Exercise and Blood Pressure

Skeletal Muscle Signaling and the Heart Rate and Blood Pressure Response to Exercise

Insight From Heart Rate Pacing During Exercise With a Trained and a Deconditioned Muscle Group

Stefan P. Mortensen, Jesper H. Svendsen, Mads Ersbøll, Ylva Hellsten, Niels H. Secher, Bengt Saltin

Abstract—Endurance training lowers heart rate and blood pressure responses to exercise, but the mechanisms and consequences remain unclear. To determine the role of skeletal muscle for the cardioventilatory response to exercise, 8 healthy young men were studied before and after 5 weeks of 1-legged knee-extensor training and 2 weeks of deconditioning of the other leg (leg cast). Hemodynamics and muscle interstitial nucleotides were determined during exercise with the (1) deconditioned leg, (2) trained leg, and (3) trained leg with atrial pacing to the heart rate obtained with the deconditioned leg. Heart rate was 15 bpm lower during exercise with the trained leg ($P<0.05$), but stroke volume was higher ($P<0.05$) and cardiac output was similar. Arterial and central venous pressures, rate-pressure product, and ventilation were lower during exercise with the trained leg ($P<0.05$), whereas pulmonary capillary wedge pressure was similar. When heart rate was controlled by atrial pacing, stroke volume decreased ($P<0.05$), but cardiac output, peripheral blood flow, arterial pressures, and pulmonary capillary wedge pressure remained unchanged. Circulating [norepinephrine], [lactate] and [K+] were lower and interstitial [ATP] and pH were higher in the trained leg ($P<0.05$). The lower cardioventilatory response to exercise with the trained leg is partly coupled to a reduced signaling from skeletal muscle likely mediated by $K^+$, lactate, or pH, whereas the lower cardiac afterload increases stroke volume. These results demonstrate that skeletal muscle training reduces the cardioventilatory response to exercise without compromising O$_2$ delivery, and it can therefore be used to reduce the load on the heart during physical activity. (Hypertension. 2013;61:1126-1133.) ● Online Data Supplement

Key Words: cardiac output • exercise • skeletal muscle • sympathetic activity

A difference in training status of the exercising skeletal muscle, such as obtained by 1-legged training or immobilization, leads to a markedly lower heart rate (HR) and blood pressure response when 1-legged exercise at the same workload is performed with the best trained leg. These observations indicate that training-induced changes in HR and blood pressure responses to exercise are sensitive to other factors than changes in the central circulation (ie, heart size and left ventricular function) and that factors within the skeletal muscle play a role in the altered cardioventilatory response to exercise with exercise training. However, the regulatory mechanisms and consequences for the training-induced changes in central and peripheral hemodynamics remain unclear.

During exercise, sympathetic nervous activity is increased by influence from a central feed forward mechanism (central command) and contracting skeletal muscle (the exercise pressor reflex), resulting in an intensity-dependent increase in HR, ventilation, blood pressure, and vasoconstriction in the inactive tissues. Afferent fibers located within the skeletal muscles respond to mechanical distortion (group III) and changes in the chemical environment (group IV), and are thought to contribute to the cardioventilatory response to exercise. Physical training attenuates the increase in sympathetic nerve activity during exercise and leads to local adaptations, such as an elevated number of capillaries and a higher mitochondrial capacity affecting both aerobic and anaerobic metabolism. Interstitial potassium ($K^+$), pH, lactate, and adenosine have been suggested to contribute to the exercise pressor reflex by stimulating and sensitizing group IV fiber afferents in skeletal muscle, although their individual contributions remain controversial. Recently, ATP has been suggested to stimulate group IV afferents and a role for ATP is supported by the tight coupling between interstitial ATP concentrations and exercise intensity and the relation between interstitial ATP and norepinephrine (NE) concentrations during exercise.

This study investigated the role of the training status of skeletal muscles on cardiovascular responses to exercise. A
secondary aim was to evaluate whether muscle interstitial ATP affects afferent feedback from the contracting muscle. We measured hemodynamics and muscle interstitial nucleotides and adenosine concentrations at rest and during exercise with a control leg, a trained leg, and a deconditioned leg. A large difference in muscle training status within the same individual was obtained by training 1 leg for 5 weeks, while the contralateral leg was immobilized for 2 weeks before the final experimental day. The importance of training-induced difference in HR response to exercise was evaluated by increasing HR during exercise with the trained muscle to the same HR as established during exercise with deconditioned leg. Our hypothesis was that the training status of the contracting muscles influences cardioventilatory response to exercise at a given workload by lowering the relative exercise intensity of skeletal muscle and sympathetic activation. Furthermore, our hypothesis was that exercise training alters skeletal muscle interstitial ATP signaling, and thereby contributes to attenuated cardioventilatory response to exercise.

