

Swim Training Improves Myocardial Resistance to Ischemia in Rats

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As the relationship between training and ischemic heart disease is not yet unraveled, we test the hypothesis that, in a model free from environmental, behavioural, and neuro-hormonal factors, endurance training improves myocardial resistance to ischemia. As carbohydrate metabolism is relevant for myocardial resistance to ischemia, we also test whether hyperglycemia blunts the protective effect of training. Eight-week old rats were randomly assigned to four groups (n = 6–8): sedentary or trained (3-week swim program, up to 2 h/day), and normal or high-carbohydrate diet (50 g/l sucrose in drinking water). Excised hearts were perfused isovolumically (flow = 15 ml/min) with Krebs-Henseleit (2 mM free Ca⁺⁺, 11 mM glucose, pH 7.38 ± 0.02, PO₂ = 670 ± 6 mmHg, PCO₂ = 43 ± 1 mmHg, mean ± SE), exposed to 60 min low-flow (1.5 ml/min) ischemia, and then reperfused for 30 min (15 ml/min). In normally fed rats training increased the stroke volume index (97.5 ± 13.0 vs. 72.6 ± 6.2 μl, P = 0.05), depressed diastolic contracture (+2.3 ± 2.0 vs. +24.2 ± 6.7 mmHg, P = 0.02), improved the recovery of developed pressure · heart rate (33.8 ± 2.3 vs. 24.1 ± 3.3 mmHg/min/1000, P = 0.05), and decreased arrhythmias (P = 0.05). In high-carbohydrate-fed rats training induced myocardial hypertrophy (1.95 ± 0.08 vs. 1.67 ± 0.03 g, P = 0.02) and decreased arrhythmias but did not affect stroke volume, developed pressure · heart rate, and diastolic contracture. Thus endurance training improves myocardial resistance to ischemia but a high-carbohydrate diet partially blunts this protection. The occurrence of an inducible alteration able to modulate myocardial tolerance to ischemia may give clues to extend our knowledge of ischemic preconditioning.

Key words: Isolated hearts, reperfusion injury, myocardial performance.

Introduction

Long-standing evidence supports the conjecture that endurance training is protective with respect to morbidity and mortality associated with ischemic heart disease [29]. There may be two broad, not necessarily exclusive, reasons for this. First, endurance training may limit incidence or severity of coronary occlusions by influencing factors not strictly dependent on myocardial function. For example, training-induced habits (limitations in smoking, alcohol consumption, and diet) may favourably affect lipid metabolism, neurohormonal homeostasis, and vascular regulation. Second, endurance training specifically improves myocardial tolerance to ischemia, possibly in way(s) similar to those exerted by ischemic preconditioning. Ischemic preconditioning accounts for the greater ischemia tolerance in hearts exposed to brief episodes of ischemia before a subsequent longer ischemia period [21]: although a number of plausible hypotheses now exist for the underlying processes [10], none of them is fully satisfactory.

In the lack of epidemiological studies aimed at assessing the relationship between training and ischemic heart disease the isolated perfused heart from small animals is a useful model to gain more insight into that issue. Animals are trained at predefined intensities. Then hearts are perfused under controlled loading conditions thereby excluding interference from different ischemia times, coronary occlusion severity, or neurohormonal factors. Previous work in this field, reviewed in [3], is not fully consistent. Some studies show a beneficial effect of training on myocardial resistance to ischemia [4–6,14,20], others that this effect is evident for hypoxia but not ischemia [9,16,28], still others that there are no clear training-associated effects [7,12,18,23], or even that training has negative effects [17]. To further investigate this issue, we tested the hypothesis that a three-week swim training program is associated with improved tolerance of hearts subsequently exposed to low-flow ischemia, a condition clinically more relevant than no-flow global ischemia.

Carbohydrate metabolism is relevant with respect to myocardial resistance to ischemia. Diabetic patients are more exposed to ischemic heart disease than non-diabetic subjects [11]. In addition hyperglycemia seems to abolish the protective effect of ischemic preconditioning [15]. Furthermore insulin improves contractile function during moderate ischemia [30]. Finally glycogen plays a significant role in post-ischemic recovery [8].

As sustained aerobic training is known to increase insulin sensitivity and glycogen in heart and muscles, we also test whether alterations in carbohydrate metabolism affect ischemia tolerance.

