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

Hafstad AD\(^1\), Boardman NT\(^1\), Lund J\(^1\), Hagve M\(^1\), Khalid AM\(^1\), Wisløff U\(^2,3\), Larsen TS\(^1,2\), Aasum E\(^1\)

\(^1\) Cardiovascular Research Group, Faculty of health sciences, University of Tromsø, Norway

\(^2\) K.G. Jebsen Center of Exercise in Medicine, \(^3\)Department of Circulation and Medical Imaging, University of Science and Technology, Trondheim, Norway

Running head: Cardiometabolic effects of exercise

Corresponding author:
Anne D Hafstad
Postdoctoral fellow
Cardiovascular Research Group
Institute of Medical Biology
Faculty of Health Sciences
University of Tromsø
N-9037 Tromsø
Norway
Tlf: +47 776 46341
Fax: +47 77645440
e-mail: anne.hafstad@uit.no
ABSTRACT

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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 weeks treadmill running; either high intensity interval training (HIT) or distance-matched moderate intensity training (MIT), where HIT led to a more 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 and 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 to moderate intensity training in terms of increasing aerobic capacity.
INTRODUCTION

High intensity aerobic interval training has been shown to have a more profound influence on cardiovascular function and aerobic capacity than isocaloric low and moderate intensity training both in healthy humans (22; 50) and in rodents (30), but also in patients with heart failure (53). Low aerobic capacity is an important predictor for development of cardiovascular disease (33), increased physical activity increases aerobic capacity, and exercise training has become important for prevention of heart disease, as well as for treatment and rehabilitation of patients with heart disease.

Chronic exercise training leads to a variety of systemic changes in the circulatory system and in the heart, for instance a physiological/adaptive hypertrophy with preserved or enhanced ventricular function (5; 40; 44). Although several physiological and pathophysiological conditions show a clear association between cardiac function, myocardial metabolism and cardiac energetics (20; 25; 37; 54), the cardiometabolic effect of exercise is not clear. Only a few studies have directly measured cardiac substrate utilization following exercise training, and the results diverge; both increased or unaltered glucose oxidation, increased fatty acid (FA) oxidation and/or decreased or unaltered glycolysis have been reported (14) (11). As no direct measurements of the overall training effect (i.e. aerobic capacity) were reported, it cannot be excluded that exercise duration and intensity may play a pivotal role in regulation the exercise-induced myocardial substrate utilisation and/or gene expression. There is also limited information related to the effect of exercise training on cardiac energetics. Exercise training have been shown to increased cardiomyocytes cell shortening and Ca$^{2+}$ myofilament sensitivity (28; 29; 52), to reduce mitochondrial ROS production (46) and uncoupling (7) in the heart. It is therefore reasons to believe that exercise may decrease processes known to be associated oxygen waist and cost, and thus improve cardiac efficiency. The objective of the present study was therefore to investigate the metabolic and mechanoenergetic changes in the heart following long term endurance exercise with two different exercise intensities.
METHODS

Study design and animals. 26 C57BL/J6 male mice (7 to 9 weeks of age) were subjected to high intensity interval training (HIT) or to moderate intensity continuous training (MIT) in two separate experimental series. Twelve mice were subjected to each of the exercise modalities, and 14 age- and weight-matched mice were allocated to each exercise group and used as sedentary controls (SED). The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and was approved by the Norwegian National Animal Research Committee. All mice had free access to food [standard mouse chow, RM1(E) from Special Diet Service, UK] and water, and were housed at 23 ºC on a reversed light/dark cycle.

Training and determination of aerobic capacity. Treadmill running (Modular Treadmill, Columbus Instruments, Ohio, USA at 25 º inclination) at high and moderate intensity was performed 5 days a week for 10 weeks according to a protocol slightly modified from that described by Kemi et al 2002 (31). HIT consisted of ten bouts of 4 min high intensity running, corresponding to 85-90% of VO2max, interspersed by 2 min of active rest. The interval pace was increased gradually from 16 to 26 m/min over 8 weeks and maintained at this value for the rest of the exercising period. MIT consisted of continuous running, of where the distance covered was matched to that of HIT, corresponding to 65-70% of VO2max, the average running time was close to 2 hours. The pace during MIT was increased gradually from 9 to 13 m/min over 8 weeks, and maintained at this value for the rest of the exercising period. Maximal oxygen consumption (VO2 max) was measured using a treadmill (25º inclination) in a metabolic chamber (Modular treadmill with Oxymax open circuit calorimeter, Columbus instruments, USA). The speed was gradually increased until oxygen consumption leveled off despite increased running speed and respiratory quotient (RER) approximated 1, where VO2 max was defined. The running speed at which VO2 max was obtained was defined as speedmax.

