Aerobic training prior to myocardial infarction increases cardiac GLUT4 and partially preserves heart function in spontaneously hypertensive rats

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Keyword:
- acute myocardial infarction, exercise training < exercise, GLUT4, cardiac function, SHR
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Aerobic training, myocardial infarction and cardiac GLUT4

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Abstract

We assessed cardiac function (echocardiographic) and GLUT4 expression (Western Blot) in response to a 10-week aerobic training (treadmill) prior to acute myocardial infarction by ligation of the left coronary artery (AMI) in spontaneously hypertensive rats. Animals were allocated to sedentary+sham, sedentary+AMI, training+sham and training+AMI. Aerobic training prior to AMI partially preserves heart function. AMI and/or aerobic training increased GLUT4 expression. However, those animals trained prior to AMI showed a greater increase in GLUT4 in cardiomyocytes.

Keywords: acute myocardial infarction, exercise training, GLUT4, SHR.
Introduction

Cardiovascular diseases are associated to risk factors including systemic arterial hypertension (Ishitani et al. 2006). About 50% of deaths caused by stroke and 40% by cardiac ischemic disease, mainly acute myocardial infarction (AMI), result from hypertension (Alpert et al. 2000).

Tucci (2011) investigated pathophysiological characteristics of post-AMI in rats and showed a decrease in contractile and functionality parameters with subsequent heart failure, which have been found in our laboratory (Lehnen et al. 2014). It is of note a study by Liepinsh et al. (2014) that demonstrated cardiac remodeling and functionality was improved in a model of ischemia in fed rats (Liepinsh et al. 2014). These findings indicate that postprandial or fed-state physiology, which is characterized by insulin-activated glucose, and glucose transporter 4 (GLUT4) expression is protective against myocardial infarction.

Huang et al. (2009) showed in diabetic rats of ischemia/reperfusion that animals exhibited high mortality rate (65% vs. 33%) and worse hemodynamic outcome than the insulin-treated group (Huang et al. 2009). Cardiac contractile dysfunction in these animals was caused by a defect in insulin-stimulated p-Akt/GLUT4 axis activation and protein expression. Insulin therapy partially improved diastolic function and increased p-Akt and GLUT4 protein levels. In addition to the improvement in insulin signaling, other mechanisms are associated with increased GLUT4 in the plasma membrane: increased gene expression (Slc2A4) and adjustments in the GLUT4 cycling (increased exocytosis and/or lower endocytosis) (Wijesekara et al. 2006). Thus, therapeutic approaches such as exercise training that can increase insulin-stimulated glucose transport in cardiac tissue may improve cardiac function and reduce cardiac workload.
Evidence has shown that exercise favorably affects contractility and cardiac function after AMI (Yengo et al. 2012). Furthermore, as discussed above, increased GLUT4 expression in cardiomyocytes may be an adjuvant therapy post-AMI. In a previous study from our group, we demonstrated that exercise training was able to increase cardiac expression of GLUT4 in spontaneously hypertensive rats (SHR) (Lehnen et al. 2010). However, these benefits have been widely investigated post-AMI, and little is known about exercise training prior to AMI. Moreover, the few results available are controversial (Dayan et al. 2005; Veiga et al. 2011). Thus, our study aimed to assess the effects of exercise training performed prior to myocardial infarction on cardiac function and cardiac GLUT4 expression in SHR.

Material and methods

Our study complied with the ethical principles of the Use of Laboratory Animals and Canadian Council on Animal Care and approved by the Research Ethics Committee at Instituto de Cardiologia do RS, Brazil (protocol #4582/10). All animals provided a standard rat chow and water ad libitum, and maintained in a controlled 12Dh light/12Dh dark cycle (6 am/6 pm) under 20–25°C conditions.

Thirty-two SHR (3 months) were assigned to (n=8/group): S-Sham (sedentary+sham), S-AMI (sedentary+AMI), T-Sham (training+sham) and T-AMI (training+AMI).

Maximum capacity for physical effort was measured by the maximal exercise test (ET), as a standard procedure in our laboratory (Lehnen et al. 2010). Later, exercise training was prescribed at low-moderate intensity (~50–70% of ET), 1 h/day (7–8 am) and 5 days/week for 10 weeks.

