Aerobic Fitness Is Associated With Cardiomyocyte Contractile Capacity and Endothelial Function in Exercise Training and Detraining

Ole Johan Kemi, MSc*; Per Magnus Haram, BSc*; Ulrik Wisløff, PhD; Øyvind Ellingsen, MD, PhD

Background—Physical fitness and level of regular exercise are closely related to cardiovascular health. A regimen of regular intensity-controlled treadmill exercise was implemented and withdrawn to identify cellular mechanisms associated with exercise capacity and maximal oxygen uptake ($V_O_2$ max).

Methods and Results—Time-dependent associations between cardiomyocyte dimensions, contractile capacity, and $V_O_2$ max were assessed in adult rats after high-level intensity-controlled treadmill running for 2, 4, 8, and 13 weeks and detraining for 2 and 4 weeks. With training, cardiomyocyte length, relaxation, shortening, $Ca^{2+}$ decay, and estimated cell volume correlated with increased $V_O_2$ max ($r=0.92, -0.92, 0.88, -0.84, 0.73; P<0.01$). Multiple regression analysis identified cell length, relaxation, and $Ca^{2+}$ decay as the main explanatory variables for $V_O_2$ max ($R^2=0.87, P<0.02$). When training stopped, exercise-gained $V_O_2$ max decreased 50% within 2 weeks and stabilized at 5% above sedentary controls after 4 weeks. Cardiomyocyte size regressed in parallel with $V_O_2$ max and remained (9%) above sedentary after 4 weeks, whereas cardiomyocyte shortening, contraction/relaxation- and $Ca^{2+}$-transient time courses, and endothelium-dependent vasorelaxation regressed completely within 2 to 4 weeks of detraining. Cardiomyocyte length, estimated cell volume, width, shortening, and $Ca^{2+}$ decay and endothelium-dependent arterial relaxation all correlated with $V_O_2$ max ($r=0.85, 0.84, 0.75, 0.63, -0.54, -0.37; P<0.01$). Multiple regression identified cardiomyocyte length and vasorelaxation as the main determinants for regressed $V_O_2$ max during detraining ($R^2=0.76, P=0.02$).

Conclusions—Cardiovascular adaptation to regular exercise is highly dynamic. On detraining, most of the exercise-gained aerobic fitness acquired over 2 to 3 months is lost within 2 to 4 weeks. The close association between cardiomyocyte dimensions, contractile capacity, arterial relaxation, and aerobic fitness suggests cellular mechanisms underlying these changes. (Circulation. 2004;109:2897-2904.)

Key Words: exercise ▪ myocytes ▪ hypertrophy ▪ contractility ▪ endothelium

Several lines of evidence suggest that regular physical exercise not only improves fitness and aerobic capacity but also reduces morbidity and mortality. Maximal oxygen uptake ($V_O_2$ max) has emerged as an important clinical referent after epidemiological studies identified it as a major independent predictor of cardiovascular morbidity and mortality. For successful implementation into standard prevention and therapy, cellular and molecular mechanisms of positive health effects need to be identified.

The present study is based on the notion that $V_O_2$ max is closely related to myocardial function. Accordingly, changes in cardiomyocyte size and function parallel those observed in $V_O_2$ max when an exercise regimen is implemented and withdrawn. Whereas regular exercise is known to increase cardiomyocyte function and dimensions as well as endothelium-dependent arterial relaxation, the response to detraining has yet to be defined. In humans, detraining decreases $V_O_2$ max, but myocardial and arterial effects remain unclear. Reported regression of training-induced hypertrophy ranges from none to 20% over a period of 3 weeks. Experimental data suggest hypertrophy regression at varying time courses with no account on cardiomyocyte or arterial function. Thus, the aim of the present study was to assess the cardiomyocyte contractile capacity and endothelium-dependent arterial relaxation changes in animals undergoing a controlled program of exercise training and detraining. We report $V_O_2$ max and associated changes in cardiomyocyte contractile capacity and endothelial function during detraining after 10 weeks of regular exercise and analyze correlations derived from previous 2- to 13-week training experiments in our laboratory.
between 8 minutes at 85% to 90% of V\textsubscript{O2max} and 4 weeks (DETR4, V\textsubscript{O2max} stabilized) with corresponding sedentary controls (SED10, SED2, SED4). Time course of training was similar for all groups. Data are mean\pm SD. TR/DETR vs sedentary: *P<0.01, †P<0.05. End point vs baseline: ‡P<0.01, §§P<0.05.