Plasma parameters. Blood samples were taken from fasted (4 hours) and fed mice at 13:00 hours. 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.

**Transcriptional changes.** Left ventricular pieces from perfused hearts were immersed in RNAlater (Qiagen, Hilden, Germany), and total RNA was extracted according to the RNeasy Fibrous Tissue kit Protocol (Qiagen Nordic, Norway). Quantification and purity of RNA was measured spectrophotometrically. Real-time PCR (qPCR) was performed in an ABI PRISM 7900 HT Fast real-time thermal cycler (19). House-keeping genes were selected on the basis of the average expression stability determined with Normfinder (3) from a pool of five candidate genes, and mRNA expression of the genes of interest was adjusted to housekeeping genes. Primer/probe sequences for housekeeping genes, transcription regulators and PPAR\(\alpha\) target genes are given in Haftsad et al. (19). Primer sequences for antioxidant enzymes are given in Khalid et al (34). Where probes are not specified, cyber green was used. Forward and reverse primer and probe sequences (5’-3’), b-type natriuretic peptide (\(bnp\)): forward: CCA-GTC-TCC-AGA-GCA-ATT-CAA, reverse: GCC-ATT-TCC-TCC-GAC-TTT-T and probe TGC-AGA-AGC-TGG-AGC-TGA-TAA-GA, atrial natriuretic factor (\(anf\)): forward: AGT-GCG-GTG-TCC-AAC-ACA, reverse: CTT-CAT-CGG-TCT-GCT-CGC and probe: TCT-GAT-GGA-TTT-CAA-GAA-CCT-GCT-AGA-CCA, calcium adenosine triphosphatase 2 (\(serca2\)): forward: TCG-ACC-AGT-CAA-CAC-CAC-CAT-CAG-G, reverse: GGG-ACA-GGG-TCA-GTA-TTC-TT and probe: # 94 in Roche Universal ProbeLibrary, \(\alpha\)-myosin heavy chain isoform (\(\alpha mhc\)): forward: TGG-TCA-CCA-ACA-ACC-CAT-ACG-AC and reverse: TGT-CAG-CTT-GTA-GAC-ACC-AGC-CTT, \(\beta\)-myosin heavy chain isoform (\(\beta mhc\)): forward: GCC-AAC-ACC-AAC-CTG-TCC-AAG-TTC and reverse: TGC-AAA-GGC-TCC-AGG-TCT-GAG-GGC, lactate dehydrogenase (\(ldh\)): forward: CAT-TGT-CAA-GTA-CAG-TTC-CTA-AGC-ATCT and reverse: TTC-CAA-TTA-CTC-GGT-TTT-TGG-GA, vascular endothelial growth factor (\(vegf\)): forward: CAA-GCC-AAG-GAG-AGC-GAG-GT-AGG-CTA and reverse: TCT-GCC-GGA-GTC-AGC-CTT-GTC-CCC-TC.

**Ex-vivo working hearts.** Myocardial glucose and palmitate oxidation was measured in isolated perfused hearts (1), and expressed as oxidation rates per gram dry weight, using a dry to wet weight ratio of 10.220.32.246 on October 14, 2017 http://jap.physiology.org/ Downloaded from
ratio of 1 to 5. Values of left ventricular contractile function, total cardiac work (pressure-volume area, PVA) and myocardial oxygen consumption (MVO₂) were then assessed by using a 1.0 F micromanometer-conductance catheter (Millar Instruments, Houston, TX) was inserted into the left ventricle through the apex and fibre-optic oxygen probes (FOXY-AL300, Ocean Optics Ltd., Duiven, Netherlands) were placed in the left atrial cannula (adjacent to the heart) and in the pulmonary trunk (20; 24). MVO₂ was calculated by the following equation: MVO₂ = [PO₂ (oxygenated perfusate) - PO₂ (coronary effluent)] • Bunsen solubility coefficient of O₂ • coronary flow. In order to determine cardiac efficiency, electrically paced hearts were exposed to different workloads (24). Steady-state values of PVA and MVO₂ were obtained at each workload in order to perform regression analysis of the relationship between PVA and MVO₂. The PVA-MVO₂ regression allows the myocardial oxygen cost to be separated in two parts: work-independent MVO₂ (y-intercept of the PVA-MVO₂ relationship) and work-dependent MVO₂ (contractile efficiency i.e the inverse slope of the PVA-MVO₂ relationship) (48). Work-dependent MVO₂ is a measure of the energy cost of excitation-contraction coupling and basal metabolism, while contractile efficiency reflects the amount of metabolic energy which is converted into mechanical work. MVO₂ was also measured in unloaded retrogradely perfused hearts before (MVO₂ unloaded) and after KCl-arrest to measure oxygen cost for basal metabolism (MVO₂ BM) (8). Oxygen cost for excitation-contraction coupling (MVO₂ ECC) was defined as the value obtained by subtracting MVO₂ BM from MVO₂ unloaded.

