

## Effect of interval versus continuous training on cardiorespiratory and mitochondrial functions: relationship to aerobic performance improvements in sedentary subjects

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<sup>1</sup>CHRU of Strasbourg, Physiology and Functional Explorations Department, Civil Hospital, Strasbourg, France and University Louis Pasteur, Faculty of Medicine, Physiology Department, Strasbourg, France; and <sup>2</sup>Cardiology Department, Civil Hospital, Colmar, France

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**Daussin FN, Zoll J, Dufour SP, Ponsot E, Lonsdorfer-Wolf E, Doutreleau S, Mettauer B, Piquard F, Geny B, Richard R.** Different effect of interval versus continuous training on mitochondrial function in sedentary subjects: relation to aerobic performance improvements. *Am J Physiol Regul Integr Comp Physiol* 295: R264–R272, 2008. First published April 16, 2008; doi:10.1152/ajpregu.00875.2007.—The goal of the study was to determine the effects of continuous (CT) vs. intermittent (IT) training yielding identical mechanical work and training duration on skeletal muscle and cardiorespiratory adaptations in sedentary subjects. Eleven subjects (6 men and 5 women,  $45 \pm 3$  years) were randomly assigned to either of the two 8-wk training programs in a cross-over design, separated by 12 wk of detraining. Maximal oxygen uptake ( $\dot{V}O_{2\max}$ ) increased after both trainings (9% with CT vs. 15% with IT), whereas only IT was associated with faster  $\dot{V}O_2$  kinetics ( $\tau$ :  $68.0 \pm 1.6$  vs.  $54.9 \pm 0.7$  s,  $P < 0.05$ ) measured during a test to exhaustion (TTE) and with improvements in maximal cardiac output ( $Q_{\max}$ , from  $18.1 \pm 1.1$  to  $20.1 \pm 1.2$  l/min;  $P < 0.01$ ). Skeletal muscle mitochondrial oxidative capacities ( $V_{\max}$ ) were only increased after IT ( $3.3 \pm 0.4$  before and  $4.5 \pm 0.6$   $\mu\text{mol O}_2 \cdot \text{min}^{-1} \cdot \text{g dw}^{-1}$  after training;  $P < 0.05$ ), whereas capillary density increased after both trainings, with a two-fold higher enhancement after CT ( $+21 \pm 1\%$  for IT and  $+40 \pm 3\%$  after CT,  $P < 0.05$ ). The gain of  $V_{\max}$  was correlated with the gain of TTE and the gain of  $\dot{V}O_{2\max}$  with IT. The gain of  $Q_{\max}$  was also correlated with the gain of  $\dot{V}O_{2\max}$ . These results suggest that fluctuations of workload and oxygen uptake during training sessions, rather than exercise duration or global energy expenditure, are key factors in improving muscle oxidative capacities. In an integrative view, IT seems optimal in maximizing both peripheral muscle and central cardiorespiratory adaptations, permitting significant functional improvement. These data support the symmorphosis concept in sedentary subjects.

mitochondria; endurance training; performance

IN ORDER TO INCREASE MUSCLE oxidative capacities, as well as endurance capacities, continuous (CT) and interval training (IT) are both established exercise modalities used in the rehabilitation of patients with chronic diseases (32, 42), as well as in the preparation of elite athletes. There is considerable debate as to which training program (CT vs. IT) will have a greater effect on aerobic performance, and there are relatively few studies that have directly compared skeletal muscle metabolic adaptations to interval and continuous training (16, 21). CT is

characterized by a constant submaximal power output and  $O_2$  consumption, whereas IT alternates periods of low with periods at high mechanical power output, during which  $O_2$  consumption fluctuates. We recently demonstrated an effect of training modality per se on peak oxygen consumption ( $\dot{V}O_{2\max}$ ) and fluctuations of  $O_2$  consumption ( $\dot{V}O_2$ ) during IT sessions improved both central and peripheral components of  $\dot{V}O_{2\max}$ , whereas the constant  $O_2$  requirement during CT was mainly associated with greater oxygen extraction (12). Then, for similar energy expenditure and training duration, endurance training adaptations are different in function of training modalities. The mechanisms underlying these adaptations, together with the relative significance of central vs. peripheral factors remain, however, unclear.