Methods

Study Design

For training-detraining experiments, a total of 36 female adult Sprague-Dawley rats (Møllegaards Breeding Center Ltd, Skensved, Denmark) were included and maintained as previously described.5,6 Rats were randomized into 6 groups of either training/detraining or sedentary control, with 6 rats in each group. Groups TR10, DETR2, and DETR4 performed training until V\textsubscript{O2max} was stable at a high level for 3 consecutive weeks (which occurred after 10 weeks), and then remained sedentary (ie, detraining) for 2 or 4 weeks, respectively. TR10 rats were euthanized 24 hours after the last exercise bout. DETR2 rats were euthanized when \approx50% of the exercise-gained V\textsubscript{O2max} was lost, which occurred after 2 weeks, and DETR4 rats after 4 weeks of detraining, when V\textsubscript{O2max} had been stable at a low level for 3 consecutive weeks. Corresponding sedentary control groups were SED10, SED2, and SED4. Detrained animals were euthanized 1 week after the latest V\textsubscript{O2max} test. Thus, during detraining, animals were tested once a week, and when DETR4 rats showed an \approx50% decrease, DETR2 were not tested but euthanized. DETR4 were euthanized 1 week after no further decrease occurred. In DETR2, 2 rats were removed because of poor running, together with 2 corresponding controls. The relationships between V\textsubscript{O2max} and cellular properties during training were investigated with unexplored data from previous studies6-18 (the data appear in Figure 2). The Norwegian Council for Animal Research approved the experimental protocols.

\textbf{VO2max and Training}

V\textsubscript{O2max} was measured during treadmill running in a metabolic chamber as previously described5,6,18 at the start of every week in training/detraining animals to adjust training intensity and before and after the training period in the sedentary group. Training rats performed interval running 1 h/d, 5 d/wk, on a 25° inclined treadmill. After a 20-minute warm-up at 50% to 60% of V\textsubscript{O2max}, exercise intervals alternated between 8 minutes at 85% to 90% of V\textsubscript{O2max} and 2 minutes at 50% to 60%. Sedentary rats performed treadmill running for 15 minutes on a flat treadmill at 0.15 m/s for 2 d/wk to maintain running skills, which did not yield any training response; previous experiments indicate that this intensity corresponds to \approx45% of V\textsubscript{O2max}.

Cardiomyocyte Contractility, Calcium Handling, and Dimensions

Left ventricular myocytes were isolated as previously described with a modified Krebs-Henseleit Ca\textsuperscript{2+}-free buffer.5 Collagenase II (250 IU/mL, Worthington), BSA (Sigma Chemical), and CaCl\textsubscript{2} stepwise to 1.2 mmol/L were subsequently introduced. Ventricles were weighed after perfusion. Cells attached to laminin-coated coverslips rested 1 hour in HEPES buffer before 20 minutes of loading with 2 \mu mol/L fura 2-AM (Molecular Probes) and were placed into a cell chamber (37°C) on an inverted microscope (Diaphot-TMD, Nikon) and stimulated electrically as previously described.6,13 A 500-Hz rotating mirror alternated excitation wavelength between 340 and 380 nm, and 510-nm fluorescence emission was counted with a photomultiplier (D-104, Photon Technology International) and expressed as the ratio of the 2 excitation wavelengths. Cell shortening and relaxation were analyzed with video/edge-detection (model 104, Crescent Electronics). Ten stable, consecutive contractions at each stimulation frequency (2, 5, 7, and 10 Hz, and thereafter at 1 Hz to ensure that cells were intact) were studied in 5 to 10 cells per animal. From each animal, 150 cells not introduced to fura 2-AM and without morphological alteration were measured for length and midpoint width. Cell volume was estimated as cell length x width x 0.00759, as established by 2D light and 3D confocal microscopy.19

Vascular Function

L-shaped holders were inserted into the lumen of 2- to 4-mm segments of the common carotid arteries; one holder was connected to a force-displacement transducer and the other to a micrometer in organ baths containing Krebs buffer and indomethacin.20 After gradually increasing tension to 1000 mg and exposure to 60 mmol/L K\textsuperscript{+}, 3\times10\textsuperscript{-7} mol/L phenylephrine, and 10\textsuperscript{-4} mol/L acetylcholine to ensure reactivity, segments were equilibrated 30 minutes before experiments started. Four segments from each animal were precontracted with phenylephrine (3\times10\textsuperscript{-7} mol/L) and relaxed with cumulative doses of acetylcholine (2 segments) and Na\textsuperscript{+} nitroprusside (1 segment), whereas 1 segment was also pretreated with 10\textsuperscript{-4} mol/L N\textsuperscript{\textsuperscript{-}nitro-L-arginine methyl ester (L-NAME) before exposure to acetylcholine.