Citrate synthase activity and mitochondrial respiration. Citrate synthase activity, a common used marker of mitochondrial content (9; 10), was measured spectrophotometrically, using a slight modification of the method of Srere (45), and mitochondrial respiration was measured in saponin-permeabilised cardiac fibres 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 (V₀). Vₘₐₓ was obtained after addition of 2.5 mM ADP and Vₐ₉₁₉ was obtained after addition of 1 μg/mL oligomycin. O₂ flux was calculated from the negative time derivative of the oxygen concentration signal, using DatLab 4 software from Oroboros Instruments GmbH. Respiration was related to both fibre weight and CS activity to adjust for potential differences in mitochondrial content.
Statistical analysis. Data are expressed as mean ± SEM. Differences between groups were analysed 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 (bnp) or atrial natriuretic factor (anf) (Table 2). HIT induced an increase in cardiac mRNA expression of the α-myosin heavy chain isoform (amhc), whereas the β-myosin heavy chain isoform (βmhc) was reduced (Table 2). There were no transcriptional changes in these genes following MIT. Neither MIT nor HIT altered cardiac mRNA expression of sarcoplasmic reticulum calcium ATPase (serca2) (Table 2).

Aerobic capacity. HIT and MIT resulted in increased maximal oxygen consumption (VO₂ max) (Table 1). Normalization of VO₂ max to their corresponding controls revealed that the exercise-induced increase in VO₂ max was most pronounced following HIT (Table 1). The increased VO₂ max was associated with an increase in running speed at VO₂ 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 uL/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 pressure derivatives during baseline loading conditions. Preload-recruitable stroke work index (PRSWi), an index of contractility, was increased (Table 1), although end-systolic pressure-volume relationships (ESPVR) were unaltered (data not shown). Parameters of ventricular diastolic function (EDPVR and Tau value) were not altered by HIT.
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 (Figure 1). These changes were not related to changes in external cardiac work during these measurements, since cardiac output of the ex vivo perfused hearts from both the HIT and MIT group (119±6 and 125±7 mL/min/g wwt) was not different from that of their respective controls (114±5 and 123±6 mL/min/g wwt). Due to the somewhat unexpected shift in cardiac substrate utilization towards glucose oxidation following HIT, we investigated target genes of HIF-1α and found an up-regulation 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. A significant and a borderline (p= 0.087) reduction in the cardiac expression of ppara following HIT and MIT respectively was not accompanied by reduced cardiac expression of PPARα target genes (data not shown). HIT was also 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 work 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, Figure 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 (Figure 2). In contrast to HIT, we did not find MIT to alter cardiac efficiency, MVO₂ unloaded 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 (Figure 3, panel C). The increase in myocardial CS activity was not matched by a concomitant increase in \textit{pgc-1\textalpha} expression (Table 2), a finding which 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 \textit{pgc-1\textalpha} following high intensity training are only transient, as reported for skeletal muscle (39).

Mitochondrial respiration measured in permeabilized cardiac fibres demonstrated a 35% increase in state 3 respiration (maximal respiratory capacity, $V_{\text{max}}$, $p<0.005$) following HIT (Figure 3, panel B). State 3 respiration was also significantly increased when adjusted for CS activity in the fiber (29.9±2.3 vs. 36.9±2.1 nmol/min/iUCS, $p=0.044$), while we did not find HIT to induce changes in respiration after addition of oligomycin ($V_{\text{oligo}}$). 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 up-regulate cardiac expression of metabolic genes involved in FA uptake, glycolysis and glucose oxidation, and 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 both a simultaneous increase in glucose and FA oxidation (14) or unaltered glucose oxidation (11). One reason for these inconsistencies regarding phenotyping physiological hypertrophy following exercise may be variability in exercise protocols, as exercise intensity and the effect on aerobic capacity is not mentioned in previous studies addressing the impact of exercise on cardiac metabolism. We therefore designed a protocol to evaluate the cardiometabolic effect of exercise intensity in physiological hypertrophy.

