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## Mitochondrial Coupling and Contractile Efficiency in Humans with High and Low $\text{VO}_2$ peak

Gwenael Layec<sup>1,2,3</sup>, Aurélien Bringard<sup>4</sup>, Yann Le Fur<sup>1</sup>, Jean-Paul Micallef<sup>5,6</sup>, Christophe Vilmen<sup>1</sup>, Stéphane Perrey<sup>5</sup>, Patrick J. Cozzone<sup>1</sup>, and David Bendahan<sup>1</sup>

<sup>1</sup>Aix-Marseille University, CNRS, CRMBM UMR 7339, Marseille, France <sup>2</sup>Department of Internal Medicine, Division of Geriatrics, University of Utah, Salt Lake City, UT <sup>3</sup>Geriatric Research, Education, and Clinical Center, George E. Whalen VA Medical Center, Salt Lake City, UT <sup>4</sup>Department of Anesthesiology, Pharmacology and Intensive Care and Department of fundamental Neurosciences, University of Geneva, Switzerland <sup>5</sup>Motricity Efficiency and deficiency, EA 2991, Faculty of Sport Science UFR STAPS, Montpellier, France <sup>6</sup>INSERM ADR 08 Montpellier, France

### Abstract

**Introduction**—Endurance training elicits tremendous adaptations of the mitochondrial energetic capacity. Yet, the effects of training or physical fitness on mitochondrial efficiency during exercise are still unclear. Accordingly, the purpose of the present study was to examine *in vivo* the differences in mitochondrial efficiency and ATP cost of contraction during exercise in two groups of adults differing in their aerobic capacity.

**Method**—We simultaneously assessed the ATP synthesis and  $\text{O}_2$  fluxes with <sup>31</sup>P-Magnetic Resonance Spectroscopy and pulmonary gas exchange measurements in 7 endurance-trained (ET,  $\text{VO}_{2\text{max}}$ :  $67 \pm 8 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ ) and 7 recreationally active (RA,  $\text{VO}_{2\text{max}}$ :  $43 \pm 7 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ ) subjects during 6 min of dynamic moderate-intensity knee-extension.

**Results**—The ATP cost of dynamic contraction was not significantly different between ET and RA ( $P > 0.05$ ). Similarly, end-exercise  $\text{O}_2$  consumption was not significantly different between groups (ET:  $848 \pm 155 \text{ ml} \cdot \text{min}^{-1}$  and RA:  $760 \pm 131 \text{ ml} \cdot \text{min}^{-1}$ ,  $P > 0.05$ ). During the recovery period, the PCr offset time constant was significantly faster in ET compared to RA (ET:  $32 \pm 8 \text{ s}$  and RA:  $43 \pm 10 \text{ s}$ ,  $P < 0.05$ ) thus indicating an increased mitochondrial capacity for ATP synthesis in the quadriceps of ET. In contrast, the estimated mitochondrial efficiency during exercise was not significantly different (P/O, ET:  $2.0 \pm 1.0$  and RA:  $1.8 \pm 0.4$ ,  $P > 0.05$ ). Consequently, the higher mitochondrial capacity for ATP synthesis in ET likely originated from an elevated mitochondrial

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Corresponding author: G. Layec, VA Medical Center, Bldg 2, 500 Foothill Dr., Salt Lake City, Utah 84148, USA. gwenael.layec@utah.edu.

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volume density, mitochondria-specific respiratory capacity and/or slower post-exercise inactivation of oxidative phosphorylation by the parallel activation mechanism.

**Conclusion**—Together, these findings reveal that 1) mitochondrial and contractile efficiency are unaltered by several years of endurance-training in young adults, and 2) the training-induced improvement in mitochondrial energetic capacity appears to be independent from changes in mitochondrial coupling.

### Keywords

endurance-training;  $^3\text{P}$ -MRS; skeletal muscle; bioenergetics; ATP cost of contraction; mitochondrial function

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### Introduction

Endurance training elicits numerous physiological, biochemical and molecular adaptations that improve whole-body aerobic capacity and overall muscle performance. In particular, endurance-training is accompanied by tremendous adaptations of a crucial constituent of the skeletal muscle, i.e. the mitochondria. Indeed, morphometric analyses have previously demonstrated that mitochondrial density can rapidly increase as a result of training (11, 18) and is higher in trained as compared to untrained individuals (20). This elevated mitochondrial volume within the muscle has been linked to an increased muscle respiratory capacity after training (9, 36) and in subjects with a high level of physical activity (11, 20). Likely as a result of these adaptations, the peak rate of ATP synthesis measured *in vivo* using  $^3\text{P}$ -Magnetic Resonance spectroscopy ( $^3\text{P}$ -MRS) has been reported to be greatly enhanced after training (13) and in endurance-trained individuals (21, 29).

Despite these positive adaptations of the mitochondrial energetic capacity, the effect of training on intrinsic mitochondrial energetic function is rather equivocal. For instance, non-phosphorylating respiration measured *in vitro*, which reflects the basal rate of proton leak back into the mitochondrial matrix not coupled to ATP synthesis, has been documented to be increased (36, 42) or unaltered after training (9). Conceptually, a greater basal leak should reduce the mitochondrial efficiency (conversion of carbohydrate or free fatty acid into ATP, also defined as P/O ratio) both at submaximal and maximal respiration rates. However, mitochondrial efficiency measured in isolated mitochondria during maximal ADP-stimulated respiration was unrelated to the training status (34, 43) or unaltered after 6 weeks of high-intensity training (44). Of note, a caveat with this approach is the alteration of the mitochondrial function during the isolation procedure and the supra-physiological level of  $\text{O}_2$  surrounding the mitochondria *in vitro*, which is known to modulate mitochondrial efficiency (37). Also, the P/O ratio measured during maximal ADP-stimulated respiration reflects a theoretical peak value that may not be reached *in vivo* as the respiration rate is lower and the degree of uncoupling higher.

