High intensity interval training alters substrate utilization and reduces oxygen consumption in the heart

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High intensity interval training alters substrate utilization and reduces oxygen consumption in the heart. J Appl Physiol 111: 1235–1241, 2011. First published August 11, 2011; doi:10.1152/japplphysiol.00594.2011.—Aims: although exercise training induces hypertrophy with improved contractile function, the effect of exercise on myocardial substrate metabolism and cardiac efficiency is less clear. High intensity training has been shown to produce more profound effects on cardiovascular function and aerobic capacity than isocaloric low and moderate intensity training. The aim of the present study was to explore metabolic and mechanoenergetic changes in the heart following endurance exercise training of both high and moderate intensity. Methods and Results: C57BL/6J mice were subjected to 10 wk treadmill running, either high intensity interval training (HIT) or distance-matched moderate intensity training (MIT), where HIT led to a pronounced increase in maximal oxygen uptake. Although both modes of exercise were associated with a 10% increase in heart weight-to-body weight ratio, only HIT altered cardiac substrate utilization, as revealed by a 36% increase in glucose oxidation and a concomitant reduction in fatty acid oxidation. HIT also improved cardiac efficiency by decreasing work-independent myocardial oxygen consumption. In addition, it increased cardiac maximal mitochondrial respiratory capacity. Conclusion: This study shows that high intensity training is required for induction of changes in cardiac substrate utilization and energetics, which may contribute to the superior effects of high compared with moderate intensity training in terms of increasing aerobic capacity.

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exercising period. \( V_{O2\max} \) was measured using a treadmill (25° inclination) in a metabolic chamber (Modular treadmill with Oxymax open circuit calorimeter, Columbus Instruments). The speed was gradually increased until oxygen consumption leveled off despite increased running speed and respiratory quotient (RER) approximated 1, where \( V_{O2\max} \) was defined. The running speed at which \( V_{O2\max} \) was obtained was defined as speed\(_{\text{max}}\).

**Plasma parameters.** Blood samples were taken from fasted (4 h) and fed mice at 1300. Plasma glucose, free fatty acids, and triglycerides were analyzed using commercial kits from Boehringer Mannheim (Mannheim, Germany), Wako Chemicals (Neuss, Germany), and ABX Diagnostics (Montpellier, France), respectively.


**Ex vivo working hearts.** Myocardial glucose and palmitate oxidation were measured in isolated perfused hearts (1) and expressed as oxidation rates per gram dry weight, using a dry-to-wet weight ratio of 1 to 5. Values of left ventricular contractile function, total cardiac work (pressure-volume area, PVA), and myocardial oxygen consumption (MVO\(_2\)) were then assessed using a 1.0-Fr. micromanometer-conductance catheter (Millar Instruments, Houston, TX), which was inserted into the left ventricle through the apex. Fiber-optic oxygen probes (FOXY-AL300, Ocean Optics, Duiven, Netherlands) were placed in the left atrial cannula (adjacent to the heart) and in the pulmonary trunk (20, 24). MVO\(_2\) was calculated by the following equation: MVO\(_2\) = [PO\(_2\) (oxygenated perfusate) − PO\(_2\) (coronary effluent)]/Bunsen solubility coefficient of O\(_2\)-coronary flow. To determine cardiac efficiency, electrically paced hearts were exposed to different workloads (24). Steady-state values of PVA and MVO\(_2\) were obtained at each workload to perform regression analysis of the relationship between PVA and MVO\(_2\). The PVA-MVO\(_2\) regression allows the myocardial oxygen cost to be separated in two parts: work-independent MVO\(_2\) (\(\gamma\)-intercept of the PVA-MVO\(_2\) relationship) and work-dependent MVO\(_2\) (contractile efficiency, i.e., the inverse slope of the PVA-MVO\(_2\) relationship) (48). Work-dependent MVO\(_2\) is a measure of the energy cost of excitation-contraction coupling and basal metabolism, while contractile efficiency reflects the amount of metabolic energy that is converted into mechanical work. MVO\(_2\) was also measured in unloaded retrogradely perfused hearts before (MVO\(_2\)unloaded) and after KCl arrest to measure oxygen cost for basal metabolism (MVO\(_2\)BM) (8). Oxygen cost for excitation-contraction coupling (MVO\(_2\)ECC) was defined as the value obtained by subtracting MVO\(_2\)BM from MVO\(_2\)unloaded.