Methods

Eight recreationally active male subjects with a mean (±SD) age of 24±4 years, body weight of 77±11 kg, height of 184±7 cm, and a maximal oxygen uptake (VO₂max) of 47±5 mL/min per kilogram participated in the study. All subjects had a normal ECG and blood pressure and were not taking any medication. The subjects were informed of the risks and discomforts associated with the experiments before giving their informed consent to participate. The study was approved by the Ethical Committee of the Capital Region of Denmark (H-1-2009-081) and conducted in accordance with the guidelines of the declaration of Helsinki. No complications in connection to the invasive procedures were observed.

Experimental Protocol

The subjects completed 5 weeks of 1-legged knee-extensor exercise (3–4 times/week) and 2 weeks of immobilization with the other leg. The subjects were examined on 1 experimental day (experimental protocol 1) before the training/immobilization period and on 2 experimental days (experimental protocol 1 and 2, separated by 2 days) after the training/immobilization period (Figure S1 in the online-only Data Supplement).

Experimental Protocol 1 (Before and After the Training/Immobilization Period)

A microdialysis probe was inserted into the vastus lateralis muscle of the experimental leg(s) under local anesthesia (lidocaine). The subjects completed 10 minutes of 1-legged knee-extensions (24±4 W, ie, 35% of maximal workload (WLmax) before the training/immobilization period). Exercise with the trained and deconditioned leg was separated by 30 minutes of rest and the order was randomized. Muscle dialystate was collected for 10 minutes before the start of exercise, during exercise, and during the recovery from exercise (see Methods in the online-only Data Supplement).

Experimental Protocol 2 (After the Intervention Period)

Three catheters were placed under local anesthesia: A 20-G catheter was inserted into the radial artery of the nondominant arm, a catheter (131HF7, Edwards Lifesciences, Irvine, CA) was inserted in a left antecubital vein and advanced to the pulmonary artery under pressure guidance. A screw-in pacing electrode (Tendril ST, St. Jude Medical, Sylmar, CA) was inserted through the right internal jugular vein and advanced to the right atrium under x-ray guidance, where it was fixated in the atrial wall.

After 30 minutes of supine rest the subjects performed 3 minutes of 1-legged knee-extensor exercise with the (1) deconditioned leg (19±2, 38±4, 56±4 W), (2) trained leg (19±2, 35±4, 56±4, 75±5 W), and (3) trained leg with HR pacing (AAI mode) to elicit the
same HR as recorded during exercise with the deconditioned leg (38±4 and 56±4 W; Figure S2). Exercise bouts were separated by 10 minutes of seated rest, whereas the 3 trials were separated by 30 minutes of supine rest. Leg blood flow and blood samples (pulmonary and radial artery) were obtained simultaneously before and after 2.5 minutes of exercise.

HR was obtained from an ECG, while mean arterial pressure (MAP), mean pulmonary pressure, pulmonary capillary wedge pressure (PCWP), and central venous pressures (CVP) were monitored with transducers positioned at the level of the heart (Pressure Monitoring Kit, Baxter, IL). The PCWP was determined as at the end of expiration (PCWPend exp). Left ventricular transmural filling pressure was expressed as PCWP minus CVP.24 Pulmonary Vo2 was measured with a metabolic system (Quark CPET system, Cosmed, Italy). CO was calculated using the Fick equation (CO=Vo2/a-vO2 difference). Femoral arterial blood flow was measured with an ultrasound machine (Philips ie33, Philips Healthcare, The Netherlands) equipped with a linear probe operating at 5 MHz. Middle cerebral artery velocity was measured by transcranial Doppler (2 MHz) through the temporal ultrasound window at a depth of 48 to 60 mm (Multidop X, DWL, Sipplingen, Germany). Leg mass was calculated from whole-body dual-energy x-ray absorptiometry scanning (Prodigy, General Electrics Medical Systems, WI).

Analytical Procedures

Blood gas variables, hemoglobin, lactate, and K+ concentrations and pH were measured using an ABL725 analyzer (Radiometer, Copenhagen, Denmark) and corrected for central venous temperatures. Plasma catecholamines concentrations were determined with a radioimmunoassay (LDN, Nordhorn, Germany). Interstitial ATP, ADP, AMP, and adenosine concentrations were determined by HPLC.

Statistical Analysis

A 2-way repeated measures ANOVA was performed to test statistical significance within and between trials. After a significant F test, pair-wise differences were identified by the Tukey honestly significant difference post hoc procedure. The significance level was set at P<0.05, and data are mean±SEM for 8 subjects unless otherwise indicated.

Results

Performance

WLmax during the incremental 1-legged knee-extensor test was 67±5 W before the intervention period and increased with exercise training to 86±6 W (P<0.05), and was lowered with detraining to 61±4 W (P<0.05). Consequently, the relative workload during the experiment was 31±1%, 62±1%, and 91±1% of WLmax in the detrained leg, and 22±1%, 44±1%, 65±1%, and 87±1% of WLmax in the trained leg.

