Regular exercise is associated with a protective metabolic phenotype in the rat heart

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Burelle, Yan, Richard B. Wambolt, Mark Grist, Hannah L. Parsons, Jeffrey C. F. Chow, Christine Antler, Arend Bonen, Angelica Keller, George A. Dunaway, Kirill M. Popov, Peter W. Hochachka, and Michael F. Allard. Regular exercise is associated with a protective metabolic phenotype in the rat heart. Am J Physiol Heart Circ Physiol 287: H1055–H1063, 2004. First published April 22, 2004; 10.1152/ajpheart.00925.2003.—Adaptation of myocardial energy substrate utilization may contribute to the cardioprotective effects of regular exercise, a possibility supported by evidence showing that pharmacological metabolic modulation is beneficial to ischemic hearts during reperfusion. Thus we tested the hypothesis that the beneficial effect of regular physical exercise on recovery from ischemia-reperfusion is associated with a protective metabolic phenotype. Function, glycolysis, and oxidation of glucose, lactate, and palmitate were measured in isolated working hearts from sedentary control (C) and treadmill-trained (T: 10 wk, 4 days/wk) female Sprague-Dawley rats submitted to 20 min ischemia and 40 min reperfusion. Training resulted in myocardial hypertrophy (1.65 ± 0.05 vs. 1.30 ± 0.03 g heart wet wt, P < 0.001) and improved recovery of function after ischemia by nearly 50% (P < 0.05). Glycolysis was 25–30% lower in T hearts before and after ischemia (P < 0.05), whereas rates of glucose oxidation were 45% higher before ischemia (P < 0.01). As a result, the fraction of glucose oxidized before and after ischemia was, respectively, twofold and 25% greater in T hearts (P < 0.05). Palmitate oxidation was 50–65% greater in T than in C before and after ischemia (P < 0.05), whereas lactate oxidation did not differ between groups. Alteration in content of selected enzymes and proteins, as assessed by immunoblot analysis, could not account for the reduction in glycolysis or increase in glucose and palmitate oxidation observed. Combined with the studies on the beneficial effect of pharmacological modulation of energy metabolism, the present results provide support for a role of metabolic adaptations in protecting the trained heart against ischemia-reperfusion injury.

exercise training; cardiac hypertrophy; ischemia-reperfusion; energy metabolism

Epidemiological data clearly show that regular physical exercise exerts a protective effect against the morbidity and mortality associated with ischemic heart disease (34, 38, 44). Regular physical activity decreases the incidence of myocardial infarction (34, 38, 44). Furthermore, the survival rate after a myocardial infarction is greater in active individuals compared with sedentary ones (34).

Several studies using trained rat models have demonstrated that these epidemiological observations can be at least partly attributed to a decreased susceptibility of the heart to ischemia-reperfusion injury (4, 5). In isolated heart perfusions, this translates into an improved recovery of contractile function (4, 5) and a reduction of cytosolic enzyme release (20) during reperfusion after global ischemia. Similar protective effects are also observed in models of left coronary occlusion in vivo: myocardial infarct size is reduced (30), pressure work is maintained at higher levels during and after ischemia (16, 39), and the occurrence of premature ventricular beats during early reperfusion is reduced (39).

Currently, the mechanisms by which training exerts its beneficial effects are not well understood. Over the past decade, studies on this topic have mainly focused on training alterations in the coronary circulation (23) as well as myocardial calcium metabolism (5), antioxidant defense systems (see Ref. 39 and references therein), and heat shock protein expression (see Ref. 40 for a review). Comparatively less attention has been paid to the potential role of changes in energy substrate use and selection in protecting the trained heart against ischemia-reperfusion injury.

There is a substantial body of clinical and experimental evidence showing that modulation of energy substrate use and selection during and after ischemia is an important determinant of the recovery of myocardial function during reperfusion (19, 25, 45). In general, pharmacological interventions that increase glucose oxidation and thus the fraction of glucose-derived pyruvate oxidized improve functional recovery of the heart during reperfusion (19, 25, 45). This has led to the development of a new class of drugs called metabolic modulators, which are currently being developed to treat ischemic heart disease (19, 25, 45).

