Exercise training restores aerobic capacity and energy transfer systems in heart failure treated with losartan

Ole Johan Kemia,b,1, Morten Andre Høydala,1, Per Magnus Harama,c, Anne Garnierd,e, Dominique Fortind,e, Renee Ventura-Clapierd,e, Øyvind Ellingsena,f,*

a Department of Circulation and Medical Imaging, Faculty of Medicine, Norwegian University of Science and Technology, Trondheim, Norway
b Institute of Biomedical and Life Sciences, University of Glasgow, United Kingdom
c Department of Cardiothoracic and Vascular Surgery, University Hospital North Norway, Tromsø, Norway
d INSERM, U769, Châtenay-Malabry, F-92296, France
e Université Paris-Sud, UMR-S0769, IFR 141, Châtenay-Malabry, F-92296, France
f Department of Cardiology, St Olavs Hospital, Trondheim, Norway

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Abstract

Objective: Clinical and experimental studies demonstrate that exercise training improves aerobic capacity and cardiac function in heart failure, even in patients on optimal treatment with angiotensin inhibitors and beta-blockers, but the cellular mechanisms are incompletely understood. Since myocardial dysfunction is frequently associated with impaired energy status, the aim of this study was to assess the effects of exercise training and losartan on myocardial systems for energy production and transfer in heart failure.

Methods: Maximal oxygen uptake, cardiac function and energy metabolism were assessed in heart failure after a myocardial infarction induced by coronary artery ligation in female Sprague–Dawley rats. Losartan was initiated one week after infarction and exercise training after four weeks, either as single interventions or combined. Animals were sacrificed 12 weeks after surgery.

Results: Heart failure, confirmed by left ventricular diastolic pressure \( \geq 15 \) mmHg and by \( \geq 20 \) mmHg drop in peak systolic pressure, was associated with 40% lower aerobic capacity and significant reductions in enzymes involved in energy metabolism. Combined treatment yielded best improvement of aerobic capacity and ventricular pressure characteristics. Exercise training completely restored aerobic capacity and partly or fully restored creatine and adenylate kinases, whereas losartan alone further reduced these enzymes. In contrast, losartan reduced left ventricle diastolic pressure, whereas exercise training had a neutral effect.

Conclusion: Exercise training markedly improves aerobic capacity and cardiac function after myocardial infarction, either alone or in combination with angiotensin inhibition. The two interventions appear to act by complementary mechanisms; whereas exercise training restores cardiac energy metabolism, mainly at the level of energy transfer, losartan unloads the heart by lowering filling pressure and afterload.

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Keywords: Myocardial infarction; Heart failure; Endurance training; Losartan; Metabolism

1. Introduction

Exercise training is emerging as an important complementary intervention in heart failure [1,2]. Exercise enhances aerobic capacity, attenuates left ventricular (LV) dilatation, regresses cellular hypertrophy, and improves cardiomyocyte contractility and Ca\(^{2+}\) handling [3–6]. Losartan, an angiotensin II type 1 (AT\(_1\)) receptor antagonist, resembles angiotensin
converting enzyme (ACE) inhibitors by improving hemodynamics and cardiac afterload [7–10], and has some of the same benefits as exercise training [11–15]. Whereas angiotensin inhibition unloads the heart and reduces myocardial energy requirements, exercise requires higher energy expenditure and may challenge adaptive mechanisms.

Depressed myocardial bioenergetic characteristics in heart failure correlate with energetic failure and reduced working capacity [16,17]. Morphological and functional abnormalities in mitochondria are commonly reported in heart failure, and are associated with changes in mitochondrial biogenesis and its master transcriptional regulator, the peroxisome proliferator-activated receptor gamma co-activator 1α (PGC-1α) [18–21]. In addition, less total creatine kinase (CK) and alterations in its isoenzyme pattern contribute to depressed energy transfer and utilization [22–26]. These changes are responsible for lower PCR/ATP ratio and CK fluxes, and for reduced primary myocardial energy reserve. In particular, CKs facilitating effect on the sarcoplasmic reticulum calcium ATPase (SERCA-2a) is important for relaxation during diastole and consequently on contractility [22]. Although exercise improves symptoms and partly restores myocardial dysfunction in heart failure, little is known about the effects on myocardial energy metabolism [27]. Voluntary wheel running in heart failure induced by aortic constriction did not improve cardiac metabolism, but uncertain exercise intensity and the experimental model may have affected the outcome [16].