AMI was induced 96 hours after the end of the training period by permanent occlusion of the anterior left descending coronary artery. Briefly, the rats were
anesthetized (1 mL/kg of ketamine and 0.5 mL/kg of xylazine), intubated and connected to a mechanical ventilator (Harvard Apparatus, Massachusetts, USA) with ambient air at a volume of 2.5 mL and ventilatory rate of 65 breaths/minute. A thoracotomy was performed on the left side of the thoracic cavity at the level of the third intercostal space. The left anterior descending coronary artery was permanently occluded with a single 6-0 nylon suture (Prolene, Johnson&Johnson, São José dos Campos, Brazil). The thoracic cavity was then sutured and intrathoracic pressure was restored. Other rats were submitted to a similar surgical procedure but without ligation of the coronary artery (sham-operated).

Echocardiographic analyses of left ventricle ejection fraction (LVEF), left ventricle shortening fraction (LVSF), and fractional area change (FAC) were conducted 48 hours after AMI, in addition to the measurement of infarct size. These analyses were performed using an EnVisor (Philips, Andover, USA) echocardiograph with a 12 MHz transducer. All measures and calculations of heart function parameters followed the procedures described in the literature (Peron et al. 2006).

Cardiac tissue was prepared by homogenization as performed in our laboratory (Lehnen et al. 2011). The final homogenate corresponds to the plasma membrane fraction or microsomal fraction. Protein concentration was determined using the Bradford method. GLUT4 expression was analyzed by Western blot with 50 µg/sample. An anti-GLUT4 antibody (Millipore, Billerica, USA) titrated to 1:2,000 overnight at 4°C followed by an additional 3-h incubation at 37°C was used. After, the membrane was incubated with secondary antibody (1:12,000 goat anti-rabbit IgG; Millipore, Billerica, USA). Blot intensity was quantified using Scion Image software. The results were standardized by densitometric analysis of the respective sample stained with Ponceau red (Klein et al. 1995), and expressed as arbitrary units (AU/µg protein).
Descriptive data (mean±SD) was used to characterize the animals studied. A generalized estimating equation (GEE) followed by Bonferroni’s post-hoc was used to examine the effect of exercise training on AMI according to two factors (sedentary/exercise training, sham/AMI, and the interaction between them). A Student’s t-test for independent samples was performed to analyze data on myocardial infarction size (p<0.05 for all tests).

Results

Table 1 presents the characteristics of the animals and echocardiographic assessment results. There was no change in body weight, and heart weight among the groups studied. The training protocol effectively improved exercise capacity (p=0.028) showing greater maximum running speed (ET) (S-Sham vs. T-Sham: Δ56.2%, p<0.001; S-AMI vs. T-AMI: Δ86.6%, p<0.001). The akinetic myocardial area corresponding to the infarct size in relation to the left ventricular circumference was similar between the groups (S-AMI vs. T-AMI, p=0.368), which was key to the analysis of echocardiographic findings and GLUT4 expression presented below.

LVSF was reduced after myocardial infarction in both S-AMI and T-AMI (S-Sham vs. S-AMI: Δ 49.9%, p<0.001). Exercise training increased LVSF by 54.7% in T-Sham compared to S-Sham (p<0.001). Interestingly, this effect was not observed in T-AMI (Table 1). Similarly, LVEF was reduced by ~31% in S-AMI compared to the control group (p<0.001). Furthermore, exercise training increased LVEF by ~54% in T-Sham compared to S-Sham (p<0.001), but it had no effect in T-AMI (Table 1). Lastly, FAC was reduced in both groups in response to AMI (S-Sham vs. S-AMI: Δ 52.7%, p<0.001). Exercise training increased FAC in T-AMI when compared to S-AMI (Δ15.8%, p=0.032).
Figure 1 shows microsomal and plasma membrane fractions of cardiac GLUT4. AMI increased GLUT4 expression in both microsomal and plasma membrane of untrained animals (S-Sham vs. S-AMI, 42.7% and 56.7%, respectively). However, this effect was not observed in T-AMI when compared to T-Sham (microsomal p=0.205; plasma membrane p=0.220). In addition, exercise training increased GLUT4 expression in T-Sham compared to S-Sham in both fractions (microsomal 64.5% and plasma membrane 87.1%).