**Citrate synthase activity and mitochondrial respiration.** Citrate synthase activity, a commonly used marker of mitochondrial content (9, 10), was measured spectrophotometrically, using a slight modification of the method of Srere (45). Mitochondrial respiration was measured in saponin-permeabilized cardiac fibers by high-resolution respirometry as described earlier (34). Respiration was assayed following addition of glutamate (10 mM) and malate (2 mM) as complex I substrate supply (\(V_0\), \(V_{O2\max}\) was obtained after addition of 2.5 mM ADP, and \(V_{O2\max}\) was obtained after addition of 1 μg/ml oligomycin. \(O_2\) flux was calculated from the negative time derivative of the oxygen concentration signal, using DatLab 4 software from Oroboros Instruments. Respiration was related to both fiber weight and CS activity to adjust for potential differences in mitochondrial content.

**Statistical analysis.** Data are expressed as means ± SE. Differences between groups were analyzed using an unpaired t-test. Where normality test failed (Shapiro-Wilk test), a Mann-Whitney Rank Sum Test was performed. One-Way ANOVA was used for comparison of the effect of HIT and MIT.

**RESULTS**

**Effect of exercise on body weight and plasma energy substrates.** Both HIT and MIT reduced fasting levels of circulating free fatty acid (FA) and increased fasting plasma glucose slightly. Neither MIT nor HIT influenced body weight (Table 1).

**Exercise-induced cardiac hypertrophy.** Both exercise training regimens resulted in cardiac hypertrophy, as indicated by a 10% increase in the heart to body weight ratio (Table 1). The exercise-induced cardiac hypertrophy following MIT and HIT was not associated with changes in the expression of B-type natriuretic peptide (bnpp) or atrial natriuretic factor (anf) (Table 2). HIT induced an increase in cardiac mRNA expression of the α-myosin heavy chain isoform (mhc), whereas the β-myosin heavy chain isoform (mhc) was reduced (Table 2). There were no transcriptional changes in these genes following MIT. Neither MIT nor HIT altered cardiac mRNA expression of sarcomplasmic reticulum calcium ATPase (serca2) (Table 2).

**Aerobic capacity.** HIT and MIT resulted in increased \(V_{O2\max}\) (Table 1). Normalization of \(V_{O2\max}\) to their corresponding controls revealed that the exercise-induced increase in \(V_{O2\max}\) was most pronounced following HIT (Table 1). The increased \(V_{O2\max}\) was associated with an increase in running speed at \(V_{O2\max}\) following both MIT and HIT, again with the most pronounced increase following HIT.

**Ventricular function.** Ventricular function was measured in electrically paced isolated working hearts at steady-state conditions (8 mmHg preload and 50 mmHg afterload). Absolute values of aortic flow and stroke volume were increased following HIT (13.1 ± 0.6 vs. 10.7 ± 0.6 ml/min and 38.5 ± 2.0 vs. 32.9 ± 1.3 μl/beat, respectively, both \(P < 0.05\)). These changes were related to increased heart weight, and weight-adjusted values of aortic flow and stroke volume were similar for both groups (Table 1). HIT did not alter ventricular pressure or its first derivative during derivative during baseline loading conditions. Preload-recruitable stroke work index (PRS\(_W\)), an index of contractility, was
ventricular systolic and diastolic pressure, respectively; dP/dt_max and dP/dt_min, maximal slopes of systolic pressure increment and diastolic pressure decrement, values were normalized to those of their sedentary controls. Cardiac function was measured in isolated perfused hearts paced at 7 Hz, using a 1-Fr. conductance catheter inserted into the left ventricle. Steady-state parameters were obtained with pre- and afterload settings of 8 and 50 mmHg, respectively. Pes and Ped, ILeft ventricular systolic and diastolic pressure, respectively; dp/dt_max and dp/dt_min, maximal slopes of systolic pressure increment and diastolic pressure decrement, respectively; Tau, early diastolic relaxation time; CS, citrate synthase; FA, fatty acids; TG, triacylglycerol. Functional parameters obtained by a temporary preload reduction are slope of end-diastolic-pressure-volume relationships (EDPVR) and preload-recruitable stroke work index (PRSWi). *P < 0.05 vs. SED, †P < 0.05 vs. MIT for normalized values.