The working hypothesis of the present study was that exercise training ameliorates myocardial energetic state in heart failure. Since angiotensin inhibition is part of standard therapy in heart failure after myocardial infarction (MI) [12], we studied the effects of exercise training with or without simultaneous treatment with losartan. It is conceivable that combined treatment is advantageous, as exercise training and losartan may have complementary effects on maximal oxygen uptake (VO2max), systolic and diastolic pressures, and enzymes involved in energy transfer and production.

2. Methods

2.1. Study design and animals

Left coronary artery ligation (MI) or sham-operation by thoracotomy alone was performed in adult female Sprague–Dawley rats (Mollegaards Breeding Center Ltd, Lille Skensved, Denmark) as previously described [4,28,29]. Anesthesia during surgery was 1% isoflurane mixed with 30% O2/70% N2O. Buprenorphine (0.05 mg Temgesic, Reckitt and Coleman, Hull, UK) was given subcutaneously immediately and 8 h after surgery. Seven days post-surgery, echocardiography was performed during sedation (40 mg/kg ketamine hydrochloride and 8 mg/kg xylazine intraperitoneally) to confirm MI. Briefly, the endocardial circumference was traced in 2-dimensional short axis mode, whereupon

### Table 1

<table>
<thead>
<tr>
<th>Study design and group assignment</th>
<th>Myocardial infarction</th>
<th>Losartan</th>
<th>Exercise training</th>
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<tbody>
<tr>
<td>SHAM SED PL</td>
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<tr>
<td>SHAM TR PL</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<tr>
<td>MI SED PL</td>
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<td>MI SED LOS</td>
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<td>MI TR LOS</td>
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Infarct size was estimated as percentage infarcted (non-contracting) part of total circumference, as described in detail by Litwin et al. [30]. Infarct sizes were estimated to 40±5% (data not shown). One week after the MI, losartan (Merck & Co, Whitehouse Station, NJ) or placebo was given in drinking water (2 g/L, ad libitum), whereas an eight week exercise training program or a sedentary control period was initiated 4 weeks after MI. In total, animals were assigned to 6 different groups; see Table 1. The study conforms to the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 85-23, revised 1996), and was approved by the Institutional Animal Research Ethics Council.

2.2. Maximal oxygen uptake

The rats warmed up by treadmill running at a 25° inclination for 20 min at 50–60% of VO2max. Thereafter, the treadmill velocity was increased by 0.03 m s−1 every 2 min until VO2 plateaued despite of increased workload, as previously described [31]. In the exercising animals, VO2max was measured every week and band speed was adjusted to maintain the same relative exercise intensity. In sedentary rats, VO2max was measured before and after the experimental period. VO2max was based upon empirical studies [32], normalized to body mass raised to the power of 0.75 to avoid confounding factors related to different body weights. VO2max is often related to body mass without allometric scaling; however, this underestimates VO2max in heavier individuals, as VO2max and body mass are not directly proportional to each other [33,34].

2.3. Aerobic exercise training

After 10 min of warm-up as in the VO2max test, the rats ran on a treadmill (25°) for 1.5 h, alternating between 8 min at an exercise intensity corresponding to 85–90% of VO2max, and 2 min active recovery at 50–60%. Exercise training was performed 5 days per week over 8 weeks, as previously described [31]. Exercise training was not initiated until degenerative remodeling had subsided, i.e. 4 weeks after MI, as previously reported in the same post-MI heart failure rat model [29], since starting exercise training earlier may increase scar thinning [12].
the coupled enzyme assay of glucose-6-phosphate dehydrogenase and hexokinase. Production of NADPH in the absence (AK) and in the presence (CK) of PCR was measured spectrophotometrically at 340 nm as previously described [16]. Lactate dehydrogenase (LDH) was assayed at 30 °C, pH 7.5 by measuring the disappearance of NADH according to Bergmeyer [35]. CK and LDH isoenzymes were separated using agarose (1%) gel electrophoresis performed at 250 V (for CK) and 200 V (for LDH) for 90 min. Individual isoenzymes were resolved either by incubation of the gels with the coupled enzyme system (CK), or a commercial detection system (LDH reagent kit, Sigma, France). The 2 homo-tetramers (H4 and M4) and 3 hetero-tetramers (H3M, H2M2, and HM3) of LDH were resolved. H-subunit of LDH was calculated as follows: H-LDH = H4 + 3/4 H3M + 1/2 H2M2 + 1/4 HM3. Cytochrome C oxidase (COX) was assayed by measuring the disappearance of reduced cytochrome C at 550 nm (pH 7.4, 30 °C), and citrate synthase (CS) activity was measured at 412 nm (pH 8, 30 °C) according to Srere [36].