Systemic Hemodynamics During Exercise With the Trained and Deconditioned Leg

Exercise increased cardiac output (CO) in proportion to the workload and to similar levels during exercise with the trained and deconditioned leg (Figure 1). However, HR was lower when exercise was performed with the trained leg at 38 W (105±3 and 118±3 bpm with the trained and deconditioned leg, respectively) and 58 W (118±3 and 138±6 bpm, respectively; P<0.05), whereas stroke volume (SV) was higher (56 W; P<0.05). Exercise increased radial and pulmonary arterial blood pressures, but both pressures were lower when exercise was performed with the trained leg (Figures 1 and 2; P<0.05).

During exercise with the trained leg, CVP was reduced compared with baseline (P<0.05), whereas CVP did not change during exercise with the deconditioned leg. PCWP increased from rest to exercise, and there was no difference between values obtained during exercise with the trained and
deconditioned leg. The left ventricular contractility index (dP/dtmax) increased similarly during exercise with the trained and deconditioned leg (Figure 3). The rate-pressure product increased during exercise in both conditions (P<0.05), but was lower during exercise (at 38 and 58 W) with the trained leg compared with the deconditioned leg (P<0.05). Pulmonary ventilation, tidal volume, and V̇CO₂ were higher during exercise with the deconditioned leg (P<0.05), whereas the ventilatory frequency was similar (Figure 4). The V̇e/V̇CO₂ ratio was lower at 38 W (P<0.05) and tended (P=0.066) to be lower at 19 W, whereas there was no difference at 56 W. The lower exercise induced increase in HR and MAP during exercise with the trained compared with the detrained leg was detectable from 10 seconds after the onset of exercise, but the difference was larger after 30 seconds of exercise compared with the initial 30 seconds of exercise (P<0.05; Figure S3).

There were no significant differences in blood gas variables, hemoglobin, or O₂ content between trials (Table S1). Radial and pulmonary arterial lactate and K⁺ concentrations were higher during exercise with the deconditioned leg (at 38 and 56 W; P<0.05), whereas pulmonary arterial pH was lower (at 19 and 38 W; P<0.05). Plasma NE concentrations increased during exercise in all 3 trials, but was lower during exercise with the trained leg at 56 W (P<0.05) and tended (P=0.057) to be lower at 38 W. Plasma epinephrine concentrations increased during exercise with the trained leg at 75 W only (P<0.05), but there was no difference between trials.

**Effect of Atrial Pacing on Systemic Hemodynamics During Exercise With a Trained Leg**

Atrial pacing during exercise with the trained leg increased HR to similar values as during exercise with the deconditioned leg (123±5 [pace] and 119±3 [deconditioned] bpm at 38 W and 141±7 [pace] and 138±6 [deconditioned] bpm at 58 W). CO, SV, CVP, pulmonary arterial, and diastolic blood pressures were similar during the pacing trial and during exercise with the deconditioned leg, whereas systolic blood pressure and MAP were lower.

Compared with exercise with the trained leg without pacing, CO during the pacing trial was similar because of a parallel decrease in SV (P<0.05). Mean arterial and pulmonary pressures, CVP, PCWP, dP/dtmax, and the rate-pressure product were also unchanged, but atrial pacing lowered the systolic pressure (P<0.05). Atrial pacing did not alter any blood gas variables.

**Figure 3.** Left ventricular contractility index (dP/dtmax), arterial plasma norepinephrine (NE), and rate-pressure product at rest and during exercise with a deconditioned and trained leg, with and without atrial pacing. Data are mean±SEM. *Different from rest, P<0.05. #Different from deconditioned leg, P<0.05.

**Figure 4.** Pulmonary ventilation, respiration frequency, and tidal volume at rest and during exercise with a deconditioned and trained leg with and without atrial pacing. Data are mean±SEM.*Different from rest, P<0.05. #Different from deconditioned leg, P<0.05.
Effect of Exercise With a Trained or Deconditioned Leg on Peripheral Hemodynamics

Leg blood flow was lower during exercise with the trained leg compared with exercise with the deconditioned leg at 38 W ($P<$0.05) and tended ($P=0.063$) also to be lower at 56 W, whereas there was no difference in leg vascular conductance between the 2 legs (Figure S4). Middle cerebral artery $V_{\text{mean}}$ increased during exercise at 19 W with both the trained and deconditioned leg, whereas it only tended to increase ($P=0.062–0.070$) at higher workloads. There was no difference in middle cerebral artery $V_{\text{mean}}$ or cerebral conductance index between when exercise was performed with the trained or deconditioned leg.

Muscle Interstitial Nucleotide and Adenosine Concentrations

Muscle interstitial ATP, ADP, AMP, and adenosine concentrations were similar in the 3 conditions at rest and increased during exercise, and returned to baseline concentrations in the recovery period (Figure 5 and Table S2; $P<0.001$). The ATP concentration was, however, lower during exercise with the deconditioned muscle compared with exercise with the control and trained muscles ($P<0.05$), whereas ADP, AMP, and adenosine concentrations were similar. The total interstitial nucleotide/nucleoside concentration was higher in the trained muscle compared with the deconditioned muscle ($P<0.05$). In the nonexercising muscle, ATP, ADP, AMP, and adenosine concentrations did not change from resting values during exercise with the contralateral leg. Arterial and femoral venous blood pressure, HR, and rate-pressure product from this experimental day are presented in Table S3.