At the present time, a training effect on in vitro activities of selected enzymes and transporters of key metabolic pathways

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has been observed in several studies (see Ref. 33 for a detailed review). However, the impact of training on energy substrate use and selection in the intact working heart has not yet been fully investigated under normal conditions of perfusion, and even more so in the context of ischemia-reperfusion.

The main goal of this study was, therefore, to determine whether training is associated with alterations in myocardial energy metabolism in the isolated working rat heart similar to those of cardioprotective metabolic modulators.

METHODS

Animal care and training program. Female Sprague-Dawley rats (200–250 g), obtained from an institutional breeding stock, were housed in a room with a 12:12-h light-dark cycle at 22°C and were fed regular rodent laboratory chow with water ad libitum. Exercise-trained rats (trained) were run on a treadmill at 25 m/min, 16% slope, 4 days/wk for 10 wk. Running time was increased from 30 min in the 1st wk to 45, 60, and 90 min during the 2nd, 3rd, and 4th wk, respectively. Running time was maintained at 90 min for the remaining 6 wk. Rats in the control (control) group were housed in the same cages as their trained counterparts and remained sedentary. These experiments were approved by the institutional committee on the use of laboratory animals in research and were in accordance with the Canadian Council on Animal Care.

Isolated working heart preparation and perfusion protocol. All experiments were performed 48 h after the last training session. Hearts from halothane (2–3%)-anesthetized control and trained rats were perfused with Krebs-Henseleit (KH) solution in the working heart mode at a left atrial preload of 11.5 mmHg and an aortic afterload of 80 mmHg, as previously described (1–3). The KH solution was continuously circulated through the closed perfusion system and contained 1.2 mM palmitate prebound to fatty acid-free albumin (3%), 5.5 mM glucose, 0.5 mM lactate, 2.5 mM calcium, and 100 μM/μl insulin. The solution was oxygenated with 95% O2–5% CO2 and maintained at 37°C throughout the perfusion.

A pressure transducer (Viggo-Spectramed, Oxnard, CA) inserted in the afterload line was used to measure heart rate and peak systolic pressure. Cardiac output and aortic flow were measured via extral flow probes (Transonic Systems, Ithaca, NY) on the left atrial preload and aortic afterload lines, respectively. Coronary flow was calculated as the difference between cardiac output and aortic flow. Rate-pressure product, the product of heart rate and peak systolic pressure, and hydraulic power, the product of cardiac output and peak systolic pressure, were used to measure cardiac work. These measures of heart function were assessed every 10 min throughout the working heart perfusion.

Hearts were initially perfused for 30 min under normoxic nonischemic conditions followed by a 20-min period of global ischemia that was induced by clamping both the left atrial preload and aortic afterload lines. At the end of ischemia, the clamps were removed, and hearts were reperfused for 40 min. At the end of reperfusion, hearts were removed from the perfusion apparatus, weighed, and frozen with a portion of ventricular tissue taken to determine the dry-to-wet weight tissue ratio.

In a separate set of experiments, hearts from control (n = 3) and trained (n = 2) rats were submitted to a time-matched normoxic perfusion to assess the effect of perfusion time per se on mechanical function. These experiments showed that function remained stable throughout the 90 min of perfusion in both experimental groups (results not shown).

Measurement of myocardial glucose, lactate, and palmitate metabolism. In a first series of experiments, hearts were perfused with [5-3H]glucose and [U-14C]glucose to determine rates of glycolysis and glucose oxidation, respectively, as previously described (1–3). In a second series of experiments performed under identical conditions, rates of lactate and palmitate oxidation were determined by perfusing the hearts with [U-14C]lactate and [9,10-3H]palmitate (2, 42). Rates of glycolysis and palmitate oxidation were determined by quantitatively measuring the rate of H2O production. Rates of glucose and lactate oxidation were measured by quantitative collection of 14CO2 released as a gas and dissolved in the perfusate as [14C]bicarbonate. Perfusion and gaseous samples were taken every 10 min of perfusion and were ultimately placed in vials containing scintillation cocktail and counted by standard techniques. Preischemic values for all metabolic rates were calculated based on data collected between 10 and 30 min of perfusion. Postischemic values were calculated between 10 and 40 min of reperfusion. Rates of CO2 production were calculated from substrate oxidation rates, assuming 6 CO2 were produced from glucose oxidation, 3 CO2 were produced from lactate oxidation, and 16 CO2 were produced from palmitate oxidation.