In accordance with earlier reports (22; 30; 50), high intensity training (HIT) was found to be superior to moderate intensity training (MIT) with regard to increasing aerobic capacity (VO₂ 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 αMHC and decreased expression of βMHC in the heart. As the ATPase activity in the α-isoform is nearly three times higher than of the β-isoform, this may contribute to increased shortening velocity and capacity for power generation, changes that will be advantageous for preserving heart function under stress (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 towards increased glucose oxidation, while FA oxidation rates were reduced. MIT did not alter myocardial oxidation rates, which is similar to what Broderick et al. found in hearts from exercised trained rats (11). The HIT-induced shift in substrate utilization resemble changes commonly associated with 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 with 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 over-expressing Ca2+ ATPase (SERCA), associated with increased mitochondrial calcium content and pyruvate dehydrogenase activity (6). Although mitochondrial calcium content was not measures 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 an 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 non-mechanical work. Although comparative studies on humans have demonstrated lower myocardial oxygen consumption (MVO2) in athletes than in untrained controls (21; 49), the factors contributing to the reduction in MVO2 were not revealed. In the present study, HIT did not alter contractile efficiency (work-dependent MVO2, determined from the slope of the PVA-MVO2 relationship), but reduced work-independent MVO2 (unloaded MVO2) 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\textsuperscript{2+} amplitude and increased myofilament Ca\textsuperscript{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\textsuperscript{2+} sensitivity may have been counteracted by the observed shift towards the αMHC isoform, as it is thought to be energetically more expensive than βMHC isoform (18).

Although the HIT-induced reduction in unloaded MVO\textsubscript{2} may be related to the observed switch in myocardial substrate utilization (as the P/O ratio for glucose oxidation is higher compared to 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\textsubscript{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 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 citrate synthase (CS) activity in skeletal muscle. In cardiac tissue, however, only HIT increased CS activity. This result shows that the myocardium does not respond as 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 fibres, an effect that was not only due to higher mitochondrial content but also to a
higher electron transfer chain capacity, as \( V_{\text{max}} \) was found to be increased when adjusted to CS activity. These results suggest that exercise needs to be of high intensity in order to activate the intracellular pathways responsible for these mitochondrial adaptations in cardiac muscle, which may 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 \( \text{MVO}_2 \) 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 \( \text{MVO}_2 \)) represent changes that could be considered beneficial. Our data may therefore point to potential mechanisms which 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 in accordance with previous reports, high intensity training was superior with regard to its effect on whole body maximal oxygen consumption (\( \text{VO}_{2\text{max}} \)). Although both high and moderate exercise increased \( \text{VO}_{2\text{max}} \), 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 towards a faster cardiac myosin isoform, and the ability to catabolise 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.
ACKNOWLEDGEMENTS

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DISCLOSURES

None
**Table 1:** Biometric data, aerobic capacity, plasma parameters and cardiac function in mice following 10 weeks of moderate intensity continuous training (MIT), high intensity interval training (HIT), as well as in age-matched sedentary mice (SED).

