides or integrin-linked kinase (33, 45, 51). A putative physiological substrate of PKB is glycogen synthase kinase-3 (GSK-3), a ubiquitously expressed Ser/Thr protein kinase with two related isoforms, GSK-3α (Ser37) and GSK-3β (Ser39) (56). PKB-mediated phosphorylation of GSK-3 leads to inactivation of the enzyme, thereby maintaining glycogen synthase in a dephosphorylated active state, leading to glycogen synthesis (11). It has been documented that diabetic rat hearts accumulate glycogen and that high-glycogen content diminished the physiological action of insulin (5, 12, 38).

Although the mechanism(s) contributing to impaired myocardial glucose homeostasis in the setting of diabetes remains undefined, dysregulation of PKB and GSK-3 may represent a salient pathophysiological event. In this regard, the following study tested the hypothesis that phosphorylation of the PKB/GSK-3 pathway was impaired in the myocardium of the Zucker diabetic fatty (ZDF) rat, an insulin-resistant animal model that genetically manifests characteristics of Type 2 diabetes observed in humans (8) and significant alterations in oxidative and nonoxidative cardiac carbohydrate metabolism (4). Second, exercise training was shown to reduce the risk of heart disease and improve diabetic-mediated cardiovascular abnormalities (17, 36, 43, 44). Moreover, in insulin-resistant human subjects and in the obese Zucker nondiabetic rat, exercise normalized the action of insulin and enhanced glycogen synthesis (16, 46). Based on these observations, a second series of experiments was performed to test the hypothesis that swimming exercise can ameliorate the regulation of PKB/GSK-3 in the myocardium of ZDF rats.

MATERIALS AND METHODS

All protocols were approved by the Animal Care Committee of University of Quebec in Trois-Rivières and followed the Principles of Laboratory Animal Care (NIH publication 85-23, 1985). Nine-week-old obese male ZDF (ZDF/Gmi fa/fa) and weight-matched nondia-

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Table 1. Morphological data

<table>
<thead>
<tr>
<th></th>
<th>WT Sed</th>
<th>ZDF Sed</th>
<th>WT Exe</th>
<th>ZDF Exe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>427±0.04</td>
<td>438±18</td>
<td>366±5.9</td>
<td>434±10</td>
</tr>
<tr>
<td>HW, g</td>
<td>1.33±0.04</td>
<td>1.29±0.04</td>
<td>1.31±0.03</td>
<td>1.32±0.04</td>
</tr>
<tr>
<td>HW/BW, mg/g</td>
<td>3.1±0.06</td>
<td>2.96±0.06</td>
<td>3.57±0.06</td>
<td>3.05±0.08</td>
</tr>
</tbody>
</table>

Values are averages±SEM. WT, wild type; Sed, sedentary; ZDF, Zucker diabetic fatty; Exe, exercise; HW, heart weight; BW, body weight; HW/BW, HW-to-BW ratio. *Significantly different from all groups, P < 0.01.

radish peroxidase) for 1–2 h at room temperature, and the bands were subsequently detected by autoradiography utilizing the enhanced chemiluminescence detection kit (Amersham). Films were quantified by using a flatbed scanner and Scion image (Scion, Frederick, MD).

Glycogen content and phosphofructokinase activity. Glycogen concentration in the left ventricle was determined spectrophotometrically by using sulfuric acids, as described by Lo et al. (30). Cardiac homogenates for phosphofructokinase (PFK) activity were prepared in 100 mM potassium phosphate buffer, pH 8.2, containing 10 mM glutathione, 0.5 mM ATP, 5 mM MgCl₂, and 30 mM NaF. PFK activity was measured spectrophotometrically at 30°C by using a coupling system, as described by Mansour et al. (34).

Statistics. Data are expressed as units or percent change ± SEM. Sed ZDF were compared with Sed WT, whereas the effect of swimming in either the WT or ZDF rat was calculated as fold increase vs. its appropriate Sed control. Data were analyzed by a two-way ANOVA followed by Newman Keuls post hoc test. P < 0.05 was considered statistically significant.

RESULTS

Morphological data, plasma glucose, insulin levels, and glycogen content of the Sed ZDF rat. Body weight (BW), heart weight (HW), and HW-to-BW ratio (HW/BW) were similar between WT and ZDF Sed groups (Table 1). ZDF rats were hyperglycemic at 12 wk of age, and plasma glucose levels progressively rose, whereas they remained normal in the WT Sed rats throughout the study (Table 2 and Fig. 1). At the time of sampling, plasma insulin levels were similar in the ZDF rat and WT Sed rats (Table 2). Left ventricular glycogen content was significantly elevated by 63% in the left ventricle of the Sed ZDF rats, compared with the WT rat (Table 2).