Materials and Methods

Animals

Male outbred Sprague-Dawley rats (Nossan, Correzzana, Italy) were admitted to our study at the age of 8 weeks (weight = 260–280 g). All rats had unlimited access to food (standard chow, Teklad, Harlan Nossan, Correzzana, Italy) and water. Animals were randomly assigned to either the untrained or the trained groups. Training consisted in a three-week swim program in 40 cm deep, 50 cm diameter pools with water at $37 \pm 2^\circ\text{C}$ where rats exercised in groups of two. The duration of exercise progressively increased from 30 min/day in the first week to 2 h/day in the last week. Untrained rats were left in their cages for the equivalent period of time. Each group was further divided into two subgroups: normal diet (ND) and high-carbohydrate diet (CHO, 50 g/l sucrose added to drinking water). At the end of the program all rats were anesthetized (i.p. heparinized sodium thiopental, 100 mg/kg) and sacrificed.

Heart perfusion

Excised hearts were immediately mounted on the perfusion system [26]. The medium was a Krebs-Henseleit buffer containing 2.0 mM free Ca^{++} and 11 mM glucose (pH 7.38 ± 0.02 , mean \pm SE, 37°C , $\text{PO}_2 = 670 \pm 6$ mmHg, $\text{PCO}_2 = 43 \pm 1$ mmHg). A peristaltic pump (Gilson, France) delivered the medium at selected flow to the filter (8 μm pore size, 47 mm diameter, Nucleopore Corp., Pleasanton, CA), the pre-heater (37°C), and the aortic cannula. A latex balloon introduced into the left ventricle was connected to a pressure transducer (model 52-9966, Harvard Apparatus, Natick, MA) to monitor cardiac performance. A cannula was inserted into the pulmonary artery to collect the venous return and to monitor venous PO_2 by an O_2 -sensing electrode (Yellow Springs Inc. model 5300 Oxygen Monitor, Yellow Springs, OH). Hearts were stabilized for 20 min at flow = 15 ml/min. During this period we measured the intraventricular balloon volume needed to increase the end-diastolic pressure (EDP) from 0 to 10 mmHg ($V_{0 \rightarrow 10}$). The balloon volume was then kept constant afterwards. At the end of the stabilization baseline measurements were taken and flow was reduced to 1.5 ml/min for 60 min. At the end of low-flow ischemia hearts were reperfused for 30 min at flow = 15 ml/min. At the end of this time measurements were taken again and compared to baseline values.

Measurements

The performance was monitored by a LabView system (National Instruments, Austin, TX) running on Macintosh Quadra 700 (Apple, Cupertino, CA). The measured parameters included EDP, heart rate (HR), left-ventricle developed pressure (LVDP), and O_2 uptake ($\dot{V}\text{O}_2$, calculated from venous PO_2 and coronary flow). In addition we monitored the incidence of arrhythmia during both the stabilization and the reperfusion periods. Arrhythmia was evaluated according to the established parameters (19) by counting the occurrences of premature beats and

of major arrhythmia episodes. Blood hemoglobin, glycerol, free fatty acids, and triglycerides were measured by standard laboratory methods.

Statistics

Data are expressed as mean \pm SE. To detect the interactions between training or diet and the recovery of post-ischemic hearts, we used a factorial 4-group ANOVA test. If this test was significant, the differences between selected pairs of data were tested using the Bonferroni-Dunnnett multiple comparison procedure, i.e. by correcting the significance of the test for the number of comparisons. Arrhythmia was evaluated by comparing their incidence during reperfusion with that during the stabilization period (paired Student's *t*-test). The significance level was set at $P = 0.05$.

Animal use statement

This study has been conducted in conformance with the policy statement of the American College of Sport Medicine on research with experimental animals.

Results

Animals and baseline performance

Table 1 summarizes the changes induced by training in whole animal homeostasis and in myocardial performance during baseline perfusion. The masses of gastrocnemius, kidney, and liver were omitted because they were not significantly different in the various groups. The sugar intake was 1.4–1.7 and 5.5–6.3 g sugar/day in ND and CHO animals, respectively. In trained ND animals the volume of the intraventricular balloon needed to increase EDP from 0 to 10 mmHg ($V_{0 \rightarrow 10}$) was larger than in untrained animals. The higher ratio $V_{0 \rightarrow 10}$ /myocardial mass (45.8 ± 8.0 vs. 66.3 ± 4.7 $\mu\text{l/g}$, in untrained and trained animals, respectively, $P = 0.05$) is supportive of higher stroke volume in trained ND animals. In contrast in CHO animals, despite lower food intake than in ND animals, training induced higher myocardial mass without affecting $V_{0 \rightarrow 10}$. The $V_{0 \rightarrow 10}$ /myocardial mass ratio was 44.5 ± 4.6 and 40.2 ± 2.1 $\mu\text{l/g}$ in untrained and trained animals, respectively ($P = \text{NS}$), supportive of myocardial hypertrophy without increased stroke volume. At the end of the stabilization the only significant difference among the various groups was the slightly higher $\dot{V}\text{O}_2$ in trained vs. untrained ND hearts.