Discussion

Several findings in this study support an important role of the training status of contracting skeletal muscles for the central hemodynamic response to exercise. First, HR was lower during exercise with the trained leg, whereas CO remained unaltered because of a parallel increase in SV. Second, arterial, pulmonary, and central venous pressures were lower during exercise with the trained leg, whereas PCWP was similar. Third, plasma NE concentrations were lower during exercise with the trained leg. Fourth, interstitial ATP and venous pH were higher during exercise with the trained leg, whereas mixed venous lactate and $K^+$ concentrations were lower. Collectively, these results demonstrate that the central response to exercise is tightly coupled to the training status of the contracting skeletal muscle and the relative workload performed and that these changes can occur independently of adaptations within the central circulation and without compromising CO and $O_2$ delivery. Reduced afferent feedback from contracting skeletal muscle resulting in a lower sympathetic nerve activity in the trained state seems to play an important role for the attenuated HR and blood pressure response to exercise. Altered lactate and $K^+$ concentrations and pH, but not interstitial nucleotides and adenosine, can contribute to the training-induced attenuated sympathetic activation.

Central Response to Exercise With a Trained and Deconditioned Leg

To differentiate between the contribution of local and central adaptations for the training-induced lowering of HR, we used a set-up that created a large difference in the training status of the leg muscles within the same central circulation. We found that HR, blood pressure, and ventilation were lower when the trained leg was exercising at the same workload, suggesting lower activation of the sympathetic nervous system as confirmed by the attenuated NE response during exercise with the trained leg.$^{13}$ These differences were related to the changes within the skeletal muscle that result in an increased $WL_{\text{max}}$, because the responses were similar when expressed at the same relative workload (Figure 6). Changes in HR and blood pressure during exercise are tightly coupled to improvements in $V_{O_2\text{max}}$ and thus relative exercise intensity.$^4$ Here, we demonstrate that similar changes in SV, MAP, and HR can occur independent of the central adaptations to exercise training (ie, an increase in $V_{O_2\text{max}}$, maximal CO, heart size, left ventricular function, and blood volume).$^3,4$ Importantly, the attenuated HR and MAP response to exercise did not affect CO, because of a parallel reverse change in SV. The unaltered CO during exercise with a trained and detrained muscle is consistent with the tight coupling between $O_2$ delivery and metabolic demand,$^{25}$ but the regulatory mechanisms increasing SV when HR is reduced in the trained state have been unclear. In the trained leg, the higher SV seems to be coupled to a lower cardiac afterload (arterial pressure) during exercise at a given workload, because the left ventricular contractility index and filling pressures were similar when exercise was performed with the trained and deconditioned leg at the same workload. The similar contractility index suggests that myocardial force production during exercise with the trained leg relies on cardiac filing (Frank-Starling mechanism) or altered cardiac cycle length (interval–force relationship).$^{26}$ Consequently, the rate-pressure product was lower during exercise with the trained leg, indicating that the myocardial oxygen consumption was reduced by $\approx20\%$ during exercise with the trained leg, despite a similar CO.$^{27}$

To evaluate the importance of the lower HR response to exercise with the trained leg, HR was increased by atrial pacing during exercise with the trained leg to elicit the same HR as during exercise with the deconditioned leg. The increase in HR did not change CO or arterial pressures, suggesting that the attenuated HR response with exercise training is not of
Collectively, these observations suggest that CO and O₂ delivery was associated with lower mixed venous K⁺ and lactate concentrations and a higher pH. Interstitial lactate concentrations increase with exercise intensity²⁶ and blockade of acid sensitive ion channels attenuates the pressor response in cats.³¹ In support of a role of K⁺ and pH for the exercise pressor reflex, a relationship between K⁺ concentrations and MAP³⁵ and between muscle pH and sympathetic activity has been reported.³² Second, differences in central command are also likely to have contributed to the observed change in responses due to differences in the relative exercise intensity between the 2 legs³⁰ and consequently increased motor unit recruitment and perceived exertion. Third, differences in circulating substances released from skeletal muscle may also have contributed by stimulating peripheral chemoreceptors. Also, the higher pH and lower Pco₂ levels may have contributed to the lower ventilation during exercise with the trained leg. Last, increased venous distension coupled to the higher femoral venous blood pressures in the exercising detrained leg may also have contributed by increasing afferent signaling.³³ Although the relative contribution of each of these variables is unclear, the difference in HR and MAP during exercise with the 2 exercising legs was delayed such that it was larger after 30 seconds of exercise compared with at the onset of exercise. Group IV afferent fibers show a 5- to 15-second delay in response from the onset of exercise reflecting their activation by metabolic substances.¹⁰,¹² Altered afferent feedback therefore seems to account for part of the observed differences in cardioventilatory response.