Effect of exercise on morphological data, glycemia, insulin levels, and glycogen content in the WT and ZDF rat. Exe WT animals showed a significantly lower BW, causing an increase in HW/BW (Table 1). By contrast, swimming had no effect on BW and HW/BW in the ZDF rat (Table 1). Glycogen content was lower in both the WT (~3%) and the ZDF Exe (~20%) rats (Table 2). Plasma glucose levels in the ZDF rat were significantly lower in the Exe group, by 12 ± 2% (Table 2 and Fig. 2). There was a tendency for a decrease in plasma insulin levels in the Exe WT rat compared with Sed WT, but this did not reach statistical significance (Table 2). By contrast, exercise increased plasma insulin levels in the ZDF rat by 70 ± 28% (Table 2).

Regulation of PKB and GSK phosphorylation in the left ventricle of the Sed ZDF rat. In the left ventricle of Sed ZDF rats, a decreased phosphorylation state of PKB Thr308 (~67%...
By contrast, the phosphorylation state of GSK-3/β (P<0.001) and PKB Ser\(^{473}\) (−63%; \(P<0.0001\)) residues was observed, compared with WT rats (Fig. 2). However, total PKB protein content was similar in ZDF and WT rats (Fig. 2). GSK-3α Ser\(^{21}\) phosphorylation was significantly reduced (−64%; \(P<0.01\)) in the ZDF rat, compared with WT (Fig. 3). By contrast, the phosphorylation state of GSK-3α Ser\(^{473}\) was only modestly reduced in the ZDF rat. However, total GSK-3 protein content was similar in ZDF and WT rats (Fig. 3).

Effect of exercise on regulation of PKB and GSK-3 phosphorylation in the left ventricle of WT and ZDF rats. In the WT rat, exercise was associated with a disparate pattern of PKB regulation, as PKB Thr\(^{308}\) phosphorylation was significantly decreased (−41%; \(P<0.01\)), whereas PKB Ser\(^{473}\) phosphorylation was unaffected, compared with Sed WT rats (Fig. 2). Interestingly, GSK-3α Ser\(^{21}\) phosphorylation was unaffected in the WT rats after swimming, whereas GSK-3β Ser\(^{473}\) phosphorylation was significantly enhanced (68%; \(P<0.05\)), compared with Sed WT (Fig. 3). In the ZDF rats, the decreased PKB Ser\(^{473}\) phosphorylation of the Sed animals was partially reversed after 13 wk of swimming (Fig. 2). Exercise training reduced glycogen content in both Exe groups, and a significant negative correlation was observed between glycogen content and PKB Ser\(^{473}\) in all groups (\(r = −0.65; \ P<0.05\)). Interestingly, PKB Thr\(^{308}\) phosphorylation of the Exe ZDF rat was also significantly increased, compared with Sed ZDF, and the level of phosphorylation was equivalent to that observed in the Exe WT rat (Fig. 2). The significant reduction in GSK-3α Ser\(^{21}\) phosphorylation in Exe ZDF was partially normalized. The modest reduction of GSK-3β Ser\(^{473}\) phosphorylation in the Sed ZDF rat was normalized by exercise (Fig. 3).

Regulation of PKB in the skeletal muscle of the Sed ZDF rat. In the rectus femoris of Sed and Exe ZDF rats, the phosphorylation state of PKB Ser\(^{473}\) increased by 111 and 136%, respectively, compared with that in WT rats (Fig. 4). Interestingly, the latter increase was associated with a 54% mean decrease in PKB total protein expression. PKB protein expression and phosphorylation were unaffected in the rectus femoris after swimming.

Regulation of HSP72 and PFK in Sed ZDF. HSP72 protein expression, as well as PFK activity (Table 2), were not different in the left ventricle of Sed ZDF and WT.

**DISCUSSION**

Abnormal myocardial glucose homeostasis represents an early pathophysiological event in diabetes and occurs before both contractile and pathological changes (1). The insulin-resistant ZDF rat manifests numerous significant alterations in oxidative and nonoxidative cardiac carbohydrate metabolism (4). The early progression of the disease in the ZDF rat is associated with elevated plasma insulin levels. During the latter stages of the disease, pancreatic beta cells do not respond to elevated plasma glucose, leading to a reduction of insulin secretion and subsequent plasma levels (9). In the present study, elevated plasma glucose in the ZDF rat was accompanied by a modest elevation of plasma insulin levels, compared with WT, thereby supporting the premise that pancreatic beta cell production of insulin was impaired at the time of death.