2.6. Real-time quantitative RT-PCR

Real-time reverse transcription-polymerase chain reaction (RT-PCR) was used to quantify mRNA expression levels of PGC-1α, and of the COX subunits I (COX-I, encoded by the mitochondrial genome) and IV (COX-IV, encoded by the nuclear genome) as previously described [37]. The housekeeping gene cyclophilin A (CycA) was used for normalization. Total cardiac RNA was isolated from frozen tissue samples (10–20 mg) using the Trizol reagent technique. Oligo-dT first-strand cDNA was synthesized from 2 mg total RNA using SuperscriptTM II reverse transcriptase (Invitrogen, France). Real-time PCR was performed using the SYBR®Green technology on a rapid thermal cycler (LightCycler, Roche Diagnostics, France) as previously described [37]. The following primers were used: PGC-1α, NM_031347, forward primer GCAGTGAAGCCGATGAAGAAC; and CycA, forward primer TGGGAGTGTTGTGAAGAGTGA, reverse primer TGAGAGAAGTAGTAGGACGGC; COX-IV, X15029, forward primer AGCAGGAATAGTAGGGACAGC, reverse primer GCAGGAAGTATGGAGCAGGC; COX-IV, X15029, forward primer TGGGAGTGTTGTGAAGAGTGA, reverse primer GCAGGAAGTATGGAGCAGGC; and CycA,

### Table 2

<table>
<thead>
<tr>
<th>SHAM-control</th>
<th>Post-MI heart failure</th>
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<tr>
<td>LVEDP</td>
<td>SED PL</td>
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<tr>
<td>mmHg</td>
<td>4±1</td>
</tr>
<tr>
<td>LVSP</td>
<td>mmHg</td>
</tr>
<tr>
<td>+dp/dtmax</td>
<td>mmHg/ ms</td>
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<tr>
<td>−dp/dtmax</td>
<td>mmHg/ ms</td>
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Data are mean±SEM with 8 animals per group. LVEDP: left ventricle end-diastolic pressure; LVSP: left ventricle peak systolic pressure; dp/dtmax: peak rate of left ventricular pressure rise (+: contraction) and fall (−: relaxation); MI: myocardial infarction; SED: sedentary; PL: placebo; LOS: losartan. Kruskal–Wallis test indicated difference from SHAM-control groups: *P<0.01; difference from MI SED PL: †P<0.001, and ‡P<0.05.
NM_017101, forward primer GAGCAGTGGGGAGAAAGGAT, reverse primer CTTGCCATCCCGCCACTCAG. Fluorescence representing each gene was normalized to CycA mRNA content to compensate for variation in input RNA amounts and efficiency of reverse transcription, and corrected for the amount of RNA relative to muscle weight [37].

2.7. Statistics

Data are presented as mean ± SEM. The significance level was set to \( P < 0.05 \). Kruskal–Wallis with appropriate post-hoc testing evaluated observations between groups, whereas associations were assessed with Pearson’s correlation coefficients.

3. Results

3.1. Maximal oxygen uptake and cardiac performance

Four weeks after the MI, aerobic capacity, measured as \( V_O^{2max} \), was 40% reduced. Aerobic exercise increased \( V_O^{2max} \) by 70% in both healthy sham-operated and heart failure animals. Thus, aerobic capacity in heart failure was restored to levels above sham-operated sedentary healthy animals (Fig. 1). Losartan alone led to a 20% improvement, whereas combined treatment with both losartan and exercise training yielded the same effect as exercise alone.

Heart failure was confirmed by increased LV end-diastolic pressure (LVEDP), decreased LV peak systolic pressure (LVPSP)

Fig. 2. Enzyme activity of citrate synthase (A), cytochrome c oxidase (B), and mRNA expression levels of cytochrome c oxidase I (C), cytochrome c oxidase IV (D), and peroxisome proliferator-activated receptor gamma coactivator 1 \( \alpha \) (PGC1\( \alpha \); E). SHAM: healthy controls; MI: myocardial infarction; SED: sedentary; TR: endurance training; PL: placebo; LOS: losartan. Data are means ± SEM. Significantly different from MI SED PL: \( *P < 0.01 \) and \( P < 0.05 \); significantly different from SHAM SED PL: \( #P < 0.01 \) and \( #P < 0.05 \). PGC1\( \alpha \) data revealed a strong trend, \( P = 0.10 \), towards decreased levels in MI SED PL and MI SED LOS by Kruskal–Wallis omnibus test. Lower \( P \) values on multiple comparisons by Kruskal–Wallis post-hoc test are therefore not valid. Nonetheless, MI SED LOS (\( P < 0.01 \)) and MI SED PL (\( P < 0.05 \)) were found different from SED PL. A regular independent sample \( t \)-test between SHAM SED PL and other groups supports the trend of decreased activity in MI SED PL and MI SED LOS (\( \ddagger P < 0.01 \)). In both of the trained MI groups, there was no difference compared to SHAM animals.
and reduced maximal rates of contraction (+dP/dt\text{max}) and relaxation (−dP/dt\text{max}; Table 2). Losartan reduced LVEDP by 8 mmHg, whereas exercise training had a neutral effect. However, all of the animals still remained above the cut-off line of 15 mmHg that previously has been reported to distinguish between MIs that lead to congestive heart failure or not in rats [38]. None of the treatments affected LVSP or maximal rates of contraction and relaxation. Also, exercise training did not alter cardiac pressures in sham-operated animals. However, intraventricular pressure was recorded during anesthesia and may therefore deviate from that of conscious animals.