At the muscle level,  $O_2$  utilization depends on both capillary and mitochondrial status. Mitochondria are the main subcellular structures using  $O_2$  to produce energy required by contractile work (27). It has been demonstrated that these organelles were altered markedly by several stimuli, including exercise training (24, 25, 27). Endurance training, using an appropriate duration per day, frequency per week, and submaximal intensity per exercise session, can produce an increase in mitochondrial content, usually ranging from 50 to 100% within 6 wk (13, 19, 20). Endurance training in humans is also associated, notably, with enhancement of muscle oxidative capacities, resulting in the improvement of systemic aerobic function both at  $\dot{V}O_{2\max}$  and during endurance exercise (24, 52). Alterations of muscle redox potential and high-energy phosphate flux, brought about by muscle contraction, induce mitochondrial activation and biogenesis in muscle cells (26). Capillary density also plays a central role in  $O_2$  supply to mitochondria, and it has been established that skeletal muscle is able to adapt to endurance training by enhancing its capillary supply (9). The influence of training modality per se on mitochondrial function, capillary networks, and their role in setting aerobic performance capacity has never been explored.

Both central and peripheral adaptations to endurance training participate in the improvement of aerobic performance, but their respective contributions to the improvements of  $\dot{V}O_{2\max}$  and/or on the speeding of the  $\dot{V}O_2$ , cardiac output ( $\dot{Q}$ ), and  $O_2$  arteriovenous difference ( $D_{a-v}O_2$ ) response to constant exercise, is largely debated (40, 45). For instance, it is questioned

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whether the  $D_{a-\bar{v}}O_2$  kinetics is faster than the  $\dot{Q}$  kinetics (18, 36). Because  $O_2$  utilization by the mitochondrial pool largely contributes to the  $D_{a-\bar{v}}O_2$  kinetics, it is reasonable to assume that the mitochondrial adaptations to training may affect  $D_{a-\bar{v}}O_2$  kinetics. What would happen for the  $\dot{Q}$  response in this condition is unclear. In a comparative study between athletic, active, and sedentary subjects, we established a relationship between muscular oxidative capacities and  $\dot{V}O_{2max}$  (53). Improvements in muscle oxidative capacity appeared involved in  $\dot{V}O_{2max}$  enhancement in response to endurance training. These results are in accordance with the concept of symmorphosis, implying a tight coupling of structural parameters to functional demand (49). However, how  $\dot{Q}$  changes are involved in setting the training-induced improvement of  $\dot{V}O_{2max}$  when muscle oxidative capacity is enhanced warrants further investigation.

This study proposes, therefore, an integrative analysis, from systemic to cellular adaptations, to determine the effects of two endurance training modalities yielding similar mechanical work and training duration. We hypothesized that 1) variations of workload and  $O_2$  uptake during training sessions, associated with disturbances to cellular homeostasis is, per se, a key contributor to peripheral adaptations, including skeletal muscle mitochondrial function and capillary supply; 2) the balance between central and peripheral adaptations to training is training modality dependent, as attested by the behavior of the central and peripheral components of  $\dot{V}O_{2max}$  at peak exercise and during constant heavy-intensity exercise.

## METHODS

### Subjects

Eleven sedentary subjects (seven men and four women), who were not taking medications participated in this study (Table 1). Sedentary status was characterized by the level of physical activity evaluated by the Baecke's questionnaire (2). All subjects were informed about the potential risks associated with the experiments before giving their written consent to participate. The investigation was approved by the Consultant Committee on Human Protection from Biomedical Research of Strasbourg in accordance with French Law and with the Declaration of Helsinki.

### Study Design

Subjects were ascribed to two experimental groups: CT or IT. After 12 wk of deconditioning and using a randomized cross-over design, each subject was asked to engage in a second training period. The subjects of the CT group were thereafter ascribed to the IT program and vice versa.

In the week before and after each training period, all subjects performed three tests, which were separated by at least 24 h of rest: 1) a maximal cycling incremental test (IET), 2) a test to exhaustion (TTE), and 3) a muscle biopsy to analyze mitochondrial respiration of permeabilized fibers and to quantify capillary density. During the first IET, we determined for each subject: the power associated with the

two training programs and the physical functional capacities (peak power, ventilatory thresholds). Benefits of training were evaluated at the end of each training period. Finally, to use each subject as his/her own control, each subject performed a total of 4 IET and 4 TTE and underwent four muscle biopsies.

The third incremental test was performed after the deconditioning period, to ensure that the subject's exercise capacities had returned to the initial level, as evaluated by peak power ( $P_{max}$ ),  $\dot{V}O_{2max}$ , ventilatory thresholds, mitochondrial respiration, and capillary density.

Each test was performed at the same time of the day, in the morning after a light breakfast on an upright electronically braked cycle ergometer (Medifit 1000S, Belgium). Pedaling frequency was 60–70 revolutions per minute and was kept constant during the test.