Discussion

This study showed the partially benefits of aerobic training prior to AMI on cardiac parameters, which are largely decreased in response to ischemia process. Furthermore, AMI and aerobic training increased GLUT4 expression. However, those animals trained prior to AMI showed a greater increase in GLUT4 in cardiac tissue (microsomal and plasma membrane).

In our study, the induction of an experimental AMI led to decreased cardiac contractility evidenced through LVSF, LVEF, and FAC. LVSF was lower in the AMI groups. Dos Santos et al. (2010) found a reduction in LVSF after coronary occlusion, which was also reported by Tucci (2011) and Veiga et al. (2011). Besides, AMI effects were all together similar in both groups (S-AMI and T-AMI) showing consistency of the procedure.

Aerobic training significantly improved LVSF and LVEF in sham animals as expected. These parameters are lower in hypertensive sedentary animals when compared to trained animals without AMI (S-Sham vs. T-Sham). However, when we assessed FAC, it was already high in sedentary animals (S-Sham, 75.5%). Thus, a 10-week aerobic training did not promote an increase in FAC above normal. In AMI animals (S-AMI and T-AMI), we found significant reductions in all echocardiographic
parameters as demonstrated before (de Castro et al. 2014). In our study, it seems that preventive aerobic training prevented major losses only in FAC to a significant degree. The effect of preventive physical training on infarct size and cardiac function has been reported in the literature, though it is still controversial. Grans et al. (2014) reported that a 12-week physical training did not lead to any significant changes in ventricular function. Yet, another study did find reduced infarct size and improved cardiac function during remodeling after a 7-week aerobic training prior to AMI (Freimann et al. 2005). This finding may be explained by different exercise modalities used. Grans et al. developed a resistance training in their study while Freimann et al. an aerobic training. Our protocol is closer to that developed by Freimann et al. and this may reasonably explain why our results are consistent with theirs. In another study, female rats underwent swimming training followed by AMI induction or sham procedures and then remained sedentary for 1 week for study assessments (Veiga et al. 2011). The authors concluded that prior exercise training did not improve contractile and functional parameters including LVSF, LVEF and FAC. This finding could be attributed to gender difference. We experimented with male rats whereas Veiga et al. used female rats. It is known there are gender differences regarding development and progression of AMI (Ostadal et al. 2009).

An important adaptation of exercise training is an increase in glucose uptake in insulin-sensitive tissues (Reichkendler et al. 2013) in response to increased GLUT4 expression (Lehnen et al. 2010). Regarding the energy source for cardiac muscle contractility, under steady-state conditions (aerobic perfusion and normal workload), the heart produces energy mainly by oxidizing non-esterified fatty acids and energy from glucose is no more than 40% (Taegtmeyer et al. 1980). In ischemic conditions such as AMI, changes in metabolic pathways lead to reduced oxidation of non-esterified fatty
acids (Dyck et al. 2004). Hence, an increase in GLUT4 expression and glucose uptake by cardiomyocytes is a viable alternative. This has been observed up to 24 hours after AMI (Nishino et al. 2004) as seen in our study (S-Sham vs. S-AMI), as well as within 48 h post-AMI (Lehnen et al. 2014).

Our results showed that exercise training increased GLUT4 expression, enabling thus increased glucose uptake and producing a cardioprotective effect by changing the energy source in response to AMI (Dyck et al. 2004). Still, GLUT4 expression was increased both microsomal (intracellularly) and in plasma membrane (cell signaling mechanisms) in response to a 10-week physical training (S-AMI vs. T-AMI). Numerous changes support this benefit including greater activation of the AMPK and CaMKII (Witczak et al. 2007); TBC1D1, TBC1D4/AS160 and p38 MAPK remain phosphorylated for hours after exercise; greater phosphorylation of IRS1 and IRS2 substrates (Luciano et al. 2002); increased transcription factors (MEF2 and GEF) involved in GLUT4 gene expression (Lima et al. 2009); and, adjustments in the GLUT4 cycling (increased exocytosis and/or lower endocytosis) (Wijesekara et al. 2006).