<table>
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<tr>
<th></th>
<th>SED n=13</th>
<th>MIT n=12</th>
<th>SED n=14</th>
<th>HIT n=12</th>
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<tr>
<td><strong>Biometric data</strong></td>
<td></td>
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<tr>
<td>Body weight (BW, g)</td>
<td>29.2 ± 0.5</td>
<td>28.1 ± 0.6</td>
<td>25.8 ± 0.4</td>
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<tr>
<td>Heart /body weight ratio (%)</td>
<td>0.45 ± 0.01</td>
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<td>0.46 ± 0.01</td>
<td>0.50 ± 0.01*</td>
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<td>CS activity muscle (IU/g wwt)</td>
<td>14.4 ± 0.8</td>
<td>20.9 ± 1.7*</td>
<td>13.8 ± 1.0</td>
<td>18.4 ± 1.6*</td>
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<td><strong>Aerobic capacity and running speed</strong></td>
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<tr>
<td>VO2 max (mL/min/kg)</td>
<td>116±2</td>
<td>130±1*</td>
<td>117±1</td>
<td>140±2*</td>
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<tr>
<td>Speed at VO2 max (m/min)</td>
<td>18.2 ± 0.2</td>
<td>24.1 ± 1.0*</td>
<td>20.0 ± 0.7</td>
<td>32.3 ± 0.8*</td>
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<tr>
<td>Normalized VO2 max</td>
<td>1.00 ± 0.01</td>
<td>1.12 ± 0.01*</td>
<td>1.00 ± 0.01</td>
<td>1.19 ± 0.01*†</td>
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<tr>
<td>Normalized speed at VO2 max</td>
<td>1.00± 0.01</td>
<td>1.33 ± 0.01</td>
<td>1.00± 0.01</td>
<td>1.62 ± 0.01*†</td>
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<td><strong>Plasma parameters</strong></td>
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<tr>
<td>Glucose fasted (mmol/L)</td>
<td>8.5 ± 0.4</td>
<td>10.5 ± 0.2*</td>
<td>9.1 ± 0.3</td>
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<td>Glucose fed (mmol/L)</td>
<td>10.9 ± 0.2</td>
<td>10.3 ± 0.4</td>
<td>10.9 ± 0.6</td>
<td>11.7 ± 0.4</td>
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<tr>
<td>FA fasted (μmol/L)</td>
<td>923 ± 41</td>
<td>673 ± 46*</td>
<td>1180 ± 43</td>
<td>957 ± 56*</td>
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<td>TG fasted (mmol/L)</td>
<td>0.64 ± 0.02</td>
<td>0.60 ± 0.03</td>
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<td><strong>Cardiac function</strong></td>
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<td>(n = 8)</td>
<td>(n = 9)</td>
<td>(n = 8)</td>
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<td>Aortic flow (mL/min/g wwt)</td>
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<td>Coronary flow (mL/min/g wwt)</td>
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<td>Pes</td>
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<td>Ped</td>
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<td>dP/dtmax (mmHg/sec)</td>
<td>4789±64</td>
<td>4471±274</td>
<td>5419 489</td>
<td>5360±589</td>
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</table>
CS (citrate synthase), FA (fatty acids) and TG (triacylglycerol). In order to compare the effect of MIT and 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 1Fr conductance catheter inserted into the left ventricle. Steady state parameters were obtained with pre- and afterload settings of 8 mmHg and 50 mmHg, respectively. Pes and Ped (left ventricular systolic and diastolic pressure), dP/dtₘₐₓ and dP/dtₘᵋₙ (maximal slopes of systolic pressure increment and diastolic pressure decrement) and Tau (early diastolic relaxation time). Functional parameters obtained by a temporary preload reduction are EDPVR (slope of end-diastolic-pressure-volume relationships) and PRSWi (preload-recruitable stroke work index). Values are mean ± SEM, *p<0.05 vs. SED.

†p<0.05 vs. MIT for normalized values.
<table>
<thead>
<tr>
<th>Genes</th>
<th>SED</th>
<th>MIT</th>
<th>SED</th>
<th>HIT</th>
</tr>
</thead>
<tbody>
<tr>
<td>pparα</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>
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<tr>
<td>pgc1α</td>
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<td>0.79±0.08</td>
<td>1.00±0.13</td>
<td>1.12±0.15</td>
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<tr>
<td>vegf</td>
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<td>0.87±0.04*</td>
<td>1.00±0.04</td>
<td>1.20±0.07*</td>
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<tr>
<td>ldh</td>
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<td>1.11±0.06</td>
<td>1.00±0.05</td>
<td>1.16±0.04*</td>
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<tr>
<td>hk</td>
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<td>0.97±0.02</td>
<td>1.00±0.11</td>
<td>1.11±0.04*</td>
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<tr>
<td>βmhc</td>
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<td>0.64±0.08*</td>
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<tr>
<td>amhc</td>
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<td>0.93±0.04</td>
<td>1.00±0.01</td>
<td>1.12±0.02*</td>
</tr>
<tr>
<td>serca2</td>
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<td>0.97±0.05</td>
<td>1.00±0.02</td>
<td>1.00±0.02</td>
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<tr>
<td>sod</td>
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<td>0.97±0.04</td>
<td>1.00±0.02</td>
<td>1.06±0.01*</td>
</tr>
<tr>
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<td>0.95±0.05</td>
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<tr>
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<td>0.71±0.11</td>
<td>1.00±0.20</td>
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<tr>
<td>bnp</td>
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<td>1.06±0.14</td>
<td>1.00±0.08</td>
<td>1.08±0.10</td>
</tr>
</tbody>
</table>