To circumvent these issues, mitochondrial function of the plantar flexor muscles has then been assessed *in vivo* using combined  $^3\text{P}$  saturation transfer- and  $^{13}\text{C}$ -MRS (2). With this approach, it has been reported that endurance-trained athletes exhibited a  $\sim 40\%$  lower mitochondrial efficiency at rest (2). However, conflicting results have been reported

on the basis of  $^{31}\text{P}$ -MRS and optical spectroscopy measurements (6). Specifically, a higher mitochondrial efficiency at rest and a lower uncoupled protein (UCP3) content was observed in the quadriceps muscle of endurance-trained subjects (6). Interestingly, both studies were conducted at rest and, under such condition, the P/O ratio might differ substantially from exercise given that mitochondrial coupling is known to increase along with the respiration rate (34). Based upon the aforementioned conflicting results and the specific experimental conditions employed, we are still missing some important information regarding the influence of endurance-training or the training status on mitochondrial efficiency during exercise.

Given that both mitochondrial and contractile efficiency (conversion of ATP into mechanical work) are key physiological factors of the muscle exercise capacity, the purpose of the present study was to compare *in vivo* mitochondrial efficiency and ATP cost of contraction during exercise between two groups of adults with different aerobic capacities. To this aim, we simultaneously assessed the ATP synthesis and  $\text{O}_2$  fluxes using  $^{31}\text{P}$ -MRS and pulmonary gas exchange during a moderate-intensity knee-extension exercise. Based upon previous findings (31, 44), we hypothesized that the quadriceps of endurance-trained subjects would exhibit a higher mitochondrial capacity for ATP synthesis but a similar mitochondrial efficiency and ATP cost of contraction in comparison to recreationally active individuals.

## Methods

### Subjects

Following the informed written consent procedures, 7 endurance trained (ET) men and 7 recreationally active (RA) (1 woman and 6 men) subjects (mean  $\pm$  SD weight, ET:  $66 \pm 9$  kg and AT:  $73 \pm 11$  kg ; height, ET:  $177 \pm 6$  cm and AT:  $177 \pm 11$  cm; age, ET:  $31 \pm 4$  years and AT:  $33 \pm 7$  years;  $\text{VO}_{2\text{max}}$ , ET:  $67 \pm 8$   $\text{ml}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$  and AT:  $43 \pm 7$   $\text{ml}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$ ) participated in this study. Subjects were classified as “endurance-trained” based on their participation to running or cycling activities at a competitive level (ET,  $>4$  times a week for several years). The recreationally active subjects had no regular and structured physical activities above that required for activities of daily living for more than twice a week (RA). The physical status of the subjects for each group was further confirmed by the aerobic capacities measured during the cardio-pulmonary assessment before being incorporated into the analysis. All experimental procedures were undertaken with the approval of the Timone University-Hospital Ethics Committee. In the 24 h before all visits, subjects abstained from intense physical activity, caffeine and alcohol consumption.

### Experimental protocol

**Cardio-pulmonary assessment**—Subjects initially performed on a cycle ergometer (Ergoline 800S, Hoechberg, Germany) an incremental exercise test to exhaustion in order to determine the maximal whole-body rate of  $\text{O}_2$  consumption, *i.e.*  $\text{VO}_{2\text{max}}$ . Throughout the exercise, ventilation, inspired and expired gases were continuously recorded (ZAN 680, ZAN Messgerate, Oberthulba, Germany), then ventilation parameters and pulmonary oxygen uptake ( $\text{VO}_2$ ) were calculated on a breath-by-breath basis. Baseline exercise intensity was 60W and after a 3-min exercise period, intensity was incremented by 30 W

every min until volitional exhaustion or achievement of at least two of the following criteria: maximal heart rate (heart rate max >90% age predicted), respiratory exchange ratio (RER) values (>1.15), and  $\text{VO}_2$  plateau according to the ATS/ACCP guidelines. The pedalling rate was kept constant at 70 rpm during the whole exercise period.  $\text{VO}_{2\text{max}}$  was averaged over the last 30 s of exercise.

### Metabolic assessment

After a familiarization session, voluntary maximum isometric force (MVC) was determined during three repeated isometric leg extensions of each leg using a dedicated dynamometric ergometer that can either be used for isometric or dynamic contraction protocols. Subjects lay prone with the knee flexed at  $\sim 40^\circ$ . Each contraction was maintained for 3 s and was repeated after a resting period of at least 30 s. The MVC was defined as the average of the three peak measurements. If the contraction intensity was  $\pm 10\%$  different from the previous contraction, subjects were asked to complete an additional contraction. The leg weight was subtracted from the MVC measurement. On a different day, subjects performed constant-load sub-maximal double-leg alternate knee-extension-flexion at  $\sim 25\%$  of MVC in the whole body 1.5 T MRI system (Siemens Vision Plus system, Siemens AG, Erlangen, Germany). Specifically, after 3 min of rest, subjects exercised for 6 min followed by 6 min of recovery. The exercise was performed at a rate of 60 repetitions per min with the subjects lifting and lowering the mass over a distance of  $\sim 17$  cm. Repeated leg extensions were gated to the MR recordings using an audible signal so that MR spectra were always recorded at the same time of the contraction-relaxation process. The displacement of the weight by each leg was measured with a potentiometer (35905-002-102, Bourns, Mexico) while the applied forces were measured by a force transducer (N2A-13-T026P-350, Vishay Micro-Measurements, Germany) before being amplified (OP 497, ST Microelectronics, gain = 1000). The force and displacement signals were analog-to-digital converted (PCI-6023E, National Instruments, France) with a sample frequency of 100 Hz and recorded on a personal computer (winATS software 6.5, SYSMA, France).

### $^{31}\text{P}$ MRS

$^{31}\text{P}$  MRS data were recorded with a dual-tuned  $^{31}\text{P}$ - $^1\text{H}$  surface coil with linear polarization (Rapid biomedical GmbH, Rimpfing, Germany) positioned under the right quadriceps muscle. The  $^{31}\text{P}$  single-loop coil diameter was 140 mm surrounded by a 270 mm  $^1\text{H}$  coil loop. An automatic localized map shimming procedure was initially performed. Then, a set of multi-slice fast proton MR images was acquired in order to determine the position of the leg with respect to the surface coil. Before each experiment, three relaxed spectra were acquired at rest with 4 averages per spectrum and a repetition time of 10 s. Then, MRS data acquisitions were performed throughout the rest-exercise-recovery protocol using a pulse-acquire sequence with the following parameters (radiofrequency hard pulse duration = 500  $\mu\text{s}$ , repetition time = 2 s, sweep width = 32 kHz, 4096 data points, and 4 averages per spectrum). We quantified saturation factors from the comparison of relaxed (TR = 10 s) and partially-relaxed spectra (TR = 2s).