Samples of perfusate were also taken every 10 min to measure the accumulation of lactate in the perfusate over the course of the experiments. Lactate accumulation was determined by taking lactate initially present in the perfusate as well as perfusate volume into account. Lactate was measured by a standard spectrophotometric method.

H+ production from glucose utilization. The proton stoichiometry of glucose fermentation to lactate when coupled to ATP turnover is 2 mol H+/mol glucose (15, 37). In contrast, oxidation of glucose to CO2 yields 0 mol H+/mol glucose (15, 37). Therefore, the overall rate of H+ production derived from glucose utilization was determined as follows (3, 21, 24):

\[ J_{H^+} = 2 \times (J_{glycolysis} - J_{CO}) \]

where \( J_{H^+} \) is the net rate of proton production from glucose utilization expressed in nanomoles per minute per gram dry weight, \( J_{glycolysis} \) is the glycolytic flux, and \( J_{CO} \) is the rate of glucose oxidation.

Myocardial content of selected proteins and enzymes involved in glucose and fatty acid metabolism. Previous studies have shown that exercise training can result in a change in the capacity to use glucose and fatty acids, a finding that is in part dependent on the training protocol and the sex of the animal used (13). Thus we determined the effect of the training protocol used in the current study on the content of selected enzymes and proteins of relevance to the control of myocardial glycolysis, pyruvate oxidation, and fatty acid metabolism to determine if any observed changes in flux could be explained by alterations at the transcriptional-translational level. Protein content, as opposed to mRNA content, was assessed because it is functionally more relevant and because of observed discrepancies between mRNA and protein contents in this setting (13).

The content of the selected enzymes and proteins was determined by immunoblot analysis using a previously described method (3). Briefly, samples of frozen ventricular tissue homogenate (containing 20 μg total protein) were solubilized by boiling in reducing sample buffer, separated by electrophoresis on 10% SDS-polyacrylamide gels, and transferred by electroblotting to a nitrocellulose membrane. After nonspecific blocking, the blots were probed overnight with the following primary antibodies: rabbit anti-GLUT4 (1:1,500 dilution; Cell Signalling Technology, Mississauga, Ontario), mouse anti-gluceraldehyde 3-phosphate dehydrogenase (1:200,000 dilution; Invitrogen, Eugene, OR), rabbit anti-β- and -α-enolase (1:5,000 and 1:3,000 dilution, respectively; see Ref. 31), rabbit anti-pyruvate dehydrogenase complex (PDC, 1:4,000 dilution; see Refs. 29 and 48), and rabbit anti-medium-chain (MCAD, 1:4,000 dilution) and anti-long-chain (LCAD, 1:5,000 dilution) acyl-CoA dehydrogenases (kindly provided by Dr. Arnold W. Strauss of Vanderbilt University; see Ref. 43). Detection of fatty acid translocase (FAT)/CD-36 and plasma membrane-associated fatty acid-binding protein (FABPp) content was performed as described previously (27, 30).
28), using a monoclonal (MO25) anti-human CD36 (1:20,000 dilution) and a rabbit polyclonal anti-FABP myocardium, and its detection system. Bands were quantified by densitometry.

Data analysis. All results are expressed as means ± SE. Differences between trained and control rats and hearts before and after ischemia were compared by means of a two-way ANOVA (SPSS Base 10.0 package). Least-significant difference post hoc tests were used to identify the location of significant differences when the ANOVA yielded a significant F-ratio. Total CO₂ production and its variance were calculated as the sum of independent random variables. The overall total for each experimental group was compared using t-tests, assuming unequal variances. The Bonferroni correction was applied to the P values obtained to correct for multiple comparisons. A corrected P value <0.05 was considered significant.

RESULTS

Morphological data. No significant difference in body weight was observed between the two experimental groups [control, 308 ± 5 vs. trained, 320 ± 8 g, P = not significant (NS)]. In contrast, 10 wk of training induced significant myocardial hypertrophy, as indicated by an ∼25% increase in heart weight (control, 1.30 ± 0.03 vs. trained, 1.65 ± 0.05 g wet wt, P < 0.001) and heart-to-body weight ratio (control, 4.2 ± 0.09 vs. trained, 5.1 ± 0.07 g/kg, P < 0.001). Wet-to-dry weight ratios of heart tissue obtained at the end of perfusion were not significantly different between groups (data not shown).