### 3.2. Energy production systems

CS is important in the citric acid cycle and is commonly used as a marker of mitochondrial content. CS activity was reduced in heart failure, and was partially restored by exercise training (Fig. 2A). Losartan had no effect alone, but tended to accentuate the beneficial effect when combined with exercise training.

The activity of COX, the terminal electron acceptor in the electron transport chain, also decreased in failing hearts (Fig. 2B). A reduction occurred with either exercise training or losartan, but not with the combination. As COX subunits are encoded by both the nuclear and the mitochondrial genome, we investigated mRNA expression levels of COX-I (mitochondrial) and COX-IV (nuclear) subunits. Both subunits tended to decrease in heart failure, while none of the treatments enabled a restoration (Fig. 2C–D).

We also measured the mRNA expression of PGC-1α, an important transcriptional co-activator for mitochondrial biogenesis and a key regulator for mitochondrial function. We noticed a trend (P=0.10) to decreased levels in heart failure, and conversely, a restoration towards normal levels after exercise training (Fig. 2E).

LDH reversibly catalyzes the conversion of pyruvate to lactate, and oxidizes NADH to NAD+. In heart failure, both total LDH (−20%; NS) and the heart isoform (H-LDH, −28%, \( p<0.05 \)), which is associated with lactate utilization, tended to decrease, whereas the muscle isoform (M-LDH) remained unchanged (Fig. 3). Treatment by either exercise training or losartan alone induced a marked further reduction (20–40%) in the activity of both isoforms and in total LDH. In contrast, the combined interventions resulted in an LDH profile similar to healthy hearts.

### 3.3. Energy transfer systems

CK is important for high-energy transfer in the myocardium. As consistently reported in heart failure[22–26], total CK and specific mitochondrial (mi), MM and MB-isoform enzyme activities were reduced in heart failure (Fig. 4A–D). Exercise training alone or in combination with losartan restored the activity levels of total CK, MM-CK and MB-CK, whereas mi-CK was only increased significantly when exercise training was combined with losartan. In contrast, losartan alone had no effect on the CK system.

Energy transfer can also be supported by AK, which produces ATP (and adenosine monophosphate, AMP) by transferring a phosphate group between ADP molecules. In heart failure, activity levels of AK were decreased. Exercise training increased AK activity towards the level of healthy animals, whereas no change occurred in healthy animals (Fig. 4E). The mi-CK-to-CS ratio was also reduced in heart failure, evidencing that mi-CK is more sensitive to changes than other mitochondrial enzymes. Exercise training restored this ratio, particularly when combined with losartan (Fig. 4F).

The pattern of dysregulation, and subsequent restoration in all isoforms in the CK phospho-transfer systems correlated well with alterations in VO\text{2}\text{max} (\( r=0.82–0.92, \ P<0.03 \)), and especially with mi-CK/CS ratio (\( r=0.92, \ P=0.01 \)) and exercise training.
MM-CK ($r=0.86$, $P=0.025$). Other enzymes, mainly involved in energy production, did not correlate significantly with $VO_{2\text{max}}$.

4. Discussion

The working hypothesis of the present study was that the beneficial effects of exercise training in heart failure associate with an improved energetic state of the heart. We report that enzymes involved in energy transfer systems in the LV are increased towards the levels of healthy controls after exercise training. These changes occur in parallel with increased aerobic capacity. Our data suggest that exercise training and losartan act by different mechanisms; losartan by unloading the heart, and training by increasing metabolic capacity. Although losartan alone mildly increased $VO_{2\text{max}}$, adding losartan to exercise training had little additive effect. These observations suggest that the current exercise training program stimulates $VO_{2\text{max}}$ to adapt to maximal levels, as also indicated by the plateau after 6 weeks of training. Enhanced energy metabolism and transfer, and increased aerobic capacity may be one of several mechanisms by which exercise training reduces morbidity and mortality in heart failure patients [3,39,40].