The increase in muscle interstitial ATP concentrations during exercise was markedly lower in the deconditioned muscle. The lower interstitial ATP concentrations but higher MAP, HR, and plasma NE concentration during exercise with the deconditioned leg suggest that ATP alone or in synergy with other substances³⁴ is not an obligatory mediator of the training-induced changes in afferent signaling. ATP is released from contracting skeletal muscle cells³⁵ as well as from endothelial cells in response to mechanical stress.³⁶ The lower increase in total adenine nucleotides and adenosine in the deconditioned leg suggests that deconditioning lowered the release of ATP into the interstitial space rather than a lower resynthesis of ATP in the interstitial space from its breakdown products.

ATP can override sympathetic vasoconstrictor activity (functional sympatholysis) in a similar manner as exercise.³⁷,³⁸ In the same subjects, we have found a coupling between the muscle training status and the degree of functional sympatholysis during exercise.³⁹ 'The sympatholytic properties of ATP are not affected by the training status of the skeletal muscles.²³,³⁰ The lower interstitial ATP concentrations and impaired functional sympatholysis in the deconditioned leg is in agreement with the observation that interstitial ATP concentrations and the degree of functional sympatholysis during exercise is lower in sedentary elderly compared with young and trained elderly.²³ Taken together, the coupling between changes in interstitial ATP levels and the degree of functional sympatholysis during exercise observations open up for the possibility that the interstitial ATP plays a role in mediating functional sympatholysis during exercise, and thereby contributes by optimizing the blood flow distribution within the contracting muscle and the lowering of leg blood flow with exercise training.²³,⁴⁰,⁴¹ Although the physiological role

**Skeletal Muscle Signaling Mechanisms**

The tight coupling between the relative load on the skeletal muscles and cardioventilatory response demonstrates that the training status of the skeletal muscles was driving these changes, but the underlying signaling mechanisms are likely to include several factors. First, exercise with the trained leg was associated with lower mixed venous K⁺ and lactate concentrations and a higher pH. Interstitial lactate concentrations increase with exercise intensity²⁶ and blockade of acid sensitive ion channels attenuates the pressor response in cats.³¹ In support of a role of K⁺ and pH for the exercise pressor reflex, a relationship between K⁺ concentrations and MAP³⁵ and between muscle pH and sympathetic activity has been reported.³² Second, differences in central command are also likely to have contributed to the observed change in responses due to differences in the relative exercise intensity between the 2 legs³⁰ and consequently increased motor unit recruitment and perceived exertion. Third, differences in circulating substances released from skeletal muscle may also have contributed by stimulating peripheral chemoreceptors. Also, the higher pH and lower Pco₂ levels may have contributed to the lower ventilation during exercise with the trained leg. Last, increased venous distension coupled to the higher femoral venous blood pressures in the exercising detrained leg may also have contributed by increasing afferent signaling.³³ Although the relative contribution of each of these variables is unclear, the difference in HR and MAP during exercise with the 2 exercising legs was delayed such that it was larger after 30 seconds of exercise compared with at the onset of exercise. Group IV afferent fibers show a 5- to 15-second delay in response from the onset of exercise reflecting their activation by metabolic substances.¹⁰,¹² Altered afferent feedback therefore seems to account for part of the observed differences in cardioventilatory response.

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of ATP-sensitive P2Y2 receptors on smooth muscle cells of human skeletal muscle remain undisclosed, the presence of these receptors and the relatively high interstitial ATP levels as compared with plasma ATP levels suggest a functional role of interstitial ATP in the regulation of the vascular tone.

Conclusion
The lower HR, blood pressure, and ventilatory responses to exercise at a given workload with a trained skeletal muscle suggest that factors within the contracting skeletal muscle contribute to a lower sympathetic activation during exercise. Changes in skeletal muscle lactate and K+ concentrations and pH are likely to contribute to these changes by altering afferent feedback, whereas the markedly lower interstitial ATP concentrations during exercise with the previously immobilized leg suggest that interstitial ATP contributes to blood flow regulation in other ways than by simulating muscle afferents. The similar CO and O2 delivery, despite an 8% to 14% lower HR and blood pressure during exercise with the trained leg, suggest that adaptations within the skeletal muscles can result in a 20% lower myocardial work during exercise without compromising O2 delivery and aerobic metabolism.

