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

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Lajoie, Claude, Angelino Calderone, François Trudeau, Nathalie Lavoie, Guy Massicotte, Sylvain Gagnon, and Louise Béliveau. Exercise training attenuated the PKB and GSK-3 dephosphorylation in the myocardium of ZDF rats. J Appl Physiol 96: 1606–1612, 2004.—Cardiac dysfunction is a severe secondary effect of Type 2 diabetes. Recruitment of the protein kinase B/glycogen synthase kinase-3 pathway represents an integral event in glucose homeostasis, albeit its regulation in the diabetic heart remains undefined. Thus the following study tested the hypothesis that the regulation of protein kinase B/glycogen synthase kinase-3 was altered in the myocardium of the Zucker diabetic fatty rat. Second, exercise has been shown to improve glucose homeostasis, and, in this regard, the effect of swimming training on the regulation of protein kinase B/glycogen synthase kinase-3 in the diabetic heart rat was examined. In the sedentary Zucker diabetic fatty rats, glucose levels were elevated, and cardiac glycogen content increased, compared with wild type. A 13-wk swimming regimen significantly reduced plasma glucose levels and cardiac glycogen content and partially normalized protein kinase B-serine473, protein kinase B-threonine 308, and glycogen synthase kinase-3α phosphorylation in Zucker diabetic fatty rats. In conclusion, hyperglycemia and increased cardiac glycogen content in the Zucker diabetic fatty rats were associated with dysregulation of protein kinase B/glycogen synthase kinase-3α phosphorylation. These anomalies in the Zucker diabetic fatty rat were partially normalized with swimming. These data support the premise that exercise training may protect the heart against the deleterious consequences of diabetes.

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, insulinopenia, 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 kinase with two related isoforms, GSK-3α (Ser21) and GSK-3β (Ser27) (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 fafa) and weight-matched nondia-

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The left ventricle of Sed ZDF rats was significantly hypertrophied (Table 2) and had a larger glycogen content (Table 2) than the ZDF Exe group (Table 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 Thr389 (−67%; Table 2).

**Table 2.** Insulinemia, glycemia, cardiac glycogen concentration, and PFK activity

<table>
<thead>
<tr>
<th></th>
<th>WT Sed (±SE)</th>
<th>ZDF Sed (±SE)</th>
<th>WT Exe (±SE)</th>
<th>ZDF Exe (±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma glucose, mM</td>
<td>8.5 ± 0.3</td>
<td>32.2 ± 0.8*</td>
<td>7.9 ± 0.4</td>
<td>28.4 ± 0.9**</td>
</tr>
<tr>
<td>Plasma insulin, pmol/l</td>
<td>229 ± 49</td>
<td>277 ± 71</td>
<td>105 ± 16</td>
<td>472 ± 73†</td>
</tr>
<tr>
<td>Cardiac PFK, μmol/g·min⁻¹</td>
<td>13.5 ± 0.6</td>
<td>14.8 ± 0.3</td>
<td>18.9 ± 0.4†</td>
<td>13.6 ± 0.9</td>
</tr>
<tr>
<td>Cardiac glycogen, μg/mg wet wt of LV</td>
<td>1.8 ± 0.01</td>
<td>3.0 ± 0.01‡</td>
<td>1.2 ± 0.01‡</td>
<td>2.4 ± 0.02‡</td>
</tr>
</tbody>
</table>

Values are averages ± SE. PFK, phosphofructokinase; LV, left ventricle.
*Significantly different from WT Sed and WT Exe, P < 0.001. †Significantly different from ZDF Sed, P < 0.05. ‡Significantly different from all groups, P < 0.01.
By contrast, the phosphorylation state of GSK-3β/H11002 (64%; estingly, PKB Thr 308 phosphorylation of the Exe ZDF rat was after swimming.
sion and phosphorylation were unaffected in the rectus femoris
decrease in PKB total protein expression. PKB protein expres-
sion, as well as PFK activity (Table 2), were not differ-
antly in the left ventricle of Sed ZDF and WT.

Interestingly, GSK-3β/H9251 phosphorylation was unaffected, compared with Sed WT rats (Fig. 2). The signi-
sifi cant reduction in GSK-3β/H9251 phosphorylation in the ZDF rat was normalized by exercise (Fig. 3).
By contrast, the latter increase was associated with a 54% mean
crease in PKB total protein expression. PKB protein expres-
protein content was similar in ZDF and WT rats (Fig. 2).

Interestingly, the latter increase was associated with a 54% mean
crease in PKB total protein expression. PKB protein expres-
polymerase was only modestly reduced in the ZDF rat. However, total GSK-3β/H9252 phosphorylation in the left ventricle of WT and ZDF rats.

In the WT rats after swimming, whereas GSK-3β/H9251 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 Ser21 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 phosphor-
ylation 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 Thr308 phosphorylation was significantly
decreased (−41%; P < 0.01), whereas PKB Ser473 phosphor-
ylation was unaffected, compared with Sed WT rats (Fig. 2).
Interestingly, GSK-3α Ser21 phosphorylation was unaffected in
the WT rats after swimming, whereas GSK-3β Ser3 phosphory-
ylation was significantly enhanced (68%; P < 0.05), compared
with Sed WT (Fig. 3). In the ZDF rats, the decreased PKB Ser473 phosphor-
ylation of the Sed animals was partially re-
served 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 Ser473 in all groups (r = −0.65; P < 0.05). Inter-
estingly, PKB Thr308 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 Ser21
phosphorylation in Exe ZDF was partially normalized. The
modest reduction of GSK-3β Ser3 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 phosphor-
ylation state of PKB Ser473 increased by 111 and 136%,
respectively, compared with that in WT rats (Fig. 4). Interest-
ingly, the latter increase was associated with a 54% mean
decrease in PKB total protein expression. PKB protein expres-
sion 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 differ-
ent 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 accompa-
nied 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 Thr308 and Ser473
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 was

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Fig. 1. Effect of exercise on glucose concentration over weeks. Values are averages ± SE. WT, wild type; ZDF, Zucker diabetic fatty; Sed, sedentary; Exe, exercise. *P < 0.05, ZDF Sed vs. ZDF Exe.

**Fig. 2.** Effect of diabetes and exercise on PKB phosphorylation in WT and ZDF rats. A: Western blots of PKB protein expression and PKB Thr308 and Ser473 phosphorylation site in WT and ZDF with or without exercise. B: percent change from control levels for each blot. Values are averages ± SE; n = 5–7/group. *P < 0.05 vs. WT Sed. †P < 0.05 vs. ZDF Sed. ‡P < 0.001 vs. WT Exe.
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-3α Ser$^{21}$ and GSK-3β Ser$^{9}$ residues was partially reversed with swimming. Thus swimming training in the diabetic ZDF rat ameliorated the phosphorylation of the PKB/GSK-3 pathway, which may, in part, improve glucose homeostasis in the myocardium.

PFK is an enzyme associated with the rate of glycolytic flux, which can be upregulated with exercise training (39). We investigated PFK activity to verify the effect of the training protocol. PFK 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 PFK 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 myoccardial 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 glycolysis 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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