Mechanical function. The impact of training on mechanical function measured before and after ischemia is summarized in Table 1 and Fig. 1. Before ischemia, no significant differences were observed between control and trained hearts for rate-pressure product, heart rate, peak systolic pressure, and coronary flow. Hydraulic power was ∼25% higher in trained hearts (14.7 ± 0.5 vs. 11.7 ± 0.5 mW/P, P < 0.05), primarily as a result of an increased cardiac output in trained compared with control hearts (60.1 ± 2.2 vs. 48.3 ± 1.6 ml/min/P, P < 0.05). However, these differences were no longer observed when values were normalized to heart weight (Table 1).

During reperfusion, rate-pressure product was higher in trained than in control hearts at all time points. After 40 min of reperfusion, relative recovery of rate-pressure product was significantly better in trained than in control hearts (88 ± 3 vs. 59 ± 6% of preischemic values, respectively, P < 0.05). This improvement was the result of a better recovery of heart rate and to a lesser extent of peak systolic pressure in trained hearts. A similar beneficial effect of training was observed with hydraulic power, which recovered to 56 ± 3% of preischemic values in trained hearts compared with 33 ± 6% in control hearts, mainly as a result of an improved recovery of cardiac output. As for coronary flow during reperfusion, no significant differences in absolute values were observed between the two groups. However, when expressed relative to preischemic values, the recovery of coronary flow was significantly higher in trained than in control hearts.

Myocardial substrate use and selection. Rates of glycolysis and of oxidation of glucose, lactate, and palmitate observed in both experimental groups before and after ischemia are summarized in Fig. 2. Before ischemia, rates of glycolysis were ∼25% lower in trained hearts than in control hearts (1,977 ± 153 vs. 2,608 ± 257 nmol·min⁻¹·g dry wt⁻¹, P < 0.05; Fig. 2A). During reperfusion, rates of glycolysis were slightly but insignificantly lower than preischemic values in both experimental groups. As before ischemia, an ∼30% lower glycolytic flux was observed in trained hearts than in control hearts (1,337 ± 99 vs. 1,956 ± 203 nmol·min⁻¹·g dry wt⁻¹, P < 0.05).

Glucose oxidation before ischemia was ∼45% higher in trained hearts compared with control hearts (316 ± 21 vs. 221 ± 25 nmol·min⁻¹·g dry wt⁻¹, P < 0.05; Fig. 2B). During reperfusion, rates of glucose oxidation recovered to preischemic levels in control hearts. In contrast, rates of glucose

<table>
<thead>
<tr>
<th>Heart rate, beats/min</th>
<th>Control Preischemia</th>
<th>Control Reperfusion</th>
<th>Control Recovery (%)</th>
<th>Trained Preischemia</th>
<th>Trained Reperfusion</th>
<th>Trained Recovery (%)</th>
</tr>
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<tbody>
<tr>
<td>228 ± 6</td>
<td>168 ± 7*</td>
<td>80 ± 4</td>
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<td>242 ± 4</td>
<td>209 ± 10†</td>
<td>94 ± 3†</td>
</tr>
<tr>
<td>Peak systolic pressure, kPa</td>
<td>15.2 ± 0.3</td>
<td>12.0 ± 0.4*</td>
<td>85 ± 5</td>
<td>15.2 ± 0.1</td>
<td>14.1 ± 0.1†</td>
<td>94 ± 1</td>
</tr>
<tr>
<td>Cardiac output, ml·min⁻¹·g dry wt⁻¹</td>
<td>221 ± 12</td>
<td>90 ± 14*</td>
<td>38 ± 5</td>
<td>218 ± 9</td>
<td>132 ± 10†</td>
<td>60 ± 4†</td>
</tr>
<tr>
<td>Hydraulic power, mW/g dry wt</td>
<td>54.7 ± 2.9</td>
<td>19.7 ± 3.6*</td>
<td>33 ± 6</td>
<td>54.3 ± 2.2</td>
<td>30.7 ± 2.4†</td>
<td>56 ± 3†</td>
</tr>
<tr>
<td>Coronary flow, ml·min⁻¹·g dry wt⁻¹</td>
<td>96.5 ± 6.7</td>
<td>43.5 ± 7.5*</td>
<td>44 ± 7</td>
<td>76.7 ± 4.1</td>
<td>53.7 ± 7.7*</td>
<td>67 ± 7†</td>
</tr>
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Values are means ± SE; n = 13–18/group. Preischemia and reperfusion are values at 30 and 90 min of perfusion, respectively. Significantly different from preischemia value in corresponding experimental group with †P < 0.05 and *P < 0.01. Significantly different from control group at corresponding period with §P < 0.05 and ‡P < 0.01.