The relative concentrations of [PCr], inorganic phosphate [Pi], and [ATP] were obtained by a time-domain fitting routine using the AMARES algorithm incorporated into the CSIPO

software (32). Intracellular pH was calculated from the chemical shift difference between the Pi and PCr signals. The free cytosolic [ADP] was calculated from [PCr] and pH using the creatine kinase equilibrium constant ( $K_{CK} = 1.66 \times 10^9 \text{ M}^{-1}$ ) and assuming that phosphocreatine represents 85% of the total creatine content. The resting concentrations were calculated from the average peak areas of the relaxed spectra recorded at rest and assuming ATP concentration = 8.2 mM. Cyttoplasmic free energy of ATP hydrolysis ( $G_{ATP}$ ) was calculated using the following equation and constants (24):

$$\Delta G_{ATP} = \Delta G_{ATP}^0 + RT \ln \frac{[ADP] \cdot [Pi]}{[ATP]}$$

$$\Delta G_{ATP}^0 = RT \left[ \ln \left( K_{ATP}^{\text{true}} \right) + \ln \left( \frac{f_{ATP}}{f_{ADP} \cdot f_{Pi}} \cdot \frac{1}{[H^+]} \right) \right]$$

With

$$K_{ATP}^{\text{true}} = 0.722$$

(14),  $R = 8.3145 \text{ K}^{-1}\text{M}^{-1}$ ,  $T = 310 \text{ K}$ . As  $G_{ATP}$  depends on [ADP], and therefore on the assumed equilibrium of the creatine kinase reaction, the free energy of ATP hydrolysis must be assumed to be equal to that of PCr breakdown (16):

$$\Delta G_{ATP} = \Delta G_{PCr} = -RT \cdot \ln (K_{ATP} + K_{CK}) - RT \cdot \ln \left( \frac{f_{PCr}}{f_{Pi}} \right) + RT \cdot \ln \left( [Pi] \cdot \frac{[Cr]}{[PCr]} \right)$$

Where  $f$  is the fraction of [PCr] or [Pi] taking into accounts correction related to  $K^+$ ,  $Mg^{2+}$  and  $H^+$ ), according to the method of Harkema and Meyer (17), modified to include  $K^+$  binding, as previously described by Kemp et al. (24)

Changes in pH and in the concentration of phosphorus metabolites during contraction and recovery phases were used in order to calculate muscle phosphorylation capacity (PCr offset time constant) and the rates of ATP synthesis through the creatine kinase reaction (ATPck), oxidative phosphorylation (ATPox), and anaerobic glycolysis (ATPgly) as previously described (23). Briefly, the rate of ATP production from the breakdown of PCr through the CK reaction ( $\text{mM} \cdot \text{min}^{-1}$ ) was calculated from the change in PCr for each time point of the exercise period:  $ATP_{CK} = dPCr/dt$ . The rate of mitochondrial ATP production (ATPox) was calculated based on the sigmoid relationship between the oxidative ATP production rate and free cytosolic ADP concentration:  $ATP_{ox} = V_{max} / (1 + (K_m/[ADP])^{2.2})$  in which  $K_m$  (the [ADP] at half-maximal oxidation rate) is  $\sim 30 \mu\text{M}$  in skeletal muscle, 2.2 is the Hill coefficient for a sigmoid function and  $V_{max}$  is the inferred peak rate of mitochondrial respiration *in vivo*. Throughout the exercise period, glycogen breakdown to pyruvate and

lactate, proton efflux, buffering capacity, protons produced by oxidative phosphorylation and the consumption of protons by the CK reaction lead to changes in intramuscular pH. Assuming that the glycogenolytic production of 1 mole of  $H^+$ , when coupled to ATP hydrolysis, yields 1.5 moles of ATP,  $ATP_{gly}$  can be calculated from the total number of protons (P) produced throughout exercise:  $P = H^+_{CK} + H^+_{\beta} - H^+_{ox} + H^+_{efflux}$ . To improve the precision of the estimation of  $H^+_{efflux}$ , we use a modified version of this calculation in which the total proton disappearance (i.e.,  $\int E dt$ ) is estimated cumulatively from the start of recovery, then fitted to an exponential function to obtain the initial efflux recovery rate. The total rate of ATP generated from aerobic and anaerobic pathways were scaled to the power output (watts) from the leg investigated by the MR coil to estimate the ATP cost of contraction. Methodological considerations addressing the validity and the reproducibility of the calculation of the rates of ATP synthesis have been previously addressed and can be found in published reports (25, 30). The steady-state rate of oxidative ATP synthesis measured by  $^{31}P$ -MRS and  $VO_2$  from the gas exchange measurements were used to estimate the P/O ratio. Briefly, the thigh's rate of oxidative ATP synthesis ( $mM.L^{-1}.min^{-1}$ ) was considered homogeneous within the quadriceps and multiplied by the quadriceps muscle mass converted from the anatomical images of the right leg assuming muscle density = 1.049 kg/L, 1 mole of  $O_2 = 25.5 L$  and 75% of the quadriceps was recruited for both legs (38). Based upon prior studies, basal skeletal muscle oxidative ATP synthesis was considered negligible. However,  $VO_2$  from non-exercising muscles was taken into account by subtracting baseline pulmonary  $VO_2$  from the steady-state pulmonary  $VO_2$ .

The PCr recovery kinetics was determined by fitting the time-dependent changes during the recovery period to a mono-exponential curve described by the following equation:

$$Y(t) = Y_{end} + Y_{res} \left(1 - e^{-(t/\tau p)}\right)$$

where  $Y_{end}$  is the level of [PCr] measured at end-of-exercise and  $Y_{res}$  refers to the amount of PCr resynthesized during the recovery, and  $\tau$  reflects the time constant of the offset kinetics, a relative measure of muscle phosphorylation capacity (23).