increased (Table 1), although end-systolic pressure-volume relationships (ESPVR) were unaltered (data not shown). Parameters of ventricular diastolic function (EDPVR and Tau) were not altered by HIT.

Table 2. mRNA expression of genes in cardiac tissue from mice following MIT and HIT, and from age-matched SED

<table>
<thead>
<tr>
<th>Gene</th>
<th>SED n = 10</th>
<th>MIT n = 10</th>
<th>SED n = 10</th>
<th>HIT n = 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>ppara</td>
<td>1.00 ± 0.05</td>
<td>0.84 ± 0.07</td>
<td>1.00 ± 0.03</td>
<td>0.85 ± 0.06*</td>
</tr>
<tr>
<td>pgc1a</td>
<td>1.00 ± 0.09</td>
<td>0.79 ± 0.08</td>
<td>1.00 ± 0.13</td>
<td>1.12 ± 0.15*</td>
</tr>
<tr>
<td>vegf</td>
<td>1.00 ± 0.04</td>
<td>0.87 ± 0.04*</td>
<td>1.00 ± 0.04</td>
<td>1.20 ± 0.07*</td>
</tr>
<tr>
<td>ldh</td>
<td>1.00 ± 0.04</td>
<td>1.11 ± 0.06</td>
<td>1.00 ± 0.05</td>
<td>1.16 ± 0.04*</td>
</tr>
<tr>
<td>hkk</td>
<td>1.00 ± 0.03</td>
<td>0.97 ± 0.02</td>
<td>1.00 ± 0.11</td>
<td>1.11 ± 0.04*</td>
</tr>
<tr>
<td>bmrn</td>
<td>1.00 ± 0.22</td>
<td>0.70 ± 0.11</td>
<td>1.00 ± 0.08</td>
<td>0.64 ± 0.08*</td>
</tr>
<tr>
<td>amhc</td>
<td>1.00 ± 0.04</td>
<td>0.93 ± 0.04</td>
<td>1.00 ± 0.01</td>
<td>1.12 ± 0.02*</td>
</tr>
<tr>
<td>servc2</td>
<td>1.00 ± 0.05</td>
<td>0.97 ± 0.05</td>
<td>1.00 ± 0.02</td>
<td>1.00 ± 0.02</td>
</tr>
<tr>
<td>sod</td>
<td>1.00 ± 0.04</td>
<td>0.97 ± 0.04</td>
<td>1.00 ± 0.02</td>
<td>1.06 ± 0.01*</td>
</tr>
<tr>
<td>cat</td>
<td>1.00 ± 0.03</td>
<td>0.95 ± 0.05</td>
<td>1.00 ± 0.02</td>
<td>1.10 ± 0.02*</td>
</tr>
<tr>
<td>anf</td>
<td>1.00 ± 0.13</td>
<td>0.71 ± 0.11</td>
<td>1.00 ± 0.20</td>
<td>0.14 ± 0.14</td>
</tr>
<tr>
<td>bnp</td>
<td>1.00 ± 0.10</td>
<td>0.60 ± 0.14</td>
<td>1.00 ± 0.08</td>
<td>1.08 ± 0.10*</td>
</tr>
</tbody>
</table>