Perspective
In cardiovascular diseases associated with an impaired muscle perfusion, the exercise pressor reflex is augmented and physical inactivity is therefore likely to aggravate the sympathetic response to exercise. In chronic heart failure, excessive ventilation coupled to increased skeletal muscle signaling impairs exercise tolerance.44,45 Previous studies have demonstrated that factors within the contracting skeletal muscle contribute to a lower sympathetic activation during exercise at a given workload with a trained skeletal muscle compared with plasma ATP levels suggest a functional role of interstitial ATP in the regulation of exercise pressor reflex. In chronic heart failure, excessive ventilation coupled to increased skeletal muscle signaling impairs exercise tolerance.44,45 Previous studies have demonstrated that factors within the contracting skeletal muscle contribute to a lower sympathetic activation during exercise.

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Disclosures
None.

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### Novelty and Significance

**What Is New?**

- A local difference in the training status of the contracting muscle alters the heart rate and blood pressure response to exercise independently of the central circulation

**What Is Relevant?**

- Small muscle mass exercise training can lower the strain on the heart during daily physical activity

**Summary**

Local adaptations in skeletal muscle with exercise training can lower the heart rate, blood pressure, and ventilatory response to exercise without compromising tissue oxygen delivery.
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Skeletal muscle signaling and the heart rate and blood pressure response to exercise:
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Expanded online methods and results section

Training and immobilization period
On the first visit to the laboratory, the subjects became accustomed to one-legged knee-extensor exercise and an incremental test was performed to exhaustion to determine the maximal workload (WL_{max}) for each leg. The training was randomized between the right and left leg and included a 5 min warm-up followed by 10 intervals of two minute exercise duration separated by a one minute of recovery. The workload was adjusted such that the subjects were exhausted upon completion of each training session. During the first training session the mean workload was 42±5 W and during the last session it was 80±7 W. After three weeks of training, the contralateral leg was immobilized using a lightweight fiber cast from above the malleoli to below the groin. The day before the first experimental day, the cast was removed and the leg was passively moved to confirm full range of motion.

Atrial pacing (experimental protocol 2)
Atrial pacing was performed in the AAI mode. In the AAI mode, the pacemaker withholds pacing when it senses an intrinsic atrial electrical activation. If the pacing rate is higher than the intrinsic rate a pacing stimulus will be delivered and this impulse will be conducted to the ventricles through the normal conduction system, ensuring normal physiologic activation and relaxation of the ventricles.

Measurements

Microdialysis (experimental protocol 1)
The microdialysis probes were perfused at a rate of 5 µl min^{-1} with ringer acetate and to determine the relative exchange of nucleotides and adenosine across the membrane, a small amount (2.7 nM) of [2-^{3}H]ATP was added to the perfusate for calculation of probe recovery. The molecular probe recovery (PR) was calculated as \[PR=\frac{dpm_{\text{infusate}}-dpm_{\text{dialysate}}}{dpm_{\text{infusate}}}\], where dpm denotes disintegrations per minute (Scheller & Kolb, 1991; Jansson et al 1994). The [2-^{3}H]ATP activity (in dpm) was measured on a liquid scintillation counter (Tri-Carb 2000; Copenhagen) after addition of the perfusate to 3 ml of Ultima Gold scintillation liquid (Perkin Elmer). After collection of samples, the microdialysate was weighed, and the actual flow rate was calculated to estimate any loss of fluid or abnormal decrease in perfusion rate.

Femoral arterial blood flow (experimental protocol 2)
Femoral arterial blood flow (LBF) was measured with ultrasound Doppler (Philips Ie33, Philips Healthcare, The Netherlands) equipped with a probe operating an imaging frequency of 9 MHz and Doppler frequency of 5.0 MHz. The site of blood velocity measurements in the common femoral artery was distal to the inguinal ligament but above the bifurcation into the superficial and profound femoral branch to avoid turbulence from the bifurcation. All recordings were obtained at the lowest possible insonation angle and always below 60°. The sample volume maximized according to the width of the vessel, and kept clear of the vessel walls. A low-velocity filter (velocities <1.8 m/s) rejected noises caused by turbulence at the vascular wall. Doppler tracings and B-mode images were recorded continuously and doppler tracings were averaged over 8 heart cycles. The arterial diameter of was determined after each doppler recording and averaged over three cardiac cycles. Arterial diameter measures were assessed during the systole from arterial B-mode images with the transducer parallel to the vessel.

Cardiac output (experimental protocol 2)
Q was calculated using the Fick principle \(Q=\text{VO}_{2}/(a-v\text{VO}_{2} \text{ difference})\). Pulmonary VO_{2} was measured online (Quark CPET system, Cosmed, Italy). Pulmonary arterial blood samples were withdrawn over 20 seconds and the measured VO_{2} values were averaged for the same time period. Heart rate (HR) was obtained from a 3-lead electrocardiogram.
Blood pressures (experimental protocol 2)
Radial arterial mean (MAP), systolic (SBP) and diastolic (DBP) blood pressure, mean pulmonary (PAP), pulmonary capillary wedge pressure (PCWP) and central venous pressures (CVP) were monitored with transducers positioned at the level of the heart (Pressure Monitoring Kit, Baxter) and connected to a hemodynamic monitor (Dialogue 2000, Danica Elektronic, Copenhagen, Denmark). Determination of PCWP was performed immediately after blood sampling.