Ischemia-reperfusion

At the end of the ischemia-reperfusion challenge EDP increased by 2.3 ± 2.0 mmHg in ND trained animals as opposed to > 20 mmHg increase in the other groups (Fig. 1). The integrated index of myocardial performance LVDP·HR was 33.8 ± 2.3 mmHg·1000/min in ND trained animals as opposed to < 25 mmHg·1000/min in the other groups. Table 2 summarizes the recoveries of HR, LVDP, and $\dot{V}\text{O}_2$ in the four groups expressed as percent of the baseline values. Although $\dot{V}\text{O}_2$ was essentially maintained in all the groups, post-ischemic myocardial performance was improved in ND trained hearts with respect to the other groups.

Table 1 Effects of the three-week swim training on whole animal homeostasis, myocardial function and metabolism during baseline perfusion (coronary flow = 15 ml/min, arterial PO₂ = 670 ± 6 mmHg, PCO₂ = 43 ± 1 mmHg, pH 7.38 ± 0.02, 37 °C.) Data expressed as mean ± S.E.

	Normal diet		High-carbohydrate diet	
	Untrained	Trained	Untrained	Trained
n	8	6	6	8
Body mass, g	404 ± 18	355 ± 11 [§]	361 ± 9*	380 ± 5*
Food intake/body mass, g/kg/day	72.0 ± 2.3	68.3 ± 0.8	38.2 ± 1.8*	42.6 ± 0.9 [§] *
Myocardium mass, g	1.72 ± 0.21	1.44 ± 0.13	1.67 ± 0.03	1.95 ± 0.08*
Hemoglobin, g/dl	7.7 ± 0.3	8.3 ± 0.3	7.7 ± 0.8	8.8 ± 0.6
Glycerol, μM	133 ± 10	91 ± 25	63 ± 26*	56 ± 27
Free fatty acids, μM	0.43 ± 0.04	0.22 ± 0.06 [§]	0.48 ± 0.07	0.55 ± 0.04*
Triglycerides, μM	103 ± 22	54 ± 13	128 ± 21	179 ± 23*
V _{0→10} , μl	72.6 ± 6.2	97.5 ± 13.0 [§]	76.3 ± 6.6	77.7 ± 2.8*
Heart rate, min ⁻¹	239 ± 16	243 ± 13	248 ± 14	250 ± 8
End-diastolic pressure, mmHg	11.2 ± 0.6	9.9 ± 0.3	9.6 ± 0.5	9.8 ± 0.5
O ₂ uptake, μmoles/min	9.4 ± 0.8	11.3 ± 0.2 [§]	8.0 ± 0.6	8.7 ± 0.5*
Developed pressure, mmHg	122.4 ± 5.5	132.2 ± 10.6	124.2 ± 7.5	111.6 ± 8.3
Developed pressure · heart rate, mmHg · 1000/min	28.9 ± 1.7	31.8 ± 2.4	30.8 ± 2.6	27.7 ± 2.0

[§] Significant difference vs. respective untrained controls (effect of training); * Significant difference vs. respective normal diet animals (effect of the high-carbohydrate diet); V_{0→10} = volume of the intraventricular balloon needed to increase the end-diastolic pressure from 0 to 10 mmHg