Values are means ± SE. To compare the effect of moderate intensity training (MIT) and high intensity training (HIT) on aerobic capacity and running speed, values were normalized to those of their sedentary controls. Cardiac function was measured in isolated perfused hearts paced at 7 Hz, using a 1-Fr. conductance catheter inserted into the left ventricle. Steady-state parameters were obtained with pre- and afterload settings of 8 and 50 mmHg, respectively. Pes and Ped, ILeft ventricular systolic and diastolic pressure, respectively; dp/dt_max and dp/dt_min, maximal slopes of systolic pressure increment and diastolic pressure decrement, respectively; Tau, early diastolic relaxation time; CS, citrate synthase; FA, fatty acids; TG, triacylglycerol. Functional parameters obtained by a temporary preload reduction are slope of end-diastolic-pressure-volume relationships (EDPVR) and preload-recruitable stroke work index (PRSWi). *P < 0.05 vs. SED, †P < 0.05 vs. MIT for normalized values.

MIT did not alter steady-state baseline ventricular function or any of the load-independent functional parameters (Table 1).

Myocardial substrate utilization and gene expression. HIT induced a shift in myocardial substrate utilization, as indicated by a 1.4-fold increase in myocardial glucose oxidation and a concomitant 37% decrease in FA oxidation (Fig. 1). These changes were not related to changes in external cardiac work, since cardiac output of the ex vivo perfused hearts from both the HIT and MIT group (119 ± 6 and 125 ± 7 ml·min⁻¹·g wet wt⁻¹) was not different from that of their respective controls (114 ± 5 and 123 ± 6 ml·min⁻¹·g wet wt⁻¹). Due to the somewhat unexpected shift in cardiac substrate utilization toward glucose oxidation following HIT, we investigated target genes of HIF-1α and found an upregulation of cardiac gene (mRNA) expression of lactate dehydrogenase (ldh), hexokinase (hk), and vascular endothelial growth factor (vegf) following HIT (Table 2); MIT did not increase the expression of these genes. Significant and borderline (P = 0.087) reductions in the cardiac expression of ppara following HIT and MIT, respectively, were not accompanied by reduced cardiac expression of PPARG target genes (data not shown). HIT was associated with increased gene expression of superoxide dismutase (sod) and catalase (cat) (Table 2).

Cardiac MVO₂ and efficiency. Cardiac efficiency was assessed by regression analysis of the relationship between MVO₂ and cardiac work (pressure-volume area or PVA). We found that HIT increased cardiac efficiency by reducing work-independent MVO₂ (given by the y-intercept of the PVA-
MVO2 regression line, Table 3), indicating that HIT reduced the oxygen costs for non-contractile processes. Contractile efficiency (1/slope of the PVA-MVO2 relationship), however, was not significantly altered (Table 3). The reduced work-independent MVO2 following HIT was also supported by direct measurement of MVO2 in mechanically unloaded and retrograde perfused hearts (MVO2 unloaded; Fig. 2). By electrically arresting these hearts, we also found a reduced MVO2 for basal metabolism (MVO2 BM), while oxygen cost for excitation-contraction coupling (MVO2 ECC) was unaltered (Fig. 2). In contrast to HIT, we did not find MIT to alter cardiac efficiency, MVO2 unloaded, MVO2 BM, or MVO2 ECC.

Citrate synthase activity and mitochondrial respiration. Although both HIT and MIT increased citrate synthase (CS) activity in skeletal muscle (Table 1), only HIT was found to increase CS activity in the heart (Fig. 3C). The increase in myocardial CS activity was not matched by a concomitant increase in pgc-1α expression (Table 2), a finding that could probably be explained by the fact that tissue samples were harvested 24 h following the last training session, while changes in the mRNA level of pgc-1α following high intensity training are only transient, as reported for skeletal muscle (39).

Mitochondrial respiration measured in permeabilized cardiac fibers demonstrated a 35% increase in state 3 respiration (maximal respiratory capacity, Vmax, P < 0.005) following HIT (Fig. 3B). State 3 respiration was also significantly increased when adjusted for CS activity in the fiber as (29.9 ± 2.3 vs. 36.9 ± 2.1 nmol-min⁻¹·g⁻¹, P = 0.044), although we did not find HIT to induce changes in respiration after addition of oligomycin (Voligo). MIT did not alter mitochondrial respiration rates.