Model variables were determined with an iterative process by minimizing the sum of squared residuals (RSS) between the fitted function and the observed values. Goodness of fit was assessed by visual inspection of the residual plot and the frequency plot distribution of the residuals, Chi square values, and the coefficient of determination ( $r^2$ ), which was calculated as follows:

$$r^2 = 1 - (SS_{reg}/SS_{tot}) \quad (4)$$

with  $SS_{reg}$ , the sum of squares of the residuals from the fit and  $SS_{tot}$ , and the sum of squares of the residuals from the mean..



## **<sup>1</sup>H-MRI**

MRI was performed using the same whole body 1.5 T MRI system. T1-weighted images were acquired at rest in a supine position using a spin echo sequence (slice thickness = 5 mm, gap thickness = 10 mm, TR = 490 ms; TE = 12 ms; field of view = 25×25 cm; matrix size = 512×512) with 9-12 axial slices covering the region from the greater trochanter to the knee (thigh). The cross-sectional area of each slice was determined using the CSIAPO software (32). On the basis of a signal-intensity threshold, muscle was segmented from other tissues. Muscle volume was then calculated by summing the areas of all the slices, taking into account the slice thickness and the inter-slice space.

### **Measurements of pulmonary oxygen uptake**

VO<sub>2p</sub> during exercise was measured on a breath-by-breath basis (ZAN 680, ZAN Messgerate, Oberthulba, Germany). Subjects breathed through a low dead space (40 ml) low resistance mouthpiece and flow sensor assembly. Gases were continuously drawn from the mouthpiece through a 6.5-m capillary line (2 mm diameter), and analyzed for O<sub>2</sub> and CO<sub>2</sub> concentrations by electrochemical and infrared absorption analyzers, respectively. Expiratory volume was determined by a flow sensor calibrated before each test using a known volume syringe (1 L, ZAN 680, Oberthulba, Germany). Gas analyzers were calibrated before each test from both the ambient air (O<sub>2</sub>: 20.93% and CO<sub>2</sub>: 0.03%) and a gas mixture of known composition (O<sub>2</sub>: 16% and CO<sub>2</sub>: 5% in normoxia). Pulmonary oxygen uptake was measured inside the MR system simultaneously to the MR measurements as previously described (5).

### **Statistical analyses**

The assessment of differences between endurance-trained and recreationally active subjects was performed with either paired t-tests or nonparametric Wilcoxon tests, after testing for the normality of the distribution (Statistica, version 5.5; Statsoft, Tulsa, Oklahoma). The normality of the distribution was verified from the skewness and kurtosis values transformed into Z-scores. Then non-parametric tests were employed when an absolute value >1.96 or <-1.96 (significance above  $P < 0.05$ ) was observed. For variables evolving with respect to time during exercise, the effect of training status on the overall time course was determined using a two-way ANOVA with repeated measurements (GROUP × TIME) (Statistica software, Statsoft Inc.). When a significant interaction effect was detected, post hoc comparisons (Bonferroni Test) were used in order to compare values between ET and AT for each time point. Statistical significance was accepted at  $P < 0.05$ . Results are presented as mean ± SD in tables and figures.

## **Results**

### **Muscle volume and mechanical measurements**

The muscle volume was not significantly different between groups (ET: 2.0 ± 0.3L and RA: 2.2 ± 0.5L,  $P > 0.05$ ). The relative contribution from both legs to the total power output was remarkably similar (ET: 0.5 ± 0.6 and RA: 0.5 ± 0.13) during the knee-extension exercise. As illustrated in figure 1, the power output from the leg sampled by the MR coil was



constant throughout the exercise and was similar between groups (ET:  $31 \pm 21$  watts and RA:  $31 \pm 9$  watts,  $P > 0.05$ )

### Muscle metabolites and intracellular pH

The group mean changes in PCr, pH, Pi and ADP during the knee-extension exercise are illustrated for both groups in figure 2. Specifically, there was no significant difference between ET and RA for all the variables. The amount of PCr consumed at the end of exercise was not significantly different between groups (Mann-Whitney U:  $P > 0.05$ ), reaching  $28 \pm 2$  % in ET and  $27 \pm 2$  % relative to baseline in RA. Changes between minute 3 and the last point of the exercise (PCr<sub>(6-3)</sub>) were not significantly different between groups (Mann-Whitney U:  $P > 0.05$ ). Despite a more pronounced alkalosis at the onset of exercise (rate of pH rise over the first 24 s of exercise, ET:  $0.17 \pm 0.11$  pH unit.min<sup>-1</sup> and RA:  $0.07 \pm 0.06$  pH unit.min<sup>-1</sup>, Mann-Whitney U:  $P = 0.07$ ), the pH drop calculated as the difference between the baseline and the end-of-exercise values was not significantly different between groups (ET:  $0.04 \pm 0.06$  pH and RA:  $0.05 \pm 0.03$  pH, Mann-Whitney U:  $P > 0.05$ ). Changes between minute 3 and the last point of the exercise (pH<sub>(6-3)</sub>) were not significantly different between groups (Mann-Whitney U:  $P > 0.05$ ). G<sub>ATP</sub> was also not significantly different between groups at rest (ET:  $-61.2 \pm 0.4$  kJ.mol<sup>-1</sup> and RA:  $-60.9 \pm 0.7$  kJ.mol<sup>-1</sup>, Mann-Whitney U:  $P > 0.05$ ) and during the last min of the exercise (ET:  $-58.2 \pm 1.2$  kJ.mol<sup>-1</sup> and RA:  $-57.7 \pm 0.9$  kJ.mol<sup>-1</sup>, Mann-Whitney U:  $P > 0.05$ ).

### Rates of ATP synthesis during exercise

The rates of ATP synthesis from glycolysis, the creatine kinase reaction, and oxidative phosphorylation during the last min of the knee-extension exercise are presented in figure 3 and were not significantly different between groups (Mann-Whitney U:  $P > 0.05$ , ATP<sub>gly</sub>, t-test:  $P > 0.05$ ). At the end of exercise, the ATP cost of dynamic contraction was not significantly different between ET and RA ( $P > 0.05$ , figure 4A).