PKB activation by insulin via a PI3K-dependent pathway requires the dual phosphorylation of the Thr\(^{308}\) and Ser\(^{473}\) residues, which are critical to achieve a high level of PKB activity (2). The role of PKB in glucose homeostasis includes the inactivation of GSK-3 (11). The phosphorylation level of both residues of PKB in the left ventricle of Sed ZDF rats was...
significantly decreased, without a change in total PKB protein content. Consistent with these data, reduced PKB activity and/or phosphorylation was observed in the myocardium and skeletal muscle of various other diabetic rat models and patients with Type 2 diabetes (16, 19, 22, 23, 27, 50). It should, however, be mentioned that other studies have shown no reduced PKB activity or phosphorylation in muscles of patients with Type 2 diabetes (21). Interestingly, we observed an increase in PKB Ser473 phosphorylation in the rectus femoris muscles of the ZDF animals, accompanied by a decrease in total PKB protein. It could be speculated that these observations are interrelated, representing a compensatory mechanism for the decreased protein expression.

In the present study, these changes occurred, despite a similar plasma insulin concentration in the ZDF and control animals, suggesting an alteration in insulin reactivity or in the signaling pathway before PKB. Thus compromised PKB regulation in the myocardium and skeletal muscle of the ZDF rat may, in part, contribute to abnormal glucose homeostasis.

GSK-3 is a Ser/Thr kinase consisting of two isoforms (GSK-3α and GSK-3β) and phosphorylated after exposure to insulin (11). Phosphorylation of GSK-3 is facilitated by PKB, resulting in inactivation of the enzyme and a subsequent increase in glycogen synthesis via increased activity of the enzyme glycogen synthase (11). In the streptozotocin diabetic rat heart, insulin stimulation of endogenous GSK-3 phosphorylation via PKB is impaired (27). Consistent with the decreased PKB phosphorylation reported in the present study, GSK-3α/β phosphorylation was reduced in the myocardium of the ZDF rats, compared with WT rats, thereby suggesting increased enzymatic activity. This latter finding would appear to be inconsistent with the elevated glycogen content in the left ventricle of the Sed ZDF rat. Indeed, increased GSK-3 activity would promote glycogen synthase phosphorylation, thereby decreasing enzyme activity and subsequent glycogen synthesis (11). It is well known that diabetes provokes an increase in heart glycogen content, despite a decline in the amount of active glycogen synthase present (26). High cardiac concentrations of glycogen alone can result in inactivation of glycogen synthase (48). In the myocyte, an inverse relationship has been shown between glycogen concentration and the percentage of glycogen synthase in the active form (18). It is possible that high-glycogen content may act as a regulator of GSK-3, to
limit further accumulation. We observed a significant negative correlation between glycogen and both GSK-3 residue phosphorylation \((r = -0.57; \ P < 0.05)\).

In the WT rat, a 13-wk swimming regimen significantly decreased BW, plasma glucose levels remained normal, and a modest decrease in plasma insulin concentration was observed. By contrast, BW remained unchanged, plasma glucose levels were significantly decreased, and plasma insulin concentration increased in the Exe ZDF rat, compared with Sed ZDF. These data indicate that swimming improved plasma glucose levels in the ZDF rat. The increased concentration of plasma insulin is consistent with previous reports highlighting a similar observation with physical training in human and rat models of Type 2 diabetes (24, 52). Whether the increase in plasma insulin concentration was associated with the improved PKB/GSK-3 phosphorylation in the myocardium of the Exe ZDF rat remains undefined.