DISCUSSION

Pathological hypertrophy is associated with a distinct cardiac phenotype with contractile dysfunction and altered myocardial substrate utilization, reflected by reduced FA oxidation and increased carbohydrate utilization (2, 13, 15, 37). In contrast, assessment of the exercise-induced cardiac phenotype has revealed inconsistent results. In a comprehensive study on rats, exercise was found to upregulate cardiac expression of metabolic genes involved in FA uptake, glycolysis, and glucose oxidation, and where improved myocardial ability to oxidize glucose was inferred based on observed transcriptional changes (47). However, in another study, enhanced FA utilization was suggested based on cardiac transcriptional changes (26). Direct measurements of myocardial oxidation rates following exercise training have shown a simultaneous increase both in glucose and FA oxidation (14) or unaltered glucose oxidation (11). One reason for the inconsistencies in metabolic phenotype following exercise may be variability in exercise protocols, as exercise intensity and the effect on aerobic capacity is not mentioned in previous studies. We therefore
designed a protocol to evaluate the cardiometabolic effect of exercise intensity in physiological hypertrophy.

In accordance with earlier reports (22, 30, 50), HIT was found to be superior to MIT with regard to increasing aerobic capacity (V\(_{O_2}\)max) and running speed. Although both exercise protocols induced a similar physiological hypertrophy based on increased heart mass, cardiac function was unaltered by MIT. The effect of HIT on contractile function was subtle and may primarily be due to the increase in cardiac size (5, 40). HIT modestly increased ventricular contractility (as indicated by an increase in the preload recruitable stroke work index), which was accompanied by a switch in the myosin heavy chain (MHC) isoform, as indicated by increased gene expression of MHC isoform (18).

In contrast to the modest effect of exercise on ventricular function, the present study showed that high intensity exercise induced a substantial shift in myocardial substrate utilization toward increased glucose oxidation, while FA oxidation rates were reduced. MIT did not alter myocardial oxidation rates, which is similar to what Broderick et al. (11) found in hearts from exercise-trained rats. The HIT-induced shift in myocardial substrate utilization resembles changes commonly associated with those of a “stressed” heart (42) and may therefore represent an important metabolic adaptation of cardiac muscle to repeated exposure to high intensity workloads. It is known from studies on skeletal muscle that carbohydrates is the major fuel for oxidative metabolism during exercise of high intensities (12). Some of the cardiac transcriptional changes observed following HIT are also similar to those induced by increased load and hypoxia, including increased gene expression of HIF 1-α target genes and decreased expression of ppara (35, 36, 43), which may suggest that high workloads during HIT can be associated with episodes of reduced oxygen tension in the cardiac tissue, activating pathways commonly associated with pathological hypertrophy. Interestingly, increased glucose oxidation and reduced FA oxidation have previously been documented in hearts from mice overexpressing Ca\(^{2+}\) ATPase (SERCA), which was associated with increased mitochondrial calcium content and pyruvate dehydrogenase activity (6). Although mitochondrial calcium content was not measured in the present study, increased myocardial SERCA content and activity in rodents following aerobic interval training is well documented in the literature (27, 28, 52) and could therefore be a contributing factor to the metabolic shift observed following HIT.

Another novel effect of HIT described for the first time in the present study is increased cardiac efficiency due to reduced myocardial oxygen consumption for nonmechanical work. Although comparative studies on humans have demonstrated lower MVO\(_2\) in athletes than in untrained controls (21, 49), the factors contributing to the reduction in MVO\(_2\) were not revealed. In the present study, HIT did not alter contractile efficiency (work-dependent MVO\(_2\), determined from the slope of the PVA-MVO\(_2\) relationship), but it reduced work-independent MVO\(_2\) (unloaded MVO\(_2\)), which includes oxygen cost associated with basal metabolism and excitation-contraction (E-C) coupling. In further experiments, HIT did not influence the oxygen cost for E-C coupling but reduced the component for basal metabolism. As previous studies have reported HIT to increase cardiomyocyte shortening accompanied by reduced Ca\(^{2+}\) amplitude and increased myofilament Ca\(^{2+}\) sensitivity (28, 29, 52), unaltered oxygen cost for E-C coupling and contractile efficiency was unexpected. In addition to the obvious differences between a work-loaded heart and unloaded cardiomyocytes with respect to energy consumption, expected changes in contractile efficiency due to increased myofilament Ca\(^{2+}\) sensitivity may have been counteracted by the observed shift toward the αMHC isoform, as it is thought to be energetically more expensive than βMHC isoform (18).