### Gas exchanges during exercise

Steady-state VO<sub>2</sub> measured during the last min of the exercise was not significantly different between ET and AT (ET:  $848 \pm 155$  ml.min<sup>-1</sup> and RA:  $760 \pm 131$  ml.min<sup>-1</sup>,  $P > 0.05$ ). Similarly, the steady-state ventilation rate (ET:  $21 \pm 3$  L.min<sup>-1</sup> and RA:  $20 \pm 4$  L.min<sup>-1</sup>) and respiratory exchange ratio (Mann-Whitney U: ET:  $0.88 \pm 0.05$  and RA:  $0.91 \pm 0.07$ ) measured during the last min of the exercise were both not significantly different between ET and RA ( $P > 0.05$ ).

### Mitochondrial function

The group mean PCr resynthesis response in both ET and RA is illustrated in figure 5. During the post-exercise period, the PCr offset time constant was significantly faster in ET compared to RA (Mann-Whitney U: ET:  $32 \pm 8$  s and RA:  $43 \pm 10$  s,  $P < 0.05$ , figure 5). In contrast, the mitochondrial efficiency estimated from the combined steady-state rates of oxidative ATP synthesis and systemic VO<sub>2</sub> was not significantly different (Mann-Whitney U:  $P > 0.05$ , figure 4b).

## Discussion

Using an *in vivo* approach combining simultaneous measurement of systemic O<sub>2</sub> consumption and muscle oxidative ATP synthesis during moderate-intensity knee-extension, this study sought to shed some light on the differences in mitochondrial efficiency and ATP cost of contraction during exercise in endurance-trained and recreationally active individuals. Consistent with our hypothesis, despite a greater mitochondrial capacity for ATP synthesis in the quadriceps, endurance-trained subjects exhibited a similar mitochondrial efficiency and ATP cost of contraction at steady-state during knee-extension in comparison to recreationally active individuals. These findings suggest that several years of endurance-training, resulting in an elevated VO<sub>2max</sub> and muscle phosphorylation capacity, do not appear to influence the two main physiological determinants of muscle efficiency, i.e. mitochondrial and contractile efficiency.

### Mitochondrial efficiency in skeletal muscle of endurance-trained individuals

With the tenet that mitochondrial coupling is more efficient at supra-basal respiration rate and that this state better reflects the mitochondrial properties, the major goal of this study was to provide some insights into the effect of endurance training on mitochondrial efficiency in the skeletal muscle during exercise. In accordance with previous *in vitro* studies assessing the P/O ratio during ADP-stimulated respiration (34, 42, 43), *in vivo* mitochondrial efficiency estimated at steady-state during dynamic contractions of the quadriceps was not significantly different between endurance-trained and recreationally active subjects (figure 4B). This result was also indirectly confirmed by the comparable ADP and Pi levels in both groups, two well-known regulators of mitochondrial respiration, at the end of exercise (figure 2). Indeed, a hypothetical improvement in mitochondrial coupling would have translated into lower levels of ADP or Pi for a given work rate or respiration rate, as mitochondrial respiration sensitivity for ADP is independent of the level of physical fitness (46). Notably, these concentrations were achieved for the same absolute work rate (figure 1) thereby implying that the activation of some mitochondrial enzymes by intracellular Ca<sup>2+</sup> was also similar between groups. Therefore, considered together, these results provide support to the conclusion that mitochondrial efficiency was unaffected by the level of aerobic physical fitness.

Our results are apparently conflicting with two previous studies conducted *in vivo* and indicating either a lower (2) or a greater resting mitochondrial efficiency (6) in the skeletal muscle of endurance-trained athletes. The reasons for these discrepancies are unclear and might be related to the ambiguity in the interpretation of the oxidative ATP synthesis rates using <sup>31</sup>P saturation transfer-MRS or the experimental conditions employed (rest versus exercise). Indeed, the contribution of glycolysis to the Pi→ATP flux measured by <sup>31</sup>P saturation transfer has been advocated to confound the interpretation of mitochondrial ATP turnover with this technique and to result in an overestimation of the rate of oxidative ATP synthesis (22). Differences in experimental paradigms might also well account for the divergence in the results. One should bear in mind that both of these studies measured mitochondrial efficiency at rest, a condition for which mitochondrial membrane potential is at the highest thereby conditioning a large generation of free radicals. Interestingly, despite a

well acknowledged elevated mitochondrial volume density in trained subjects, indirectly confirmed here by a larger muscle phosphorylation capacity (figure 5), resting ATP demand from the skeletal muscle appears to be independent from the training status (2, 6). This metabolic configuration implies a lower ATP synthesis per mitochondrial mass, which, if associated with an unchanged or perhaps even improved mitochondrial efficiency at rest (6), would in turn create a metabolic imbalance. In other words, the overabundance of energetic substrates relative to cellular energy demand would lead to exaggerated free radical leak and mitochondrial stress and/or damage. Given the unequivocal benefits from physical activity on mitochondrial function and oxidative stress level in numerous populations, this scenario appears unlikely. One can instead speculate that, for a low activity state i.e. resting state *in vivo* or non-phosphorylating respiration *in vitro*, trained subjects may exhibit a lower mitochondrial efficiency in order to decrease the mitochondrial membrane potential and prevent excessive generation of free radicals at rest (2, 36, 42). In contrast, during exercise or ADP-stimulated respiration, mitochondrial coupling would be optimized for ATP synthesis regardless of the muscle aerobic capacity as suggested here *in vivo* and previously *in vitro* (34, 42, 43). Further supporting this interpretation, a lower free radical leak has been documented during non-phosphorylating respiration but not during ADP-stimulated respiration in permeabilized fibers from the skeletal muscle following endurance training (8).