**Training program.** Subjects performed three training sessions per week in the laboratory over an 8-wk period (24 sessions). The duration of the initial training session was 20 min and to maintain sufficient training stimulation, training duration was increased by 5 min every 2 wk, achieving 35 min during the last 2 wk. IT consisted of a series of blocks of 5 min, each block featuring 4 min at the power associated to the first ventilatory threshold ( $P_{VT1}$ ), followed by 1 min at 90% of  $P_{max}$ . Intensity was gradually increased and decreased during the alternation of loads. The power output used during CT ( $P_{CT}$ ) was computed from the equation below, allowing similar total energy expenditure and duration compared with the IT sessions:  $P_{CT} = [(4 \times P_{VT1} + 90\% \text{ of } P_{max})/5]$ .

**IETs.** Each subject carried out a maximal effort, according to Howley et al. (29a). Ventilatory thresholds were determined graphically (5); the first ventilatory threshold ( $VT_1$ ) was obtained from a regression analysis of the slope of carbon dioxide elimination ( $\dot{V}CO_2$ ) vs.  $\dot{V}O_2$  plot, and the second ventilatory threshold (i.e., respiratory compensation point;  $VT_2$ ) was determined as the point at which the increase of ventilation ( $\dot{V}_E$ ) becomes faster than the increase in  $\dot{V}CO_2$  (48). Heart rate (HR),  $\dot{V}_E$ ,  $\dot{V}O_2$ ,  $\dot{V}CO_2$ , and  $\dot{Q}$  were monitored continuously during the test.

**TTE.** TTE was performed at pretraining power calculated as:  $P_{\Delta 50} = \text{Power at } VT_2 (P_{VT2}) + (P_{max} - P_{VT2})/2$ . The test began by 10-min warmup at 40% of subject's  $P_{max}$  (lower than power at  $VT_1$  in all subjects). After a 5-min period of rest, the subjects were asked to cycle at  $P_{\Delta 50}$  for as long as tolerable. HR,  $\dot{V}_E$ ,  $\dot{V}O_2$ ,  $\dot{V}CO_2$ , and  $\dot{Q}$  were monitored continuously during the test.

**Skeletal muscle biopsy.** Vastus lateralis muscle was taken 48 h after TTE, and muscle was obtained by the percutaneous Bergström technique after local anesthesia as previously described (7). The muscle tissue retrieved was rinsed in ice-cold saline; one part was immediately frozen in liquid nitrogen for histology analysis, and the other part was reserved for in situ respiration studies.

**In situ study of mitochondrial respiration.** Mitochondrial respiration was studied in situ in saponin skinned fibers (44). Briefly, fibers were separated under binocular microscope in solution S at 4°C (see below) and permeabilized in solution S with 50  $\mu\text{g/ml}$  of saponin for 30 min. After being placed 10 min in solution R (see below) to wash out adenine nucleotides and creatine phosphate, skinned separated fibers were transferred into a 3-ml water-jacketed oxygraphic cell (Strathkelvin Instruments, Glasgow, Scotland) equipped with a Clark electrode as previously described (21). Solutions R and S contained: 2.77 mM  $\text{CaK}_2$  EGTA, 7.23 mM  $\text{K}_2$  EGTA (100 mM free  $\text{Ca}^{2+}$ ), 6.56

Table 1. Physical subjects characteristics

	Gender, male/female	Age, yr	Height, cm	Weight, kg	BMI, $\text{g/m}^2$	Body Fat, %	Baecke Index*
Female	n = 4	42 ± 3	167 ± 6	62 ± 3	21.4 ± 00.6	28.4 ± 00.4	5.8 ± 00.4
Male	n = 7	47 ± 4	178 ± 3	84 ± 9	26.2 ± 20.3	24.8 ± 20.3	5.9 ± 00.3
Total	n = 11	45 ± 3	174 ± 3	76 ± 7	24.4 ± 10.6	26.1 ± 10.8	5.9 ± 00.3

Values are expressed as means ± SE. \*Physical subject level was evaluated by a Baecke's questionnaire (2), index less or equal to 7.5 corresponded to a sedentary subject and was the maximal value accepted to be included in this study. Body mass index (BMI) = weight (kg)/height<sup>2</sup> (m<sup>2</sup>).

mM MgCl<sub>2</sub> (1 mM free Mg<sup>2+</sup>), 20 mM taurine, 0.5 mM DTT, 50 mM K-methane sulfonate (160 mM ionic strength), and 20 mM imidazole (pH 7.1). Solution S also contained 5.7 mM Na<sub>2</sub> ATP and 15 mM creatine-phosphate, while solution R contained 5 mM glutamate (G), 2 mM malate (M), 3 mM phosphate, and 2 mg/ml fatty acid free bovine serum. After the experiments, fibers were harvested and dried, and respiration rates were expressed as  $\mu\text{mol O}_2 \cdot \text{min}^{-1} \cdot \text{g dry weight}^{-1}$ .