Fig. 1. Function of hearts from control (control) and exercise-trained (trained) rats before and after ischemia. A: rate-pressure product before and after ischemia in trained and control hearts. B: % recovery of rate-pressure product at the end of reperfusion computed relative to preischemic values measured at 30 min into perfusion. A and B: n = 13 control hearts and 16 trained hearts. *Significantly different from control at corresponding period with P < 0.01. †Significantly different from preischemia in corresponding experimental group with P < 0.01. bpm, beats per minute.
oxidation were depressed significantly compared with preischemic values observed in the control hearts (trained, 216 ± 10 vs. control, 220 ± 24 nmol·min⁻¹·g dry wt⁻¹, P = NS).

Palmitate oxidation was 50–65% higher in trained hearts compared with control hearts before (1,200 ± 67 vs. 805 ± 53 nmol·min⁻¹·g dry wt⁻¹, P < 0.05) and after (1,262 ± 116 vs. 764 ± 53 nmol·min⁻¹·g dry wt⁻¹, P < 0.05) ischemia (Fig. 2C). In both experimental groups, postischemic rates of palmitate oxidation were similar to those observed before ischemia.

As for lactate oxidation, no significant difference was observed between trained and control hearts before or after ischemia. In both groups, ischemia led to a slight but nonsignificant increase in lactate oxidation during reperfusion.

Consistent with a training-induced reduction of glycolytic flux from exogenous glucose, net accumulation of lactate in the perfusate was lower in trained hearts than in control hearts at the end of nonischemic perfusion (34.6 ± 4.5 vs. 44.9 ± 10.0 μmol, P = NS) and the end of reperfusion (77.8 ± 8.8 vs. 107.8 ± 19.2 μmol, P = NS), although these differences did not achieve statistical significance. At 10 min of reperfusion, net accumulation of lactate, which is partly a reflection of lactate arising from glycogen degraded during ischemia, also tended to be lower in trained hearts than in control hearts (data not shown).

Figure 3 shows total CO₂ flux computed from the measured rates of glucose, lactate, and palmitate oxidation. Before ischemia, total aerobic energy production was higher in trained compared with control hearts. This difference in energy production was mostly related to the 25% greater hydraulic work performed by trained hearts (Table 1). During reperfusion, total CO₂ flux recovered to preischemic levels in trained and control hearts, indicating that a complete recovery of oxidative metabolism occurred.

**Proton production from glucose catabolism.** As a result of changes in glucose metabolism, fractional oxidation of glucose (or the rate of glucose oxidation expressed as a percentage of glycolytic flux) was nearly twofold higher in trained compared with control hearts (trained, 17 ± 2 vs. control, 9 ± 1%, P < 0.01; Fig. 4A). This difference, although less pronounced, was also observed during reperfusion, with fractional glucose oxidation being ~40% higher in trained hearts compared with control hearts (trained, 17 ± 2 vs. control, 12 ± 1%, P < 0.05). This effect of training on the fraction of glucose passing through glycolysis that is oxidized had a significant impact on the proton stoichiometry of glucose metabolism (Fig. 4B). Indeed, the net rate of proton production arising from glucose metabolism was decreased by ~33% in trained hearts before and after ischemia as a result of increased glucose oxidation and/or decreased rates of glycolysis.
Myocardial content of selected proteins and enzymes involved in glucose and fatty acid metabolism. Figure 5 summarizes the expression of GLUT4, PFK, GAPDH, and enolase-α and -β proteins, all of which are enzymes or proteins with potential relevance to the control of glycolysis, in hearts from control and trained rats. No significant change in the content of any of these proteins occurred as a result of training. Expression of PDC, which is involved in the integration of glycolysis and mitochondrial pyruvate oxidation, is shown in Fig. 6. No significant effect of training on the content of dihydrolipoamide acetyltransferase (E₂), dihydrolipoamide dehydrogenase (E₃)-binding...
protein, pyruvate dehydrogenase (E₁)-α or -β subunits of PDC was observed.