It has been consistently demonstrated that endurance-training is associated with a reduction in UCP3 mRNA expression and protein content (6, 11, 34, 41). UCP3 is a protein which has been proposed to be involved in the modulation of mitochondrial oxidative phosphorylation coupling. Indirect support to this theory has been provided by the negative correlation observed between UCP3 protein content and work efficiency during cycling exercise (34, 41) or resting mitochondrial coupling measured *in vivo* (6). Therefore, our finding that mitochondrial efficiency is unaltered in the skeletal muscle of endurance-trained individuals appears to be somewhat inconsistent with this concept. However, several other studies have provided evidence against a tight link between UCP3 content and mitochondrial efficiency. For instance, mitochondrial efficiency determined at submaximal or maximal ADP-stimulated respiration was not correlated to UCP3 content in isolated mitochondria from skeletal muscle of untrained and endurance-trained subjects (34). Also, 6 weeks of endurance-training have been shown to decrease non-phosphorylating respiration with free-fatty acid substrate and UCP3 protein content, but did not change the P/O ratio measured during ADP-stimulated respiration with carbohydrate substrates in isolated mitochondria from skeletal muscle (11, 42). Further to this point, it has been documented that changes in UCP3 protein expression and mitochondrial coupling could be dissociated under some conditions in heart tissue (3) and recent evidence suggests that both free-fatty acid and reactive oxygen species can also activate mitochondrial uncoupling (10). Collectively, these findings suggest that although UCP3 may have some role in regulating mitochondrial efficiency at rest when oxidation of free-fatty acid is predominant, its role might be relatively minor during exercise when carbohydrates become the main substrates and the mitochondria is rather tightly coupled (35). It is very likely that mitochondria use multiple mechanisms (UCP, adenine nucleotide transporters, free fatty acid transporter, fuel selection) to reduce their own production of reactive O<sub>2</sub> species, and these mechanisms may be

controlled somewhat independently from the training-induced change in oxidative phosphorylation capacity.

### **Mitochondrial capacity in skeletal muscle of endurance-trained individuals**

The PCr resynthesis time constant, an index of muscle phosphorylation capacity, was 27% faster in endurance-trained subjects in comparison to recreationally active individuals (figure 5). Such value is in agreement with previous reports documenting an increase in muscle phosphorylation capacity ranging between 14% and 103% following a training intervention or comparing trained and untrained individuals (13, 21, 29). Given that mitochondrial efficiency did not appear to be improved in endurance-trained individuals, the higher mitochondrial capacity for ATP synthesis reported herein likely stemmed from an elevated mitochondrial volume density and/or an intensification of the parallel activation of ATP supply and ATP demand (i.e. simultaneous activation of the respiratory complexes of the mitochondria and myosin ATPases). With regard to the former hypothesis, it is well established that mitochondrial volume density increases after only few days of training (8) and is superior in trained compared to untrained subjects (20). Interestingly, some studies have also documented an increase in mitochondria-specific respiratory capacity with endurance-training (36) or in subjects with high level of physical activity (20). This result is particularly interesting considering the lack of changes in mitochondrial efficiency in endurance-trained individuals suggested by our findings. Unfortunately, mitochondrial volume within the muscle was not assessed in the present study and it is thereby difficult to speculate on the contribution of this mechanism to the present findings. Further studies investigating the effects of a training program or training status on mitochondria-specific phosphorylation capacity and the coupling between electron donors and acceptors ( $H^+/e^-$ ) in the electron transport chain would be of great interest to improve our understanding of the exercise-induced adaptation of the mitochondria.

Alternatively, it has been postulated that an intensification of the parallel activation of both ATP supply and demand associated with a slower post-exercise inactivation of the oxidative phosphorylation could also increase the maximum ATP production rate by oxidative phosphorylation and accelerate the PCr resynthesis kinetics (27). For instance, 5 weeks of moderate-intensity training accelerated the  $VO_2$  onset kinetics during moderate-intensity cycling, indirectly indicating an increased muscle respiratory capacity, in the absence of a significant change in mitochondrial volume density (45). Using computer simulations, the authors suggested that a moderate increase in the parallel activation intensity accounted entirely for the reported improvement in  $VO_2$  onset kinetics (45). The same computer model also predicted that the slower the post-exercise inactivation of the parallel activation mechanism, the slower the recovery of PCr (28). Although not assessed here, such mechanism might have contributed to some extent to the augmented mitochondrial capacity in endurance-trained individuals compared to recreationally active subjects.

### **ATP cost of contraction in skeletal muscle of endurance-trained individuals**

Another interesting finding of the present study was the lack of significant difference in the ATP cost of contraction during moderate-intensity dynamic knee-extension between endurance-trained and recreationally active individuals (figure 4A). This finding was further

confirmed by the remarkably similar level of cytoplasmic free energy of ATP hydrolysis, indicating that the energy released during the hydrolysis of ATP in the skeletal muscle to accomplish the same work rate (figure 1) was unaffected by the training status. Accordingly, the pH and concentrations in various metabolites (PCr, Pi, ADP) reached at the end of the exercise performed were also comparable between groups (figure 2). These results extend to the moderate-intensity domain previous findings of similar ATP cost of contraction in the skeletal muscle of subjects with different physical fitness during high-intensity exercise (21, 31).

This consistent finding of unaltered ATP cost of contraction in subjects chronically trained is particularly intriguing as several adaptations of the skeletal muscle phenotype with endurance-training may in theory result in a lower energy cost of contraction. Indeed, the typical training-induced shift in fiber-type composition toward more oxidative type I fiber is generally thought to diminish muscle energy cost as type I fibers were initially suggested to be more energy efficient than type II fibers (7). However, the consistent finding *in vitro* that peak efficiency is actually unrelated to fiber type but dependent on shortening speed has cast some doubt on the validity of this theory (1). On this basis, although the trained subjects in the present likely exhibited some shift toward a more oxidative type I fiber phenotype, this adaptation did not seem to provide any benefit on the ATP cost of contraction during dynamic low frequency exercise. Due to the dynamic nature of our protocol, the estimated ATP cost reflected the energy demand from both the cross-bridge cycling (myosin-ATPase) and the processes of ion pumping (Ca<sup>2+</sup> ATPase and Na<sup>+</sup>-K<sup>+</sup> ATPase). Given the significant metabolic demand from the non-contractile processes of ion transport, which can represent 30 to 50% of the total energy consumed by the contracting muscle, a potential improvement in Ca<sup>2+</sup> handling may also contribute to decrease the metabolic cost during dynamic contractions. Interestingly, endurance-training has been documented to improve the levels of proteins involved in Ca<sup>2+</sup> handling such as dihydropyridine receptor, ryanodine receptor, Na<sup>+</sup>-Ca<sup>2+</sup> exchanger, sarcoplasmic reticulum Ca<sup>2+</sup>-ATPase (SERCA1 and 2) and calsequestrin in mice (12, 26). However, in humans, downregulation of sarcoplasmic reticulum Ca<sup>2+</sup>-ATPase expression and decreased Ca<sup>2+</sup> sequestration have also been reported following endurance training (19, 33). In addition, *in vitro* results from isolated myofibers of highly trained distance runners do not appear to support the hypothesis that fiber-type shift and alterations in Ca<sup>2+</sup> handling substantially affect the contractile functional properties of the skeletal muscle (15). Specifically, although some of the intrinsic properties of the myofibers were improved in trained distance runners, mainly in type I fibers, these differences in peak force and power were eliminated when accounting for differences in fiber type composition and fiber cross-sectional area (15). Therefore, based upon the above evidence, it does not appear that the training-induced changes in the contractile properties of the skeletal myofibers may influence the metabolic demand during dynamic contractions.