**Immunohistochemical assays.** Serial transverse sections, 10  $\mu\text{m}$  thick, from vastus lateralis biopsies were cut with a microtome at  $-20^\circ\text{C}$  (Leica CM3050, Cambridge, MA). The identification of capillaries was performed using monoclonal antibody CD 31 (monoclonal mouse anti-human, GeneTex, San Antonio, TX), which recognizes platelet endothelial cell adhesion molecule, a transmembranous glycoprotein strongly expressed by vascular endothelium cells. For each experiment, a control reaction with the same protein concentration of normal serum as in the reaction with the first antibody was carried out.

### Measurements

**Ventilatory parameters.** During IET and TTE, gas exchanges were measured breath-by-breath using an open-circuit metabolic cart (Sensor Medics, Yorba Linda, CA).

**Cardiovascular parameters.** HR, SV, and  $\dot{Q}$  were monitored continuously.  $\dot{Q}$  was determined by bioimpedance method (Physioflow, Manatec, France), as described in a previous study (see appendix I of Ref. 10). This method has been validated both during constant-load and maximal incremental exercises (35, 36).

**Maximal muscular oxidative capacities.** The ADP-stimulated respiration above basal oxygen consumption ( $V_{O_0}$ ) was measured by the addition of 2 mM of ADP. After the determination of basal oxygen consumption ( $V_{O_0}$ ), the maximal fiber respiration rates were measured at  $22^\circ\text{C}$  under continuous stirring in the presence of a saturating amount of ADP as phosphate acceptor and glutamate-malate as mitochondrial substrates ( $V_{\text{max}}$ ). The acceptor control ratio (ACR) representing the degree of coupling between oxidation and phosphorylation was defined as  $V_{\text{max}}/V_{O_0}$ .

**Capillary measurements.** Muscle sections were examined under a light microscope (Labophot2, Nikon, Japan), at a magnification of  $\times 10$ , and a digital image was taken of the section (digital DS-5M, Nikon, Japan). Capillaries were quantified from the digital image, and the capillary-to-fiber ratio (C/F) was determined for each subject. Briefly, capillary present in an area and fiber present in the same area were counted manually and used to determine capillary-to-fiber ratio. The number of capillaries was corrected by subtracting half of the number of capillaries in the periphery of the area (1, 9).

### Data Analysis

**Signal treatments.** Gas exchanges and  $\dot{Q}$  data, initially obtained breath-by-breath and beat-to-beat, respectively, were later reduced to 5-s averages for all tests. For each parameter, the start of the test was stamped while recording. This measured point enabled the establishment of a single synchronous database for both  $\dot{V}_{O_2}$  and  $\dot{Q}$ . We used the Fick equation to calculate the  $D_{a-\bar{v}O_2}$ , dividing  $\dot{V}_{O_2}$  by  $\dot{Q}$  values averaged over the corresponding time interval.

Systolic and diastolic arterial blood pressures were measured at the end of each stage during IET using an inflatable cuff. Mean arterial pressure (MAP) was calculated as  $[(2 \times \text{diastolic blood pressure}) + \text{systolic blood pressure}]/3$ . Measures of blood pressure were synchronized with the  $\dot{Q}$  signal to calculate systemic vascular conductance (SVC) established from the ratio between  $\dot{Q}$  and MAP.

**Kinetics modelization.** To describe the  $\dot{V}_{O_2}$ ,  $\dot{Q}$ , and  $D_{a-\bar{v}O_2}$  on kinetics during the time to exhaustion test, different mathematical models were used (3):

$$Y(t) = Yb$$

$$+ A_1(1 - e^{-(t-td_1)/\tau_1})U_1 \quad \text{Fast component} \\ + A_2(1 - e^{-(t-td_2)/\tau_2})U_2 \quad \text{Slow component}$$

where  $U_1 = 0$  for  $t < td_1$  and  $U_1 = 1$  for  $t \geq td_1$ , and  $U_2 = 0$  for  $t < td_2$  and  $U_2 = 1$  for  $t \geq td_2$ .