4.1. Energy production (CS, COX, PGC-1α, and LDH)

It is well established that increased $VO_{2\text{max}}$ at least partly relates to cardiac function [41]. At the cellular level, cardiomyocyte fractional shortening, diastolic relaxation, and calcium handling improve in both healthy [4,42–45] and heart failure [4–6] animals. Production of ATP and transfer of high-energy phosphates are important for maintenance of adequate...
cardiomyocyte function. Although exercise alone to some extent improved enzymes involved in energy production, the most pronounced effect occurred when exercise was combined with losartan. The main effect of losartan was to unload the heart in diastole, whereas it only moderately improved aerobic capacity, as previous shown [4,14,29,46]. In contrast, exercise did not affect cardiac pressures significantly, indicating that unloading the heart by angiotensin inhibition may be important to fully restore energy production.

As PGC-1α regulates mitochondrial biogenesis[18–21], correlates well with mitochondrial respiration [37], and controls cardiac function through metabolic changes [47], the exercise-induced improvement of PGC-1α status may contribute to improved energy production, cardiac contractility and VO₂max in heart failure[4–6]. Combined treatment with losartan and exercise also normalized LDH activity. Restoring energy production to normal sham levels may, however, not suffice to fully normalize contractile or metabolic function, as the myocardium also adapts to heart failure by switching from mainly utilizing fatty acids to utilizing glucose [20,24].

4.2. Energy transfer (CK and AK)

In the present study exercise, but not losartan, increased the expression of enzymes involved in energy transfer systems. VO₂max changed in parallel with alterations in the CK energy transfer systems, which suggests that cardiac bioenergetics might be related to VO₂max in heart failure. mi-CK, which is important for feedback control of mitochondrial respiration and the ability to meet increasing energetic demands [16,17], appears to be more affected by heart failure than other mitochondrial proteins. Although not proving a cause–effect relationship, the close correlation between mi-CK activity and exercise capacity in heart failure, as demonstrated after pressure overload [17] and after MI in the present study, suggests a biologically important link.

Other isoforms of CK are important for contractile force and kinetics by supporting ATP regeneration. In particular, the MM-CK-isoform controls the ATP/ADP ratio close to the myosin filaments and SERCA-2a[22,48–50]. Restored MM-CK activity toward healthy levels after exercise training may also be associated with improved pH regulation and myofilament sensitivity in heart failure [4], since CK buffers acidosis [48]. In contrast, losartan had a neutral effect on CK energy transfer in heart failure, whereas ACE inhibitors improved cardiac energy metabolism [51,52].

Changes in AK followed a similar pattern to CK in the present study. Exercise training corrected the depressed AK in heart failure, while losartan had no effect. Thus, CK and AK may both maintain an energy reserve capacity that allows for fast ATP generation on demand.

Although LV remodeling post-MI is not aggravated in hearts of CK-deficient mice [53], the myocardium is characterized by significant hypertrophy and dilation, reduced perfusion and maximal contraction velocity, and altered contractile and metabolic reserve, suggesting that adaptive mechanisms cannot fully compensate [54]. In addition, lack of AK can be compensated for in the absence of metabolic challenge, but under hypoxia AK1-knockout hearts display compromised energetic and impaired cardioprotective signaling [55]. However, none of CK, AK, or the energy production systems work alone, but in concert with other regulatory networks. Hence, multiple alterations in energy production and transfer systems in heart failure may compromise cardiac function. It is therefore not surprising that beneficial effects of exercise in the heart also encompass restoration of energy metabolism together with excitation–contraction coupling[4,56–58].

As exercise training in heart failure activates signal pathways that promote a shift towards a more physiologically consolidated heart, such as activation of the Akt pathway [56,57], it is tempting to speculate that this activation can initiate coordinated improvement of contractile and metabolic features. Also, we studied female rats; the standard model for prolonged studies in our laboratories as this minimizes confounding by e.g. changes in body mass. Hence, the possibility remains that the observed effects are sex-specific, although our experience suggests that VO₂max and the myocardium adapt independent of sex [31].

5. Conclusion

The present results demonstrate that aerobic exercise training provides an important supplement to medical treatment of heart failure by angiotensin inhibitors. The two interventions complement each other in counteracting a dysfunctional cardiac phenotype, since different mechanisms are involved. Aerobic exercise training enhances aerobic capacity and reduces the cardiac metabolic myopathy in heart failure, especially at the level of energy transfer, whereas losartan unloads the heart by lowering filling pressure and afterload.

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