Although the HIT-induced reduction in unloaded MVO\(_2\) may be related to the observed switch in myocardial substrate utilization (as the P/O ratio for glucose oxidation is higher compared with fatty acid oxidation), this cannot solely be the underlying mechanism, as a complete switch from FA to carbohydrate as energy substrate could theoretically account for maximally 12% reduction in MVO\(_2\). An additional mechanism for myocardial oxygen wasting is increased mitochondrial uncoupling induced by either FA (23) or reactive oxygen...
species (ROS) (16). HIT could potentially reduce uncoupling through its lipid-lowering effect and, in addition, transcriptional downregulation of uncoupling proteins has previously been demonstrated following exercise (47). Exercise-induced oxygen-sparing mechanisms could also include increased myocardial antioxidant capacity (7, 41), reduced mitochondrial ROS production, and thus diminished ROS-induced mitochondrial uncoupling (7, 46). In support of an exercise-induced increase in myocardial antioxidant capacity, HIT increased the myocardial gene expression of manganese superoxide dismutase and catalase.

It is well known that both prolonged exercise of moderate intensity (4) and high intensity interval training (17) induce mitochondrial biogenesis in skeletal muscle, and accordingly both high and moderate intensity training was found to increase CS activity in skeletal muscle. In cardiac tissue, however, only HIT increased CS activity. This result shows that the myocardium does not respond easily to exercise training and that it probably exhibits less metabolic plasticity than skeletal muscle. HIT was also found to markedly increase maximal mitochondrial respiration (V\textsubscript{max}) in skinned myocardial fibers, an effect that was not only due to higher mitochondrial content but also to a higher electron transfer chain capacity, as V\textsubscript{max} was found to be increased also when adjusted to CS activity. These results suggest that exercise needs to be of high intensity to activate the intracellular pathways responsible for the mitochondrial adaptations in cardiac muscle, which could be essential for myocardial ATP production during high workloads.

The fact that isocaloric moderate intensity training did not induce changes in cardiac substrate oxidation rates, mitochondrial respiration, and myocardial M\textsubscript{VO}2 illustrates that the term “exercise-induced metabolic effects” should be used with caution, as such effects clearly are dependent on exercise intensity. The present study may therefore partly explain the diversity regarding the exercise-induced effects reported in the literature (11, 14). Furthermore, as heart failure is associated with reduced mitochondrial capacity, reduced contractile function, impaired oxidative capacity, and impaired energetic status (15, 38) the presently observed HIT-induced cardiac adaptations (increased contractility, increased glucose oxidation, improved mitochondrial function, and decreased unloaded M\textsubscript{VO}2) represent changes that could be considered beneficial. Our data may therefore point to potential mechanisms that could explain the profound beneficial cardiovascular effects of HIT found both in animal models of heart failure and in post-infarcted patients (32, 51, 53).

In conclusion, we found that high intensity training was superior to moderate intensity training with regard to its effect on whole body V\textsubscript{O}2max. Although both high and moderate exercise increased V\textsubscript{O}2max, the exercise-induced metabolic and mechanoenergetic changes in the heart were only observed following high intensity training. We suggest that increased mitochondrial oxidative capacity, increased cardiac efficiency due to decreased unloaded myocardial oxygen consumption, a switch toward a faster cardiac myosin isof orm, and the ability to catabolize carbohydrates over fats are cardiac adaptations that will facilitate sustained cardiac output during maximal workloads and thereby enhance aerobic capacity. The metabolic adaptations following HIT also suggest a specific therapeutic potential for cardiovascular conditions with impaired cardiac metabolism and mechanoenergetics.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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