$Yb$  is the basal value,  $A_1$  and  $A_2$  are the asymptotic amplitudes for the first and second components, respectively;  $\tau_1$  and  $\tau_2$  are the time constants of each exponential;  $td_1$  and  $td_2$  represent the time delays of each equation. The primary component phase is not distorted by any early cardiodynamic influence (8, 50). Because we focused on the fast and slow components, the cardiodynamic phase was excluded from analysis. As a consequence, the first 20 s were removed from analysis to ensure that the early initial component did not influence the results.

**Statistics.** The bootstrap method (17) was used to obtain an accuracy of the parameters describing the kinetics. A Fisher test was used to determine whether a monoexponential or biexponential model better fitted the kinetics data during the time to exhaustion test.

Statistical analyses were performed using Sigma Stat for Windows (ver. 3.0; SPSS, Chicago, IL). After testing for data distribution normality and variance homogeneity, a two-way ANOVA with repeated measures was performed to test significance between and within training. Pearson correlation was determined with difference between pretraining and posttraining values ( $\Delta$ ). A paired Student  $t$ -test was used to compare the parameters of  $\dot{V}_{O_2}$ ,  $\dot{Q}$ , and  $D_{a-\bar{v}O_2}$  kinetics during a given exercise. The significance level was set at  $P < 0.05$ . Data are presented as means  $\pm$  SE.

## RESULTS

### Training Intensity and Basal Status

During CT, the training intensity corresponded to  $109 \pm 3$  W or 61% of  $P_{\text{max}}$ , inducing a total mechanical work equal to  $4,320 \pm 115$  kJ. With IT, subjects alternated 4 min at low intensity ( $96 \pm 2$  W or 56% of  $P_{\text{max}}$ ) with 1 min at 90% of  $P_{\text{max}}$  ( $156 \pm 5$  W), leading to a total mechanical work ( $4,277 \pm 112$  kJ) equivalent to CT.

Seven subjects (4 men and 3 women) started the first training period with IT, while four subjects (2 men and 2 women) began with CT. No significant difference appeared among the data measured in the two pretraining periods, allowing us to compare the training effect between and within modalities. Moreover, no gender effect was identified with regard to the parameters of interest, allowing us to focus our analysis on the averaged data set.

### Effect on Performance

Both training modalities enhanced peak aerobic capacity (Fig. 1A):  $\dot{V}_{O_{2\text{max}}}$  improved by 9% with CT ( $P < 0.05$ ) and by 15% with IT ( $P < 0.01$ ) without difference between modalities. However,  $\dot{V}_{O_{2\text{max}}}$  improvements resulted from different adaptations. CT was mainly associated with improvement of  $D_{a-\bar{v}O_2}$  ( $10.9 \pm 0.9$  vs.  $12.6 \pm 1.1$  ml/100 ml,  $P < 0.01$ , Fig. 1C). Conversely, IT was associated with improvement of  $\dot{Q}_{\text{max}}$  ( $18.1 \pm 1.1$  vs.  $20.1 \pm 1.2$  l/min,  $P < 0.01$ , Fig. 1B) and  $D_{a-\bar{v}O_2}$  (from  $10.8 \pm 0.8$  to  $11.9 \pm 1.0$  ml/100 ml,  $P < 0.05$ , Fig. 1C).  $\dot{Q}_{\text{max}}$  improved with IT through an increase of both HR ( $164 \pm 3$  vs.  $168 \pm 4$  beats/min,  $P < 0.05$ ) and SV ( $110 \pm 7$  vs.  $119 \pm 8$  ml,  $P < 0.05$ ), whereas no changes were observed in these parameters after CT ( $165 \pm 4$  vs.  $165 \pm 4$  beats/min for HR and  $113 \pm 8$  vs.  $112 \pm 6$  ml for SV).