Allometric Scaling

In addition to exercise, differences in cardiac muscle weight and V\textsubscript{O2} may result from altered body mass.21 According to dimensional analysis and empirical studies, V\textsubscript{O2} should be expressed in relation to body mass raised to the power of 0.75,22 whereas ventricular mass should be expressed with the scaling exponent 0.78, which empirically is the best approximation when lean body mass is unavailable.23

Statistics

Data are expressed as mean\pm SD. EC\textsubscript{50} values were obtained as previously described.24 The Friedman test, Wilcoxon paired samples t test, and appropriate procedures for multiple comparisons determined changes in V\textsubscript{O2max}, whereas the Mann-Whitney test determined different cellular data, cardiac weights, and arterial function; a univariate repeated-measures ANOVA including Scheffé post hoc tests verified the differences. Relationships were assessed by Pearson’s correlation coefficient and complementary univariate, forward and backward linear regression analyses. V\textsubscript{O2max} was modeled using cardiomyocyte volume, length, width, fractional shortening, time to half contraction and relaxation, and time to half Ca\textsuperscript{2+} peak and decay, and vasorelaxation EC\textsubscript{50} for detraining as explanatory variables, with P>0.05 as exclusion criterion.

Results

Both exercise training and detraining led to substantial increase and regression of aerobic fitness and V\textsubscript{O2max}, which closely corresponded to changes in cardiomyocyte contractile capacity, Ca\textsuperscript{2+} handling, and arterial endothelium function.

\textbf{VO2max During Training and Detraining}

As previously reported,6,18 regular high-intensity interval running increased V\textsubscript{O2max} substantially. After 8 to 10 weeks...
of training, \( \dot{V}_O2 \) max stabilized 37% above baseline and 26% above sedentary. During detraining, exercise-gained \( \dot{V}_O2 \) max decreased 50% within 2 weeks and stabilized 5% above sedentary after 4 weeks (Figure 1). In trained animals, univariate analysis of the data revealed that cardiomyocyte length, relaxation, shortening, \( Ca^{2+} \) decay, and volume correlated strongly with \( \dot{V}_O2 \) max (Figure 2). Backward multiple regression identified cell length, diastolic relaxation, and \( Ca^{2+} \) decay as the main factors for \( \dot{V}_O2 \) max; unstandardized coefficients \( b \) 0.95±0.09, \( P < 0.01 \); 0.71±0.54, \( P < 0.01 \); and -1.02±0.42, \( P < 0.02 \), respectively; residual SD=4.63, adjusted \( R^2 = 0.76 \).

### Cardiomyocyte Morphology and Function

Training increased ventricular weights and cardiomyocyte dimensions and improved contractility and \( Ca^{2+} \) handling in the heart (Figure 1). Animals randomized for detraining also increased cardiomyocyte width and length by 20% to 22% and estimated volume by 46% (Figure 4). During
detraining, the responses varied slightly; cell width regressed completely within 2 weeks, whereas length (7% to 5%) and volume (15% to 9%) remained enlarged after 2 and 4 weeks, respectively, ie, similar to $V_o_2\text{max}$. Parallel changes occurred in cardiac weights, which regressed toward sedentary within 4 weeks of detraining (Table).

Cardiomyocyte Ca$^{2+}$/H$^{+}$ handling and intrinsic contractility were assessed at physiological cell stimulation frequencies (Figure 5). Within 2 weeks of detraining, the $\approx 30\%$ increase in cardiomyocyte fractional shortening regressed almost completely. Diastolic and systolic fura $2$ Ca$^{2+}$/H$^{+}$ ratios and amplitude of Ca$^{2+}$ transient were largely unaffected by training/detraining. The increase in cardiomyocyte contractility seemed to be associated with higher myofilament Ca$^{2+}$ sensitivity. The Ca$^{2+}$ sensitivity index (cell shortening/Ca$^{2+}$ ratio amplitude) was elevated at 7 to 10 Hz electrical stimulation after 10 weeks of training and reversed to sedentary values during detraining. As shown in Figure 6, rates of both contraction and relaxation were increased, with parallel changes in Ca$^{2+}$ handling. These changes prevailed to some extent after 2 weeks of detraining but not by week 4.