### Experimental consideration

The measurement of mitochondrial efficiency necessitates the simultaneous measurement of O<sub>2</sub> consumption and ATP synthesis from the same muscle sample and therefore represents a technical challenge *in vivo*. In the present study, the ratio between the local rate of oxidative ATP synthesis and systemic VO<sub>2</sub> from the gas exchange was employed as an indirect

measurement of mitochondrial efficiency. Such an approach relies on two assumptions relative to the muscle mass activated during the exercise and the contribution from the exercising limbs to the systemic measurement of  $\text{VO}_2$ . With regard to the first assumption, Ray *et al.* have previously documented that  $\sim 50\%$  and  $\sim 100\%$  of the quadriceps muscle volume was recruited at 20 W and 40W during single-leg dynamic knee-extension within an MR-scanner (38). Accordingly, we estimated that  $\sim 75\%$  of the quadriceps was recruited during the knee-extension exercise at  $\sim 30$  W in the present study. Also, given that both endurance-trained and recreationally active subjects exercised at the same power output and exhibited similar muscle volume, it is unlikely that muscle recruitment was appreciably different between groups at such low workloads. Indeed, muscle activation inferred from the exercise-induced changes in the transverse relaxation time of the skeletal muscle measured by magnetic resonance imaging has previously been reported to be similar at low intensity or modestly decreased in only one muscle ( $\sim 3\%$ ) during high-intensity cycling exercise after training (40). Additionally, albeit carried out at a higher intensity, we did not previously detect any significant difference in the integrated electromyogram of the vastus lateralis between endurance-trained and sedentary subjects during knee-extension exercise (31). Collectively these findings suggest that, although potential inaccuracies in the estimation of the muscle mass activated during the exercise may have affected our quantification of the P/O ratio, these systematic errors would not confound a between-group comparison.

With regard to the use of systemic measurement of  $\text{VO}_2$  from gas exchanges to reflect exercise-induced changes in muscle  $\text{VO}_2$ , it has been previously documented that pulmonary  $\text{O}_2$  consumption primarily reflected muscle  $\text{O}_2$  uptake from the active muscle during knee-extension exercise (39). In addition, baseline  $\text{VO}_2$ , which represent the metabolic demand from other sources than the skeletal muscle, was subtracted to take into account the energy demand from non-exercising muscle. Also, as both groups exercised at the same power output it is unlikely that accessory muscles contributed differently to systemic  $\text{VO}_2$  between groups. Along the same line, both the steady-state ventilation rate and respiratory exchange ratio were similar between endurance-trained and recreationally active subjects thus implying similar energy demand from the ventilatory muscles and fuel oxidation in both groups. Finally, the P/O values calculated in the present study (1.8-2.0) are in reasonable agreement with the theoretical value for coupling of oxidative phosphorylation (P/O  $\sim 1.4$ -2.5 (4)). Therefore, although we acknowledge the limitation of the current approach to provide an absolute measurement of the P/O ratio *in vivo*, this is still a sound approach to estimate mitochondrial efficiency and compare endurance-trained and recreationally active subjects within the current experimental conditions.

One could suggest that the lack of differences in the ATP cost of contraction observed between endurance-trained and recreationally active subjects might be the results of a type II error. However, this specific result has to be interpreted in the context of large differences in whole body aerobic capacity ( $\text{VO}_{2\text{max}}$ , ET +55%) and muscle mitochondrial capacity ( $\tau$ , ET +26%), for which the sample size was sufficient to detect significant differences between groups. In addition, similar results have previously been reported regarding the influence of training status on the ATP cost of contraction at higher exercise intensity (21, 31). Therefore, although a type II error cannot completely be dismissed, taken together, our finding and



previous results suggest that it is unlikely that the ATP cost of contraction is affected by the training status, or if so, to an extent that is trivial and thus of limited clinical significance.

## Conclusion

In conclusion, this study indicated that endurance-trained subjects exhibited similar mitochondrial efficiency and ATP cost of contraction during moderate-intensity knee-extension in comparison to recreationally active individuals. Interestingly, given that mitochondrial efficiency did not appear to be altered, the higher mitochondrial capacity for ATP synthesis in endurance-trained subjects likely stemmed from an elevated mitochondrial volume density and/or mitochondria-specific respiratory capacity. Taken altogether, these findings suggest that 1) the training-induced change in mitochondrial energetic capacity and mitochondrial coupling during exercise are somewhat independently regulated, and 2) several years of endurance-training do not appear to influence the two main physiological determinants of muscle efficiency, i.e. mitochondrial and contractile efficiency.