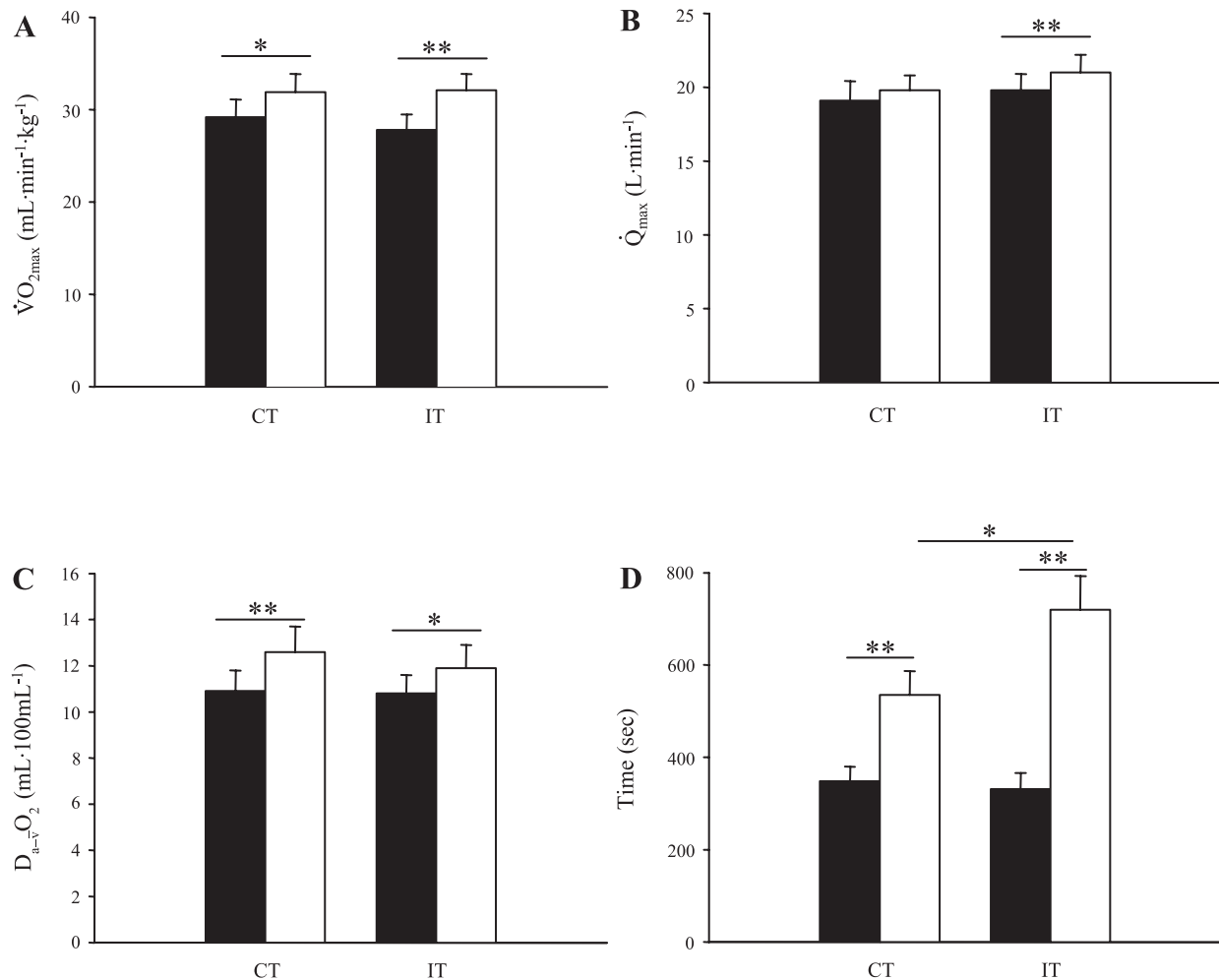


Fig. 1. Training effect on maximal values of oxygen uptake (A), cardiac output (B), arteriovenous oxygen difference (C) and time to exhaustion during all-out test (D). Values are means  $\pm$  SE. Black bars represent pretraining values and white bars are posttraining values. \*Significant difference ( $P < 0.05$ ). \*\*Significant difference ( $P < 0.01$ ).

### Cardiovascular Response to Exercise

SVC increased with CT at  $VT_1$  and peak exercise and improved only at peak exercise with IT (Table 2). A decrease of MAP at peak exercise was observed only with CT ( $113 \pm 1$  mmHg before training vs.  $103 \pm 1$  mmHg after training,  $P < 0.01$ ), and MAP was lower after CT than IT ( $103 \pm 1$  mmHg vs.  $110 \pm 1$  mmHg, respectively,  $P < 0.05$ ).

### Effect of Training Modality Upon On-Kinetics

TTE was improved after both training modalities ( $348 \pm 32$  s before training vs.  $535 \pm 52$  s after CT and  $331 \pm 35$  s

Table 2. Systemic vascular conductance

	CT		IT	
	Pre	Post	Pre	Post
Rest	$65 \pm 3$	$67 \pm 3$	$65 \pm 3$	$65 \pm 3$
$VT_1$	$128 \pm 6$	$145 \pm 11^*$	$130 \pm 8$	$143 \pm 9$
Peak exercise	$169 \pm 8$	$180 \pm 9^*$	$167 \pm 9$	$182 \pm 8^\dagger$

Values are expressed as means  $\pm$  SE and are expressed in milliliters per minute per millimeters  $\cdot$  Hg. \*Different ( $P < 0.05$ ) from pretraining values.  $^\dagger$ Different ( $P < 0.01$ ) from pretraining values. CT, continuous training; IT, intermittent training;  $VT_1$ , first ventilatory threshold.

before training vs.  $720 \pm 73$  s after IT, Fig. 1D). Interestingly, the improvement of TTE after IT was two times greater than after CT ( $P < 0.05$ ).