Endothelium-Dependent Arterial Relaxation
Endothelium-dependent arterial relaxation increased significantly after regular exercise training. After 10 weeks, the magnitude of acetylcholine-induced relaxation increased by 13% and EC$_{50}$ for agonist decreased 4-fold, whereas maximal absolute relaxation (R$_{max}$) leveled off 24% above sedentary (Figure 7). With detraining, all effects reversed within 2 weeks. The 7% and 2-fold larger EC$_{50}$ and 11% increased R$_{max}$ after cumulative Na$^{+}$ nitroprusside addition in trained animals indicate a transient enhanced sensitivity to nitric oxide (NO), because it vanished within 2 weeks of detraining.

Discussion
The present training-detraining experiments identified 2 distinctive cellular factors associated with changes in aerobic fitness, one closely correlated with cardiac myocyte size and function and another related to endothelium-dependent arterial relaxation. Although both may be important for the salutary health effects of exercise, the myocardial mechanisms seem to be more closely correlated to $V_o_2\text{max}$.

Cardiomyocyte Contractile Capacity
During long-term adaptation to regular exercise, the heart meets increased needs of peripheral tissues by matching pump capacity to afford sufficient cardiac output to transport oxygen corresponding to $V_o_2\text{max}$. Both physiological hypertrophy and changes in myocardial function may account for the required increase in stroke volume. In the present study, changes in $V_o_2\text{max}$ closely paralleled cardiomyocyte length and width, providing a cellular basis for increase and regression of right and left ventricle stroke volumes. In addition, long-term changes in myocardial function may contribute significantly by altering diastolic filling and systolic emptying, as indicated by higher
Several aspects of cardiomyocyte contractile function are likely to account for the changes in V\textsubscript{O}2 max, including cardiomyocyte shortening and relaxation representing systolic and diastolic contractile properties, respectively. Although stroke volume is acutely regulated by extracardiac factors such as venous return, neurohormonal regulation, and afterload, previous studies identified significant correlation between contractile function of isolated myocytes and integrated in vivo function, indicating a contribution of intrinsic myocardial properties as well as both the molecular\textsuperscript{29} and cellular levels.\textsuperscript{6,18,30} Both statistical correlation and parallel time courses indicate that changes in cardiomyocyte size and function are likely to account for the changes in V\textsubscript{O}2 max. Because of the high internal correlations between different measures of cardiomyocyte size and function, it is to be expected that only 1 or 2 prove to be significant in multivariate regression analysis. However, this statistical interdependence does not preclude the possibility that myocyte size, contractility, and relaxation contribute cumulatively to the total contractile capacity in vivo.

### Endothelium-Dependent Arterial Relaxation

The training-detraining experiment demonstrated substantial changes in acetylcholine-induced arterial relaxation, which were highly sensitive to inhibition of the endothelial nitric oxide synthase (eNOS) inhibitor L-NAME. These observations indicate induction and regression of endothelial function, which were significantly correlated with V\textsubscript{O}2 max in both univariate and multivariate analysis. However, the marked difference in time course of EC\textsubscript{50} for acetylcholine indicates that endothelium-dependent vasodilation may not be as directly related to V\textsubscript{O}2 max as cardiomyocyte size and function. Whereas training-induced myocardial effects gradually regress over 3 to 4 weeks, exercise-gained endothelium-dependent relaxation is completely abolished within 2 weeks. The time course of the onset of endothelium-dependent changes could not be

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**Body Mass and Cardiac Weights**

<table>
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<th>Sedentary</th>
<th>TR10</th>
<th>DETR2</th>
<th>DETR4</th>
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<td>Before</td>
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<td>1569.2±150.6</td>
<td>1697.2±55.3</td>
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<td>In mg/g</td>
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<td>In mg</td>
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<td>In mg</td>
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<td>In mg/g</td>
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<td>1.3±0.2</td>
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<tr>
<td>In mg/g\textsuperscript{1/3}</td>
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<td>4.9±0.8</td>
<td>4.7±0.7</td>
<td>4.3±0.6</td>
</tr>
</tbody>
</table>

Body mass before and after experimental period and postmortem heart weights in detrained and sedentary; heart weights after Langendorff perfusion (see Methods). Training lasted 10 weeks (TR10); detraining, 2 (DETR2) and 4 (DETR4) weeks, respectively. LV indicates left ventricle; RV, right ventricle. Data are mean±SD.