In conclusion, physical training prior to an AMI leads to improved cardiac parameters that are dramatically reduced after AIM. In addition, increased GLUT4 in the microsomal and plasma membrane fractions in response to physical training can increase glucose uptake by cardiomyocytes, producing a cardioprotective effect in the post-AMI energy environment.

Conflict of Interest Disclaimer

The authors report no conflicts of interest associated with this manuscript.
References


Table 1 – Characteristics and echocardiographic assessment results

<table>
<thead>
<tr>
<th></th>
<th>S-Sham</th>
<th>S-AMI</th>
<th>T-Sham</th>
<th>T-AMI</th>
<th>p-value (interaction)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>198 ± 5</td>
<td>205 ± 6</td>
<td>191 ± 13</td>
<td>199 ±17</td>
<td>0.717</td>
</tr>
<tr>
<td>Heart weight (g)</td>
<td>0.96 ± 0.48</td>
<td>0.99 ± 0.09</td>
<td>0.91 ± 0.04</td>
<td>1.02 ± 0.10</td>
<td>0.649</td>
</tr>
<tr>
<td>ET (km.h⁻¹)</td>
<td>1.6 ± 0.3</td>
<td>1.5 ± 0.2</td>
<td>2.5 ± 0.2*†</td>
<td>2.8 ± 0.3*†</td>
<td>0.028</td>
</tr>
<tr>
<td>Infarct size</td>
<td>----</td>
<td>41.4 ± 1.5</td>
<td>----</td>
<td>42.8 ± 6.0</td>
<td>0.368</td>
</tr>
<tr>
<td>LVSF (%)</td>
<td>50.1 ± 1.6</td>
<td>25.1 ± 6.2*</td>
<td>77.5 ± 6.4*†</td>
<td>28.2 ± 2.4*‡</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>56.4 ± 9.0</td>
<td>38.9 ± 5.9*</td>
<td>87.0 ± 3.0*†</td>
<td>39.6 ± 2.7*‡</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FAC (%)</td>
<td>75.5 ± 18.2</td>
<td>35.9 ± 13.8*</td>
<td>80.8 ± 14.4†</td>
<td>41.2 ± 19.2*‡</td>
<td>0.040</td>
</tr>
</tbody>
</table>

S: sedentary+sham; S-AMI: sedentary+AMI; T-Sham: training+sham; T-AMI: training+AMI. ET: maximal running speed during the maximal exercise test. LVSF: left ventricular shortening fraction; LVEF: left ventricular ejection fraction; FAC: fractional area change. A generalized estimating equation (GEE) was used according to two factors [sedentary/exercise training, sham/AMI, and the interaction between them – p-value (interaction)], followed by Bonferroni’s post-hoc. An independent samples t-test was performed only to infarct size. * vs S-Sham, † vs S-AMI and ‡ vs T-Sham.
Figure 1 – Changes in cardiac GLUT4 expression in response to aerobic training prior to myocardial infarction. Quantitative Western blot analysis and corresponding bands of the microsomal (Panel A) and plasma membrane fractions (Panel B). Total protein loading was controlled by Ponceau staining, as described in Methods. S-Sham: sedentary+sham; S-AMI: sedentary+AMI; T-Sham: training+sham; T-AMI: training+AMI. A generalized estimating equation (GEE) followed by Bonferroni’s post-hoc was used according to two factors [sedentary/exercise training, sham/AMI, and the interaction between them – p-value (interaction)].
A  GLUT4 (microsomal fraction)

B  GLUT4 (plasma membrane)

GLUT4 (AU/g tissue)

S-Sham  S-AMI  T-Sham  T-AMI

p=0.004  p=0.008  p=0.018

GLUT4 (AU/g tissue)

S-Sham  S-AMI  T-Sham  T-AMI

p<0.001  p=0.039  p<0.001