Before IT and CT, a biexponential model was required for two subjects to describe  $\dot{V}O_2$  kinetics. Six subjects after CT and eleven subjects after IT needed a biexponential model to explore  $\dot{V}O_2$  kinetics.  $\dot{Q}$  kinetics were always described by a biexponential model, whereas  $D_{a-v}O_2$  kinetics required a monoexponential model.  $\dot{V}O_2$  kinetics was unchanged after CT (Table 3) but was significantly faster after IT, as demonstrated by a decrease of  $\tau_1$  ( $-19\%$ ,  $P < 0.05$ , Table 4).  $\dot{Q}$  and  $D_{a-v}O_2$  kinetics were not modified after both training modalities. We can, therefore, postulate that both central and peripheral adaptations to training may influence  $O_2$  uptake kinetics.  $D_{a-v}O_2$  kinetics was faster than  $\dot{Q}$  kinetics ( $P < 0.01$ ) before and after training by both modalities and determined the rapid component of  $\dot{V}O_2$  uptake, in spite of training modality and training status.

### Capillary Density and Muscular Oxidative Capacities

As seen on Fig. 2 for a typical subject, C/F was enlarged after both trainings:  $1.7 \pm 0.1$  vs.  $2.3 \pm 0.1$  capillaries per fiber with CT ( $P < 0.01$ ) and  $1.6 \pm 0.1$  vs.  $1.9 \pm 0.1$  with IT ( $P <$

Table 3. Kinetic parameter estimates for  $\dot{V}O_2$ ,  $\dot{Q}$ , and  $D_{a-\bar{v}}O_2$  with CT

	$\dot{V}O_2$		$\dot{Q}$		$D_{a-\bar{v}}O_2$	
	Pre	Post	Pre	Post	Pre	Post
$td_1$ , s	12.4±0.7	9.6±0.8	1.3±0.7	1.2±1.3	17.1±1.2	14.8±1.2
$\tau_1$ , s	60.6±2.7	52.8±1.4	54.5±2.7	61.3±4.3	29.5±1.0	30.8±0.7
$A_1$	1.90±0.07	1.68±0.06	9.7±0.3	10.5±0.3	6.4±0.3	6.8±0.2
$A_{tot}$	1.99±0.08	1.90±0.08	18.3±0.4	17.9±0.3	10.9±0.3	11.4±0.3

Values are expressed as means ± SE.  $A_1$  and  $A_{tot}$ , amplitude terms in liters per minute for  $\dot{V}O_2$ ,  $\dot{Q}$  and in milliliters·100 ml for  $D_{a-\bar{v}}O_2$ ;  $td_1$ , time delays to onset of each component;  $\tau_1$ , time constants.

0.05). The magnitude of change in capillarization was higher with CT compared with IT (+40 ± 3% vs. +21 ± 2%, respectively,  $P < 0.01$ ).

To establish maximal oxidative capacities of mitochondria in muscles in situ, we studied the oxygen consumption rates of saponin-permeabilized fibers.  $V_0$  remained unchanged after both training modalities.  $V_{max}$  was unchanged after CT (3.0 ± 0.5 before vs. 3.4 ± 0.4  $\mu\text{mol O}_2 \cdot \text{min}^{-1} \cdot \text{g fiber dw}^{-1}$  after training), whereas  $V_{max}$  was increased after IT (3.3 ± 0.4 before vs. 4.5 ± 0.6  $\mu\text{mol O}_2 \cdot \text{min}^{-1} \cdot \text{g fiber dw}^{-1}$  after training, Fig. 3A,  $P < 0.05$ ). IT had a greater effect on  $V_{max}$  than CT (+40 ± 15% vs. +18 ± 7%, respectively,  $P < 0.05$ ). Moreover, ACR improved only after IT (+22%,  $P < 0.05$ , Fig. 2B).

#### Relationships Between Peripheral Adaptations and Exercise Performance After IT

As seen in Fig. 4A, the gain of  $V_{max}$  ( $\Delta V_{max}$ ) was correlated with  $\Delta \dot{V}O_{2max}$  ( $P < 0.01$ ,  $r = 0.84$ ) and with  $\Delta \text{TTE}$  ( $P < 0.05$ ,  $r = 0.64$ , Fig. 4B). The improvement of the central parameter, represented by  $\Delta \dot{Q}_{max}$ , was also correlated with  $\Delta \dot{V}O_{2max}$  ( $P < 0.05$ ,  $r = 0.71$ , Fig. 4C).