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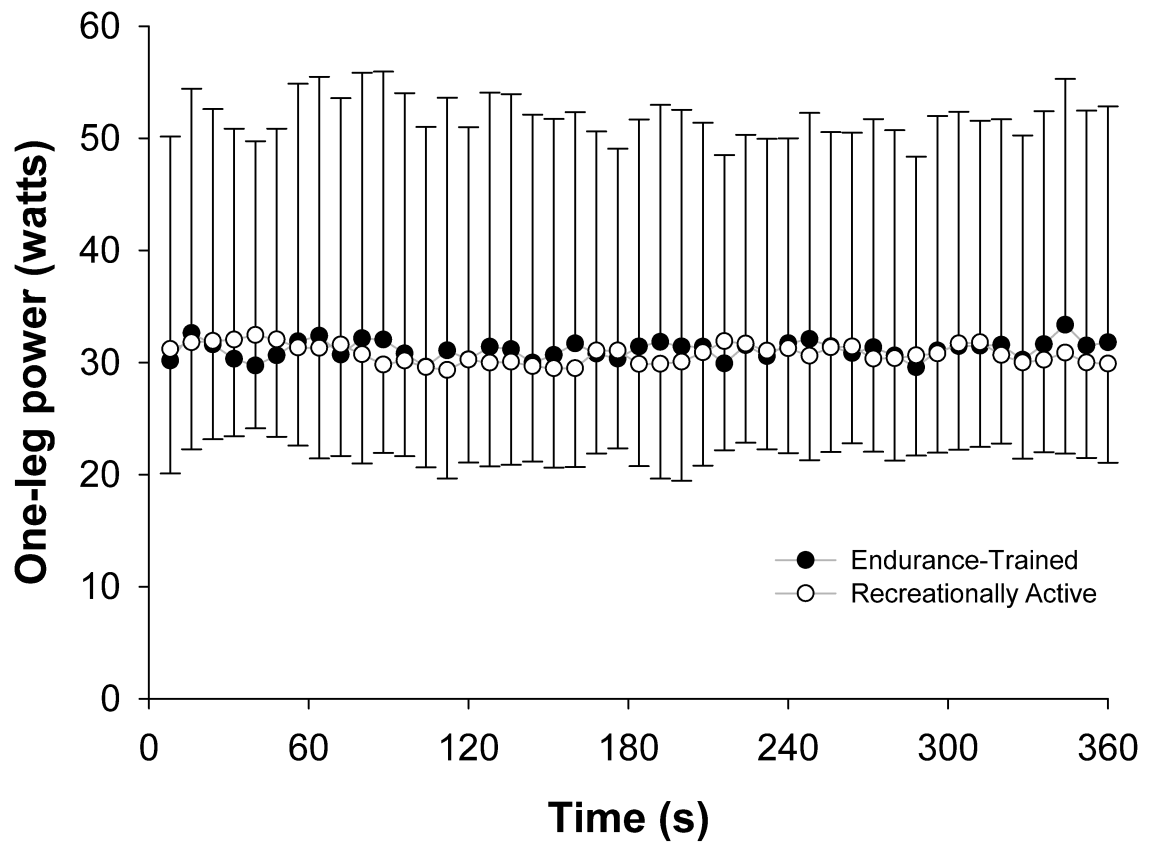
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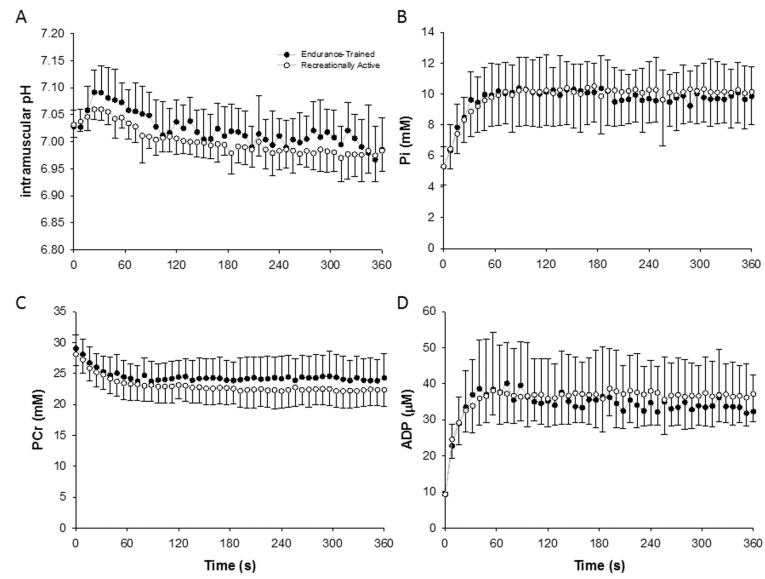
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**Figure 1.**

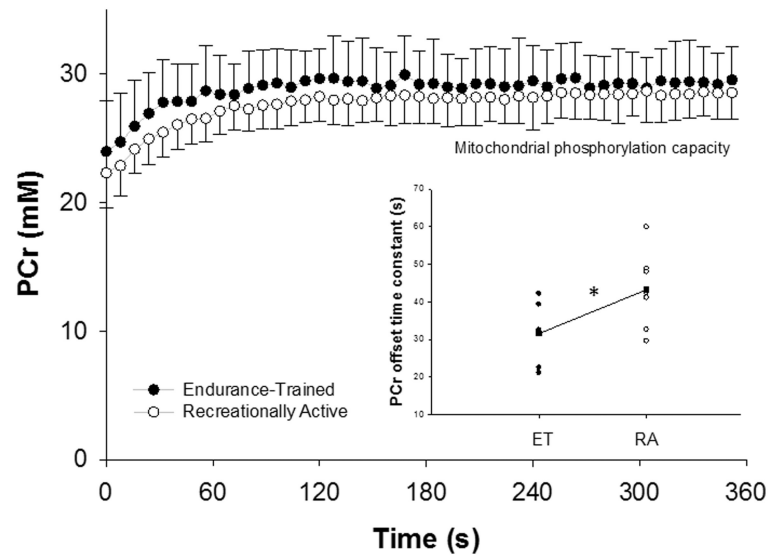
Power output from the MR sampled leg with respect to time throughout the moderate knee-extension exercise in endurance-trained (●) and recreationally active (○) subjects. Values are presented as means  $\pm$  SD. There was no significant interaction between time and group ( $P > 0.05$ ).



**Figure 2.** Changes in pH (A), inorganic phosphate (Pi, B), phosphocreatine (PCr, C) and Adenosine Di-Phosphate (ADP, D) with respect to time throughout the knee-extension exercise in endurance-trained (●) and recreationally active (○) subjects. Values are presented as means  $\pm$  SD. There was no significant interaction between time and group for each of the variables ( $P > 0.05$ ).







**Figure 5.** PCr resynthesis with respect to time following exercise in endurance-trained (●) and recreationally active (○) subjects. Data are presented as mean  $\pm$  SD. The figure insert provides the individual and the mean PCr offset time constant, which was significantly faster in endurance-trained individuals ( $P < 0.05$ ).