Calculations (experimental protocol 2)
Stroke volume (SV) and systemic vascular conductance (SVC) was calculated as the quotient of Q (ml) divided by HR and MAP, respectively. The left ventricular contractility index $\frac{dP}{dt_{\text{max}}}$ was calculated as the peak systolic value of the first derivative of the arterial pressure curve over 20 cardiac cycles. The rate-pressure rate was calculated by multiplying HR by the systolic blood pressure.
Table S1 Blood gas variables, O2 content, plasma lactate and pH at rest and during exercise

<table>
<thead>
<tr>
<th>Blood variable</th>
<th>Po2 (mmHg)</th>
<th>Hemoglobin (g/L)</th>
<th>O2 saturation (%)</th>
<th>O2 content (ml/L)</th>
<th>PCO2 (mmHg)</th>
<th>pH</th>
<th>Lactate (mmol/L)</th>
<th>Potassium (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Deconditioned</td>
<td>Trained</td>
<td>Deconditioned</td>
<td>Trained</td>
<td>Deconditioned</td>
<td>Trained</td>
<td>Deconditioned</td>
</tr>
<tr>
<td></td>
<td></td>
<td>before pace trial</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deconditioned</td>
<td>103±2</td>
<td>99±3</td>
<td>101±1</td>
<td>108±3#</td>
<td>102±2</td>
<td>107±3#</td>
<td>100±2</td>
<td>104±2</td>
</tr>
<tr>
<td>Trained</td>
<td>43±2</td>
<td>46±4</td>
<td>43±1</td>
<td>38±2</td>
<td>38±4</td>
<td>36±3*</td>
<td>35±2*</td>
<td>34±1*</td>
</tr>
<tr>
<td>Deconditioned</td>
<td>143±4</td>
<td>143±3</td>
<td>146±3</td>
<td>146±5</td>
<td>147±3*</td>
<td>148±4</td>
<td>148±3*</td>
<td>150±3*</td>
</tr>
<tr>
<td>Trained</td>
<td>143±4</td>
<td>142±3</td>
<td>145±3</td>
<td>146±5</td>
<td>145±4</td>
<td>150±4*</td>
<td>147±3*</td>
<td>150±3*</td>
</tr>
<tr>
<td>Trained with HR pacing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deconditioned</td>
<td>190±6</td>
<td>190±4</td>
<td>195±5</td>
<td>195±6</td>
<td>195±5*</td>
<td>198±5*</td>
<td>197±4*</td>
<td>200±4</td>
</tr>
<tr>
<td>Trained</td>
<td>140±4</td>
<td>140±3</td>
<td>141±4</td>
<td>117±5*</td>
<td>116±5*</td>
<td>112±4*</td>
<td>109±5*</td>
<td>108±4*</td>
</tr>
<tr>
<td>Deconditioned</td>
<td>40±1</td>
<td>37±2</td>
<td>40±0</td>
<td>41±1</td>
<td>40±1</td>
<td>39±1</td>
<td>41±1*</td>
<td>40±1</td>
</tr>
<tr>
<td>Trained</td>
<td>46±1</td>
<td>43±2</td>
<td>44±1</td>
<td>51±1*#</td>
<td>48±2*</td>
<td>54±1*</td>
<td>51±1*</td>
<td>50±2*</td>
</tr>
<tr>
<td>Deconditioned</td>
<td>7.40±0.01</td>
<td>7.42±0.01</td>
<td>7.38±0.01</td>
<td>7.39±0.00</td>
<td>7.41±0.00</td>
<td>7.39±0.00</td>
<td>7.40±0.00</td>
<td>7.38±0.00</td>
</tr>
<tr>
<td>Trained</td>
<td>7.37±0.01</td>
<td>7.41±0.02</td>
<td>7.39±0.03</td>
<td>7.33±0.01**</td>
<td>7.37±0.01*</td>
<td>7.32±0.00**</td>
<td>7.35±0.00**</td>
<td>7.33±0.01*</td>
</tr>
<tr>
<td>Deconditioned</td>
<td>1.3±0.2</td>
<td>1.6±0.3</td>
<td>1.6±0.1</td>
<td>2.5±0.2*</td>
<td>1.8±0.3</td>
<td>3.2±0.3*#</td>
<td>2.2±0.3</td>
<td>2.9±0.5</td>
</tr>
<tr>
<td>Trained</td>
<td>1.2±0.2</td>
<td>1.5±0.3</td>
<td>1.6±0.1</td>
<td>2.2±0.3*</td>
<td>1.7±0.3</td>
<td>3.0±0.3*#</td>
<td>2.1±0.3</td>
<td>2.7±0.5</td>
</tr>
<tr>
<td>Deconditioned</td>
<td>3.8±0.1</td>
<td>3.8±0.1</td>
<td>3.8±0.0</td>
<td>4.2±0.1*#</td>
<td>4.0±0.1*</td>
<td>4.5±0.1*#</td>
<td>4.1±0.1*</td>
<td>4.3±0.1*</td>
</tr>
<tr>
<td>Trained</td>
<td>3.8±0.1</td>
<td>3.7±0.1</td>
<td>3.7±0.1</td>
<td>4.1±0.2*</td>
<td>3.9±0.1*</td>
<td>4.4±0.1*#</td>
<td>4.0±0.1*</td>
<td>4.2±0.2*</td>
</tr>
</tbody>
</table>