Ppara (peroxisome proliferator-activated receptor α a), pgc1α (peroxisome proliferator activator γ coactivator-1α, vegf (vascular endothelial growth factor), ldh (lactate dehydrogenase), hk (hexokinase type 2), βmhc and amhc (α and β-myosin heavy chain isoforms), serca2 (calcium adenosine triphosphatase 2), sod (superoxide dismutase), cat (catalase), anf (atrial natriuretic factor) and bnp (B-type natriuretic peptide). mRNA levels were normalised to the geometric mean of hypoxanthine phosphoribosyltransferase, cyclophilin and succinate dehydrogenase complex subunit A. Values are mean ± SEM, n=6-8 in each group, *p<0.05 vs. SED.
Table 3: Regression analysis of the relationship between myocardial oxygen consumption (MVO$_2$) and pressure-volume work (PVA) measured in isolated working mouse hearts at varying workloads following 10 weeks of moderate intensity continuous training (MIT) and high intensity interval training (HIT). Sedentary age-matched mice were used as controls (SED).

<table>
<thead>
<tr>
<th></th>
<th>SED</th>
<th>MIT</th>
<th>SED</th>
<th>HIT</th>
</tr>
</thead>
<tbody>
<tr>
<td>contractile efficiency</td>
<td>0.38 ± 0.02</td>
<td>0.42 ± 0.14</td>
<td>0.48 ± 0.07</td>
<td>0.37 ± 0.08</td>
</tr>
<tr>
<td>work-independent MVO$_2$</td>
<td>7.5 ± 0.4</td>
<td>7.4 ± 0.7</td>
<td>7.0 ± 0.4</td>
<td>4.1 ± 0.8*</td>
</tr>
<tr>
<td>$r^2$</td>
<td>0.93 ± 0.01</td>
<td>0.90 ± 0.02</td>
<td>0.93 ± 0.02</td>
<td>0.97 ± 0.01</td>
</tr>
</tbody>
</table>

The y-intercept of the PVA-MVO$_2$ relationship represents the work-independent MVO$_2$ (expressed as mJoule/beat/g wwt), inverse of the slope of this relationship represent contractile efficiency (dimensionless) and $r^2$ is the square of the regression coefficient. Values are mean ± SEM, n=5-9 in each group. *p<0.05 vs SED.
**Figure 1:** Oxidation rates in isolated working mouse hearts following 10 weeks of moderate intensity continuous training (MIT), high intensity interval training (HIT) and in age-matched sedentary mice (SED). Bars are mean ± SEM, n= 9-11 in each group, *p<0.05 vs. SED.

**Figure 2:** Myocardial oxygen consumption measured in retrogradely perfused and unloaded hearts; (MVO$_2$ unloaded, grey bars), oxygen consumed for basal metabolism (MVO$_2$ BM, white bars) and calculated oxygen consumed for excitation-contraction coupling (MVO$_2$ ECC, black bars) following 10 weeks of moderate intensity continuous training (MIT) and high intensity interval training (HIT), and in age-matched sedentary mice (SED). Bars are mean ± SEM, n=8-11 in each group, *p<0.05 vs SED.

**Figure 3:** Mitochondrial function in saponin-permeabilized cardiac fibres obtained from mice following 10 weeks of moderate intensity continuous training (MIT, panel A, white bars) and high intensity interval training (HIT, panel B, hatched bars). Sedentary age-matched mice were used as control groups (SED, black bars) and CS activity cardiac muscle (panel C). Respiration in the presence of glutamate (10 mM) and malate (2 mM) ($V_0$), following addition of 2.5 mM ADP ($V_{max}$) and after addition of 1 μg/mL oligomycin ($V_{oligo}$). The respiratory control ratios ($V_{max}$; $V_{oligo}$) was 2.9±0.3 and 3.1±0.4 for SED and MIT respectively, and 2.4 ± 0.3 and 2.8± 0.4 for SED and HIT respectively. Bars are mean ± SEM, n=8 in each group, *p<0.05 vs. SED.
REFERENCES