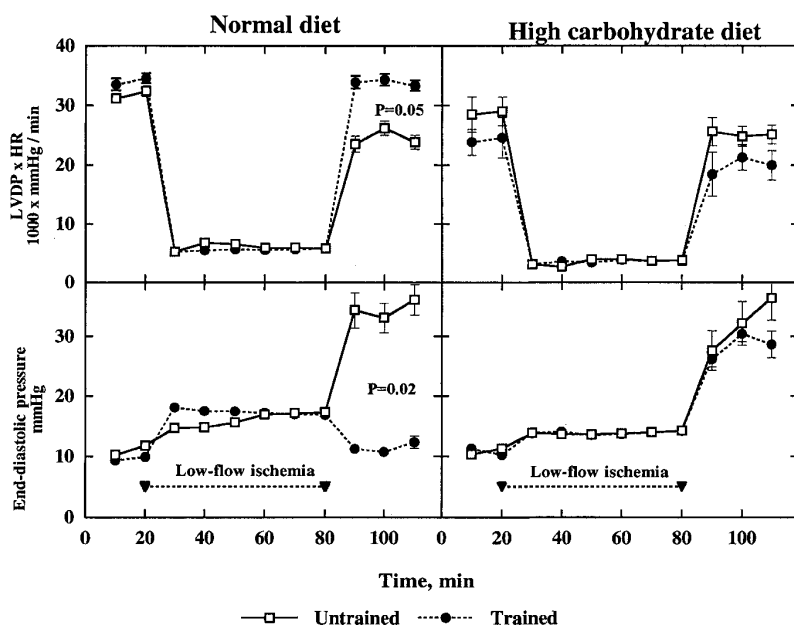


Fig. 1 Time course of developed pressure · heart rate (LVDP · HR, upper panels) and end-diastolic pressure (lower panels) during stabilization at flow = 15 ml/min, low-flow ischemia (flow = 1.5 ml/min), and reperfusion (flow = 15 ml/min), in hearts from untrained controls (solid lines) and trained (dashed lines) animals, fed with either normal (left panels) or high-carbohydrate diet (right panels). The vertical bars indicate S.E.

Arrhythmias

None of the hearts died from arrhythmic accident. Premature beats were not recorded. The main observed arrhythmias were tachycardias, bradyarrhythmias, bigeminal and trigeminal rhythms, ventricular fibrillation, and sinusual arrests. All the detected arrhythmia events were „not-sustained“ (duration < 30 s) according to the established classification [19]. Fig. 2 shows that the ischemia-reperfusion challenge increases arrhythmia incidence in hearts from untrained animals, irrespective of diet. In contrast in hearts from trained animals arrhythmia incidence is either maintained throughout ischemia-reperfusion in the CHO group or decreased in the ND group.

Discussion

A three-week swim-training program improves tolerance in hearts subsequently exposed to low-flow ischemia, and carbohydrate supplementation apparently blunts this effect.

Effects of training and diet on animal homeostasis

The changes observed in trained ND animals are comparable to those normally found in sedentary humans undergoing light aerobic exercise programs: blood glycerol, triglycerides, and free fatty acid levels keep low, probably as a result of low catecholamines [31] and maintained insulin/glucagon ratio

Table 2 Myocardial function and metabolism at the end of the reperfusion, expressed as percent of baseline value (mean \pm S.E.)

	Normal diet		High-carbohydrate diet	
	Untrained	Trained	Untrained	Trained
n	8	6	6	8
Heart rate	87 \pm 4	103 \pm 6 [§]	97 \pm 3	90 \pm 3*
End-diastolic pressure	306 \pm 55	121 \pm 19 [§]	351 \pm 71	352 \pm 57*
O ₂ uptake	103 \pm 10	111 \pm 3	106 \pm 7	112 \pm 7*
Developed pressure	91 \pm 6	105 \pm 5	88 \pm 12	90 \pm 10
Developed pressure · heart rate	81 \pm 9	107 \pm 1 [§]	83 \pm 10	79 \pm 7*

[§] Significant difference vs. respective untrained controls (effect of training);

* Significant difference vs. respective normal diet animals (effect of the high-carbohydrate diet)

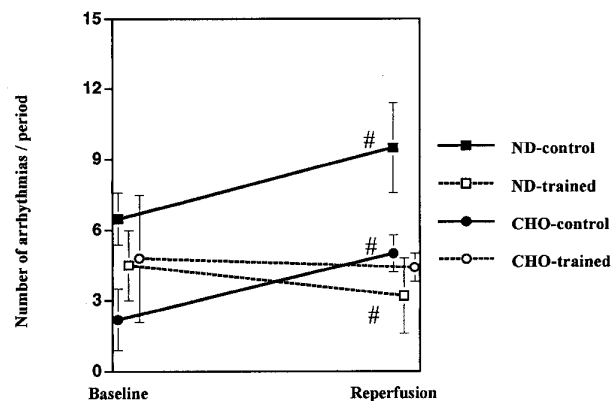


Fig. 2 Arrhythmia incidence during baseline and after the ischemia-reperfusion challenge in hearts from untrained (solid lines) and trained (dashed lines) animals, fed with either normal (ND, squares) or high-carbohydrate (CHO, circles) diet. # Significant difference vs. baseline (paired Student's t-test).