Expression of selected enzymes and proteins involved in cellular fatty acid uptake and oxidation is summarized in Fig. 7. The expression of MCAD and LCAD, which catalyze the first dehydrogenation step of the β-oxidation pathway for MCAD and LCAD, respectively, were unchanged after training. A lack of change after training was also observed for the expression of FAT/CD36 and FABPₚₘ, two sarcolemmal fatty acid transport proteins.

DISCUSSION

Results from the present study confirm that endurance treadmill training in rats confers protection against ischemia-and reperfusion-induced injury. This protective effect translated into greater heart function at all time points during reperfusion. Moreover, the protective effect was evident when contractile function was expressed as a percentage of preischemic values, indicating that the improved posts ischemic recovery seen in the trained hearts was not simply a reflection of training-enhanced cardiac pump performance. These results are in accordance with previous studies conducted on isolated working hearts from rats trained using a comparable training protocol (4, 5).

The mechanisms by which exercise training confers protection to the heart during ischemia-reperfusion remain unclear. However, it is increasingly apparent that several potential mechanisms are involved (4, 5, 23, 39, 40). At the level of the coronary vasculature, training is known to alter the control of

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**Fig. 6.** Content of pyruvate dehydrogenase complex (PDC) protein subunits in heart from control and trained rats. Each lane represents a different heart; n = 6 in each group. Subunit sizes (in Da) are as follows: E₂, 68,000; E₃ binding-protein (BP), 56,000; E₁-α, 41,000; and E₁-β, 35,000. Densitometric analysis of protein levels is expressed in arbitrary units, with values as means ± SE.

**Fig. 7.** Content of selected proteins and enzymes involved in fatty acid metabolism in hearts from control and trained rats. Representative immunoblots of fatty acid translocase (FAT/CD36 (A), plasma membrane-associated fatty acid-binding protein (FABPₚₘ, B), and long-chain (LCAD, C) and medium-chain (MCAD, D) fatty acyl-CoA dehydrogenase. Each lane represents a single heart. Densitometric analysis of protein levels is expressed in arbitrary units, with values as means ± SE; n = 6 hearts/group.
vascular resistance through a variety of mechanisms, resulting in an increased blood flow capacity (23). This kind of adaptation is thought to be responsible for the exaggerated hyperemic response observed early during retrograde reperfusion in trained hearts (4, 5). It could also contribute to the slightly improved postischemic recovery of coronary flow observed in trained hearts in the present study.

At the level of the cardiomyocyte, improved calcium handling (4, 5) and an upregulation of antioxidant defense systems (Ref. 39 and references therein) and of 72-kDa heart shock proteins (see Ref. 40 for a review) are believed to be involved in protecting the trained heart against ischemia-reperfusion injury. In the present study, we found that endurance training led to adaptations in myocardial energy metabolism that may have a role in the cardioprotective effect of exercise.

Training-induced changes in myocardial glucose catabolism. Ten weeks of treadmill running in female rats results in significant adaptations in myocardial substrate utilization, including alterations in glucose and fatty acid metabolism. Fractional oxidation of glucose was increased significantly in hearts of trained rats before and after ischemia (Fig. 4A). This phenomenon was mostly apparent before ischemia, where fractional glucose oxidation was twofold greater in trained hearts as a result of a decrease in rates of glycolysis and an increase in glucose oxidation. It was also apparent, although to a lesser extent, during reperfusion, in which the fraction of the glucose oxidized was 25% higher in trained than in sedentary control hearts. During reperfusion, this increase was primarily the result of a reduction in glycolysis in trained hearts.

Rates of glucose oxidation were increased in trained hearts before ischemia, whereas oxidation rates of lactate were similar to that observed in control hearts (Fig. 2). Although such a finding is somewhat surprising, it may reflect the potential for the fates of pyruvate derived from glucose and pyruvate derived from lactate to differ (6, 7).