RA: radial artery, PA: pulmonary artery. * different from rest, P<0.05, # different from trained muscle, P<0.05
<table>
<thead>
<tr>
<th>Nucleotide</th>
<th>Control leg exercise (24±4 W)</th>
<th>Deconditioned leg exercising (24±4 W)</th>
<th>Trained leg exercising (24±4 W)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control muscle</td>
<td>Deconditioned muscle</td>
<td>Trained muscle</td>
</tr>
<tr>
<td></td>
<td>Rest</td>
<td>Exercise</td>
<td>Recovery</td>
</tr>
<tr>
<td>ATP (µmol/L)</td>
<td>0.3±0.2</td>
<td>3.9±1.2*</td>
<td>0.3±0.2</td>
</tr>
<tr>
<td>ADP (µmol/L)</td>
<td>0.1±0.0</td>
<td>3.7±1.3*</td>
<td>0.4±0.2</td>
</tr>
<tr>
<td>AMP (µmol/L)</td>
<td>0.8±0.5</td>
<td>7.4±2.8*</td>
<td>1.4±0.5</td>
</tr>
<tr>
<td>Adenosine (µmol/L)</td>
<td>0.2±0.1</td>
<td>1.4±0.3*</td>
<td>0.3±0.1</td>
</tr>
<tr>
<td>Total adenines (µmol/L)</td>
<td>1.4±0.6</td>
<td>16.5±5.2*</td>
<td>2.4±0.7</td>
</tr>
</tbody>
</table>

* different from rest, P<0.05, □ different from control muscle, P<0.05 # different from trained muscle, P<0.05
Table S3 Arterial blood pressure, heart rate and rate-pressure product

<table>
<thead>
<tr>
<th>Hemodynamic variable</th>
<th>Control leg exercising</th>
<th>Deconditioned leg exercising</th>
<th>Trained leg exercising</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rest</td>
<td>Exercise</td>
<td>Recovery</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>94±2</td>
<td>106±3*</td>
<td>92±2</td>
</tr>
<tr>
<td>Systolic pressure (mmHg)</td>
<td>127±2</td>
<td>141±4*</td>
<td>123±6</td>
</tr>
<tr>
<td>Diastolic pressure (mmHg)</td>
<td>78±1</td>
<td>84±2</td>
<td>77±2</td>
</tr>
<tr>
<td>Femoral venous pressure (mmHg)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>71±2</td>
<td>91±3*</td>
<td>73±2</td>
</tr>
<tr>
<td>Rate-pressure product (beats/mmHg/sec)</td>
<td>8977±213</td>
<td>12913±598*</td>
<td>8901±473</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Hemodynamic variable</th>
<th>Change from rest to exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>12±3</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>15±3</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>6±3</td>
</tr>
<tr>
<td>Femoral venous pressure (mmHg)</td>
<td>-</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>20±2</td>
</tr>
<tr>
<td>Rate-pressure product (beats/mmHg/sec)</td>
<td>3936±567</td>
</tr>
</tbody>
</table>

* different from rest, $P<0.05$, □ different from control muscle, $P<0.05$ # different from trained muscle, $P<0.05$. 
Figure S1. Experimental design. Subjects completed an experimental day (experimental protocol 1) before a five week exercise training period of one leg and two experimental days (experimental protocol 1 and 2) after the training period.

Three weeks into the training period, the other leg was deconditioned (full leg cast). To ensure full range of motion, the cast was removed the day before the first experimental day and passive leg movement was performed. A knee-brace (Don-Joy) was applied at a fixed position (30°) until the second experimental day.
Figure S2. Experimental protocol 2 (experimental day 3).

Subjects performed one-legged knee extensor exercise with the deconditioned leg, trained leg and trained leg with atrial pacing to the same heart rate observed during exercise with the deconditioned leg. The workloads were selected based on the peak workload of the trained leg. Red arrows indicate when blood samples were obtained. See text for details.
Figure S3 Difference in heart rate and blood pressure response between exercise with a deconditioned and trained leg.

§ different from < 30 seconds of exercise, $P<0.05$
Online figure 4 Leg blood flow, leg vascular conductance, middle cerebral artery mean blood velocity (MCA $V_{\text{mean}}$) and cerebral conductance index (CVCi) at rest and during exercise with a deconditioned and trained leg with and without atrial pacing.

* different from rest, $P<0.05$, # different from deconditioned leg, $P<0.05$