[13]. The body weight gain is markedly less than in resting controls without signs of ventricular hypertrophy but the higher V_{0-10} /myocardial mass ratio indicates increased stroke volume in trained ND animals.

The high-carbohydrate diet significantly alters this pattern. Food intake is lower because CHO animals obtain part of their caloric intake from sucrose. Probably this keeps blood glycerol low. We cannot rule out the possibility that CHO animals are developing insulin resistance but we are unable to evaluate this possibility as blood insulin does not necessarily reflect diabetes insurgence. While higher in exercising humans [22], blood insulin was found to be lower in trained vs. untrained rats [24].

Effects of training and diet on myocardial ischemia tolerance

In the four groups under study only hearts from trained ND animals develop resistance against ischemia. First, the ischemia-induced EDP increase is blunted. In isovolumic preparations increased EDP indicates the onset of diastolic contracture. Second, contractility is better maintained as from improved recovery of HR, LVDP, and LVDP·HR. Third, arrhythmia

incidence is maintained or even decreased throughout the ischemia-reperfusion challenge.

Critique of the model

By reducing the variables to a minimum, the selected model is suitable to investigate the direct effects of training on ischemic cardiac muscle. As isolated hearts are denervated, any interference from neuro-hormonal factors is excluded. Hearts are perfused with red cell-free media, thus disturbing effects like neutrophils accumulation and thrombin-induced platelet aggregation are ruled out. Strict temperature control ($\pm 0.5^\circ\text{C}$), constant volume of the intraventricular balloon, and same coronary flows in the various groups rule out differences in the loading conditions. The volume of the intraventricular balloon is fixed at the start of the experiment to yield $\text{EDP} = 10 \pm 1$ mmHg. Thus any subsequent rise of EDP is attributed to the onset of diastolic contracture, which stems from impaired Ca^{++} sequestration into the sarcoplasmic reticulum secondary to low available ATP [1].

Mechanisms underlying ischemia tolerance

As training-induced ischemia tolerance is blunted in the CHO groups, the carbohydrate metabolism and tissue glycogen are predicted to play a significant role. The effect of glycogen is related to ischemia duration and severity [8]: While protective during moderate ischemia because more glucose is supplied to glycolysis thereby supplying more substrate level ATP, glycogen becomes detrimental during prolonged or severe ischemia because its depletion induces higher tissue H^+ , which ultimately leads to Ca^{++} overload. The down-regulation in performance in hearts perfused at coronary flow = 1.5 ml/min [25] is compatible with the concept of moderate ischemia in our hearts and therefore with protective effect of glycogen. The lack of training-induced protection in CHO animals may be due to ventricular hypertrophy, which exacerbates the ischemic injury [2], and may have exceeded the protection afforded by training in CHO animals. The training-induced ischemia tolerance may also be due to increased anti-oxidant enzyme level, in agreement with a study in rats undergoing a 9 week-swim training program (1 h/day, 5 days/wk) [14]. However, in another study with rats undergoing a 2–3 months (4 h/day, 5 days/wk) swim training the protection was not elicited by increased levels of anti-oxidant enzymes [16]. Possibly the role of this factor may be better assessed in hearts undergoing hypoxia/reoxygenation rather than ischemia/reperfusion as in the first instance the anti-oxidant enzymes are challenged to a greater extent [27].

The effects of training and diet on the incidence of arrhythmia follow a pattern different from that observed for diastolic contracture and contractility recovery. Indeed training protects from arrhythmia irrespective of the diet. This might indicate that the paths that lead to protection of myocardial contractility are different from those responsible for the protection of the conducting system.

Conclusion

This study confirms that, in a model free from environmental, behavioural, hormonal, and nervous factors, moderate endurance training improves myocardial resistance to low-flow

ischemia. High-carbohydrate supplementation impairs the protection elicited by training on myocardial contractility and development of diastolic contracture but does not have effects on the conducting system. Although the mechanism underlying protection is still unresolved, the occurrence of an inducible metabolic alteration that is able to differentially improve myocardial tolerance to ischemia may give clues to extend our knowledge of a still elusive phenomenon as ischemic preconditioning.

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