TR/DETR vs sedentary: *P<0.01, †P<0.05.
TR10 vs DETR2: ‡P<0.01, §§P<0.05, and DETR4: ¶P<0.01.

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**Figure 4.** Cardiomyocyte length (A), width (B), and estimated volume (C) in detrained and sedentary rats (SED10, SED2, SED4) in 4800 cells, 150 from each rat. Training lasted 10 weeks (TR10) and detraining 2 (DETR2) and 4 (DETR4) weeks, respectively. Data are mean±SD. TR/DETR vs sedentary: *P<0.01, †P<0.05. TR10 vs DETR2: ‡P<0.01, §§P<0.05, and DETR4: ¶P<0.01.
determined because the previous studies did not include arterial function. On the basis of pilot experiments and exercise-induced resistance to decompression, we hypothesize that endothelial function changes much more quickly than myocardial. A more rapid time course does not preclude salutary health effects. Hambrecht et al recently demonstrated training-induced improvement in myocardial oxygen supply associated with increased endothelium-dependent relaxation and upregulation of the eNOS signaling pathway.

Figure 5. Cardiomyocyte contractility and Ca²⁺ handling in detrained and sedentary rats determined in 4 to 10 cells per rat. Training lasted 10 weeks (TR10) and detraining 2 (DETR2) and 4 (DETR4) weeks, respectively. A, Cell shortening; B, Ca²⁺ ratio amplitude; C, Ca²⁺ ratio sensitivity index (cardiomyocyte relative shortening/Ca²⁺ ratio amplitude); and D, diastolic and systolic Ca²⁺ ratios. Data are mean±SD. TR10 vs sedentary: *P<0.05; TR10 vs DETR2: †P<0.05; and DETR4: ‡P<0.01, §P<0.05.

Figure 6. Time course of contraction/relaxation and Ca²⁺ transient in detrained and sedentary cardiomyocytes stimulated at increasing frequencies. Training lasted 10 weeks (TR10) and detraining 2 (DETR2) and 4 (DETR4) weeks, respectively. A and B, Time to peak contraction and peak Ca²⁺ ratio, respectively; C and D, half-time to peak contraction and peak Ca²⁺ ratio, respectively; E and F, half-time to relaxation and Ca²⁺ ratio decay, respectively. Data are mean±SD. TR10 vs sedentary: *P<0.01, †P<0.05. DETR2 vs sedentary: ‡P<0.05. TR10 vs DETR2: §P<0.01, †P<0.05, and DETR4: #P<0.01.
Conclusion:
Regular exercise induces substantial improvements in cardiovascular function and health, and increased resistance to myocardial and endothelial dysfunction and failure. Although both myocardial and endothelial factors contribute significantly to improved health and increased resistance to cardiovascular disease. The present study demonstrates that improvements in myocardial and endothelial function that are likely to contribute to improved health and increased resistance to cardiovascular disease. The present study demonstrates that improvements in myocardial and endothelial function that are likely to contribute significantly to improved health and increased resistance to cardiovascular disease. The present study demonstrates that improvements in myocardial and endothelial function that are likely to contribute significantly to improved health and increased resistance to cardiovascular disease. The present study demonstrates that improvements in myocardial and endothelial function that are likely to contribute significantly to improved health and increased resistance to cardiovascular disease.

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References:

Figure 7. Phenylephrine-precontracted carotid artery response to accumulating acetylcholine, acetylcholine + L-NAME, or Na+ nitroprusside, all in presence of indomethacin, in trained 10 weeks (TR10, A), detrained 2 weeks (DETR2, B), and detrained 4 weeks (DETR4, C), with respective controls. Dose-response curves are constructed as described in Reference 27. TR10 vs sedentary: *P<0.01, †P<0.05. Note that training-induced responses were lost within 2 weeks of detraining.
References:


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