In skeletal muscle, it has previously been shown that decreased PKB Ser\(^{473}\) phosphorylation was partially restored after chronic exercise combined with troglitazone, an insulin sensitizer that activates the peroxisome proliferator receptor-\(\gamma\), in the nondiabetic Zucker rat (16). Similarly, Luciano et al. (33) observed that 6 wk of swimming training increased PKB Ser\(^{473}\) phosphorylation and GLUT-4 expression in rat skeletal muscles after insulin infusion. Based on these observations, the present study examined whether a swimming regimen for the ZDF rat would improve the status of PKB phosphorylation in the heart. Indeed, exercise significantly increased PKB Ser\(^{473}\) and Thr\(^{308}\) phosphorylation in the ZDF rat. This novel finding was associated with increased insulinemia, decreased glycemia, and glycogen content. One possible explanation for the improved phosphorylation level of PKB in the myocardium of the ZDF rat might be a mechanism related to glycemia. Chronic hyperglycemia reduces the efficiency of the activation step from PI3K to PKB (41), and normalized glycemia has been shown to bring the phosphorylation and activity of PKB to a normal level (42), a finding similar to the effect of exercise in this study. In the WT rats, swimming did not alter PKB Ser\(^{473}\) phosphorylation but unexpectedly reduced Thr\(^{308}\) phosphorylation. A disparate pattern of PKB residue phosphorylation has been previously observed in ceramide-treated TF-1 cells, as Thr\(^{308}\) phosphorylation was decreased, whereas the phosphorylation state of Ser\(^{473}\) remained unchanged (51). The PKB activity in the Exe WT rat may thus be diminished, but probably not to the same extent as in the Sed ZDF rat, where the phosphorylation level of both residues of PKB was markedly reduced. Exercise training might have contributed to maintain glucose homeostasis, despite reduced PKB Thr\(^{308}\), by a mechanism involving increased contractile activity. Contractile activity increases plasma membrane glucose transporters even in the absence of insulin (13). Hence, PKB activity via the usual insulin-signaling pathway (PI3K) might not be fully required for glucose homeostasis. Markuns et al. (35) found that insulin and exercise decrease GSK-3 activity by different mechanisms and that deactivation of GSK-3 was induced by a PKB-independent mechanism in rat skeletal muscle. Consistent with the improved phosphorylation state of PKB in the ZDF rat after exercise, the decreased phosphorylation of GSK-3\(\alpha\) Ser\(^{34}\) and GSK-3\(\beta\) Ser\(^{9}\) residues was partially reversed with swimming. Thus, swimming training in the diabetic ZDF rat ameliorated the phosphorylation state of the PKB/GSK-3 pathway, which may, in part, improve glucose homeostasis in the myocardium.

PKF is an enzyme associated with the rate of glycolytic flux, which can be upregulated with exercise training (39). We investigated PKF activity to verify the effect of the training protocol. PKF activity in the myocardium of WT and ZDF Sed rats was similar. However, its activity increased only in the Exe WT rats. The absence of increased PKF activity in the Exe ZDF rat may, in part, be related to a variety of factors, such as the presence of Type 2 diabetes or a different body composition, which could have influenced exercise intensity. We have also measured HSP72 to evaluate the effect of training in the heart. Although it is not a classic training index, it has been reported that HSP72 increased in the heart with exercise training (31, 40), HSP72 was increased in ZDF as well as in WT animals. Exercise training may thus have protected the myocardium of both Exe groups by means of enhanced HSP72 expression. To our knowledge, it is not clear whether HSP72 expression is modified in the diabetic heart. However, studies have shown that HSP mRNA content is reduced in muscle from Type 2 diabetic patients and correlates with insulin resistance (25). Enhanced HSP72 expression could improve recovery of myocardial mechanic (47) after ischemia (32), reduce infarct size (20), and decrease myocardial apoptosis (37, 54).

As mentioned previously, hearts of diabetic rats accumulate glycogen (5, 6). In this study, heart glycogen content was much elevated in Sed ZDF compared with control rats, despite similar insulinemia. Exercise training of moderate intensity was shown to reduce glycogen synthesis in fed streptozotocin-diabetic rats, recovering from prolonged exercise (15). In the present study, exercise training reduced heart glycogen content in Exe ZDF. This was probably not due to the effect of the last bout of exercise, because measurements were done 48 h after the last training period. It has been shown (10) that glycogen content in hearts from control animals is reduced immediately after exercise, but unchanged 24 h after the cessation of work compared with the preexercise value.

Furthermore, the latter group found in the diabetic rat heart that glycogen content, which was initially twice the normal group, was not significantly altered after exercise. High blood glucose level per se is known to increase glucose uptake in peripheral tissues by a mass action effect (55), leading to enhanced glycogen content. It has been suggested that exercise-induced depletion of the muscle glycogen stores improved insulin responsiveness secondarily (49). Moreover, it has been demonstrated that insulin signaling, including PKB activation, was, in part, negatively regulated by muscle glycogen content (12, 38). Thus it is possible that the elevated content of glycogen in the myocardium of ZDF rats may have partially suppressed insulin-dependent activation of the PKB/GSK-3 pathway.

In conclusion, the present study demonstrated a decreased phosphorylation of PKB and GSK-3 in the myocardium of the ZDF rat. The dysregulation of PKB/GSK-3 could contribute to the reported abnormal glucose homeostasis in the myocardium of Type 2 diabetic rats. Training improved phosphorylation of both PKB residues and partially normalized GSK-3 phosphorylation in the ZDF rat heart. In addition, exercise training significantly reduced glycemia and heart glycogen content with a concomitant increase in plasma insulin levels. It is tempting
to suggest that the reduction of cardiac glycogen content in the Exe ZDF rat may have, at least in part, contributed to the improved phosphorylation status of PKB.

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