Our expression data (Figs. 5 and 6) indicate that, in the intact rat heart, alterations in the content of relevant proteins and enzymes cannot account for the reduction in glycolytic flux or elevation in rates of glucose oxidation observed in the heart after training. Others have also determined the effect of exercise training on the activity of a variety of glycolytic enzymes and transporter proteins and found that apparent capacity is either mildly increased or does not change (see Ref. 33 for a review). To our knowledge, little information is available on PDC capacity in hearts of exercise-trained normal rats. However, total capacity of PDC in skeletal muscle does not change with exercise training (35, 36). Of interest, these investigators found that the proportion of PDC in the active state actually decreased with exercise training in skeletal muscle, an effect they attributed to enhanced fatty acid catabolism. Taken together, these data suggest that, at least insofar as glycolysis or PDC flux is concerned, alterations in protein expression play a limited role in the metabolic adaptations to training. Other factors, such as changes in allosteric and covalent modulation, subcellular localization of enzymes and proteins (8, 45), and organization of the energy transfer networks coupling ATP-producing pathways to sites of ATP demand, are likely to be more important (9, 17, 41). The effect of exercise training on these aspects of metabolic control remains to be explored.

Potential role of altered glucose catabolism in cardioprotection. Several clinical and experimental studies show that pharmacological treatments that stimulate glucose oxidation and/or reduce glycolysis and thus increase the fractional oxidation of glucose improve functional recovery of the heart after ischemia (19, 25, 45, 47). On the basis of the different proton stoichiometry of glucose fermentation compared with oxidation (15, 37), this protective effect has been suggested to be related to a reduction in net proton production from glucose catabolism (2, 3, 21, 24, 25, 45). Such a reduction was shown to favor the recovery of intracellular pH, which could limit calcium overload by way of successive trans-sarcolemmal H⁺/Na⁺ and Na⁺/Ca²⁺ exchange and therefore reduce the energetic cost associated with the maintenance of ion homeostasis (21, 24). This would in turn lead to improved postischemic contractile function and efficiency (see Refs. 21 and 24 and references therein). These lines of evidence suggest that the training-induced increase in fractional glucose oxidation and the concomitant reduction in calculated proton production from glucose catabolism observed in the present study might partly explain the improved functional recovery of the trained heart after ischemia.

Glycogen turnover (or simultaneous synthesis and degradation of glycogen) was not determined in the present study. As such, the net rate of proton production from glucose catabolism calculated in this study does not account for the possible contribution of glycogenolysis. However, it is well established that glucose arising from glycogen is preferentially oxidized compared with exogenous glucose (1, 11, 14). An important consequence of preferential oxidation of glycogen-derived glucose is that the contribution of glycogen to proton production in the heart is very small, being less than ~5% of total H⁺ production from overall glucose catabolism (1). Furthermore, the fact that perfusate lactate generally parallels differences in glycolysis from exogenous glucose indicates that any contribution of glycogen to lactate production is small relative to exogenous glucose and suggests that major differences in glycogen metabolism between the two groups do not exist under the conditions of study. Thus, although proton production from glycogen metabolism was not accounted for, calculation of the net rate of proton production from exogenous glucose metabolism probably yields a reasonable estimate of the true value in both experimental groups, a view that will require future experiments to confirm.

Training-induced changes in myocardial fatty acid catabolism. The present study is apparently the first one to assess the effect of training on fatty acid oxidation in the intact working heart under normoxic conditions and after ischemia-reperfusion. Significant increases in oxidation rates of palmitate were observed in trained hearts before and after ischemia (Fig. 2). This result is consistent with the observation that treadmill training increases palmitoylcarnitine oxidation in homogenates of subendocardial layers of the left ventricle (18). Our expression data indicate that this increase in palmitate oxidation was achieved without any change in the expression of LCAD (and MCAD as well), the first step of palmitoyl-CoA oxidation in the β-oxidation pathway (Fig. 7). One possibility that was not investigated in the present study is if training increased the expression and/or altered the regulation by malonyl-CoA of mitochondrial carnitine palmitoyltransferase (CPT)-1, an important control point for long-chain fatty acid oxidation. In line
with this possibility, training has been reported to increase myocardial CPT-1 activity and to reduce its sensitivity to inhibition by acidic pH shifts (12).

Cellular uptake is another important site of control of fatty acid oxidation that could be affected by training. However, the expression of FAT/CDE36 and FABPpm, two proteins involved in fatty acid transport across the sarcolemma (27, 28), was similar in hearts from control and trained rats. Although this observation suggests no major upregulation of fatty uptake capacity, it does not exclude a role for FAT/CDE36 in the training-induced increase in palmitate oxidation observed in the current study. Indeed, it has been observed that subcellular distribution of FAT/CDE36 can be altered without changes in total FAT/CDE36 content by translocation of FAT/CDE36 from an intracellular depot to the sarcolemma (27). Regarding fatty acid-binding proteins, it should also be mentioned that treadmill training was shown to increase the expression of heart FABPc, a cytosolic fatty acid-binding protein (46, 49). However, the impact of this increase on fatty acid oxidation was not determined in these studies.

Potential role of altered fatty acid metabolism in cardioprotection. It is generally accepted that the high levels of plasma free fatty acids observed after acute myocardial ischemia are detrimental to the heart during reperfusion (19, 26, 45). Several free fatty acids observed after acute myocardial ischemia are byproducts of fatty acid metabolism, such as ceramides, in the cytosol and mitochondria (10), and other detrimental effects of high free fatty acids during reperfusion after ischemia. Finally, an important corollary of our findings is that evaluation of changes in the content of potentially relevant proteins or enzymes in a metabolic pathway is insufficient by itself to draw meaningful conclusions about metabolic flux in the intact organ and how it is controlled.

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14. Harachova Y, Butterworth P, and Bonen A. Exercise training-induced increase in palmitate oxidation observed in the current study. Indeed, it has been observed that subcellular distribution of FAT/CDE36 can be altered without changes in total FAT/CDE36 content by translocation of FAT/CDE36 from an intracellular depot to the sarcolemma (27). Regarding fatty acid-binding proteins, it should also be mentioned that treadmill training was shown to increase the expression of heart FABPc, a cytosolic fatty acid-binding protein (46, 49). However, the impact of this increase on fatty acid oxidation was not determined in these studies.

Potential role of altered fatty acid metabolism in cardioprotection. It is generally accepted that the high levels of plasma free fatty acids observed after acute myocardial ischemia are detrimental to the heart during reperfusion (19, 26, 45). Several mechanisms have been proposed to account for this detrimental effect, including 1) accumulation of toxic intermediates of byproducts of fatty acid metabolism, such as ceramides, in the cytosol and mitochondria (10), 2) direct detrimental effects of fatty acids on mitochondrial membranes and specific proteins (22), and 3) a reduction in the fractional oxidation of glucose caused by high rates of fatty acid oxidation (19, 26, 45).

The finding that higher rates of fatty acid oxidation in hearts from trained rats are associated with an improved outcome after ischemia supports the idea that enhanced rates of fatty acid oxidation protect these hearts from the noxious effects of fatty acids after ischemia, particularly the effects that typically accompany the high concentrations used in the present study. The higher rates of fatty acid oxidation observed in trained hearts may partially prevent the accumulation of fatty acids and activated fatty acids to limit their toxic effects in the cytosol and mitochondria. This hypothesis warrants future investigation. In addition and of interest, fractional glucose oxidation was greater in the trained hearts before and after ischemia despite higher rates of fatty acid oxidation. This observation suggests that the deleterious effect of high levels of fatty acid on glucose metabolism during reperfusion was circumvented in trained hearts, a possibility that also requires study in the future.

In conclusion, combined with the studies on the beneficial effect of pharmacological modulation of glucose metabolism, the present results provide support for a role of metabolic adaptations in protecting the trained heart against ischemia-reperfusion injury. In addition, these data support the idea that increasing fatty acid oxidation could be beneficial to the trained heart by limiting the noxious effect of high free fatty acids during reperfusion after ischemia. Finally, an important corollary of our findings is that evaluation of changes in the content of potentially relevant proteins or enzymes in a metabolic pathway is insufficient by itself to draw meaningful conclusions about metabolic flux in the intact organ and how it is controlled.