#### DISCUSSION

Our study showed that a constant workload and  $O_2$  uptake during CT induces a higher increase of muscular capillary density than IT. Only high and fluctuating workload and  $O_2$  uptake during IT improve skeletal muscle mitochondrial function, which seems to be crucial in increasing  $\dot{V}O_2$  kinetics,  $\dot{V}O_{2max}$ , and  $\dot{Q}_{max}$ , even if both training programs resulted in the same global energy expenditure and training duration.  $D_{a-\bar{v}}O_2$  was improved after both training modalities, but only IT induced a significant increase of  $\dot{Q}_{max}$ . Interestingly, relationships between  $\Delta \dot{V}_{max}$ ,  $\Delta \dot{Q}_{max}$ , and  $\Delta \dot{V}O_{2max}$  were found only after IT.

#### Exercise Performance, $D_{a-\bar{v}}O_2$ and Capillary Density Adaptations

Our data demonstrate an improvement of  $\dot{V}O_{2max}$  with CT in accordance with the literature (6, 31), resulting mainly from peripheral adaptations as demonstrated by improvements of  $D_{a-\bar{v}}O_2$  and capillary density, while  $\dot{Q}_{max}$  stayed unchanged. These adaptations, occurring mainly at the skeletal muscle level, were associated with an increase of SVC, presumably allowing for an increased muscle perfusion. Capillary density is involved in muscle conductance as demonstrated on rats, with a positive correlation between capillary density and muscle conductance (29). The beneficial effect of capillary density on SVC is thought to be mediated by a capillary-induced reduction of MAP. Moreover, Beere et al. (6) suggested that 3 mo of CT is associated with a better redistribution of  $\dot{Q}$  to the exercising locomotor muscles, which improved local  $O_2$  availability. Similarly,  $\dot{V}O_{2max}$  was also improved after IT, together with SVC, and the magnitude of this training effect was in accordance with previous studies (6, 30, 31). However, the mechanism of increase of SVC after IT is likely to be different than that after CT, as it results mainly from the enhancement of  $\dot{Q}_{max}$ . These results support the idea that most of the adaptations involved in  $\dot{V}O_{2max}$  improvement after CT occur peripherally in skeletal muscles as previously described (41), whereas, after IT, the increase of  $\dot{V}O_{2max}$  seems to result from both peripheral ( $\Delta D_{a-\bar{v}}O_2$ ) and central adaptations ( $\Delta \dot{Q}_{max}$ ).

TTE is used to test aerobic capacity and more precisely characterizes the maximal endurance capacity of the subject (14). Both training modalities displayed a significant impact on TTE, with a higher improvement observed after IT (+129%) compared with CT (+64%). This improvement was associated with faster  $O_2$  adjustments, and it has been reported that faster  $\dot{V}O_{2max}$  result in smaller  $O_2$  deficit and greater exercise capacity (34). Only capillary density improves with CT, whereas capillary density,  $\dot{Q}_{max}$  and  $V_{max}$  are enhanced by IT. Indeed, we also observed an

Table 4. Kinetic parameter estimates for  $\dot{V}O_2$ ,  $\dot{Q}$ , and  $D_{a-\bar{v}}O_2$  with IT

	$\dot{V}O_2$		$\dot{Q}$		$D_{a-\bar{v}}O_2$	
	Pre	Post	Pre	Post	Pre	Post
$td_1$ , s	8.5±0.7	12.6±0.3	1.0±0.6	0.6±0.4	16.6±1.2	16.3±1.1
$\tau_1$ , s	68.0±1.6	54.9±0.7*	55.6±4.0	50.4±2.7	39.8±2.8	40.3±2.3
$A_1$	1.90±0.07	1.72±0.06	10.2±0.4	9.5±0.2	7.5±0.2	6.7±0.2
$A_{tot}$	1.93±0.07	1.92±0.07	18.3±0.4	18.9±0.4	12.3±0.3	11.0±0.3

Values are expressed as means ± SE.  $A_1$  and  $A_{tot}$ , amplitude terms in liters per minute for  $\dot{V}O_2$ ,  $\dot{Q}$ , and in milliliters·100 ml<sup>-1</sup> for  $D_{a-\bar{v}}O_2$ ;  $td_1$ , time delays to onset of each component;  $\tau_1$ , time constants. \*Significantly different ( $P < 0.05$ ) from pretraining values.

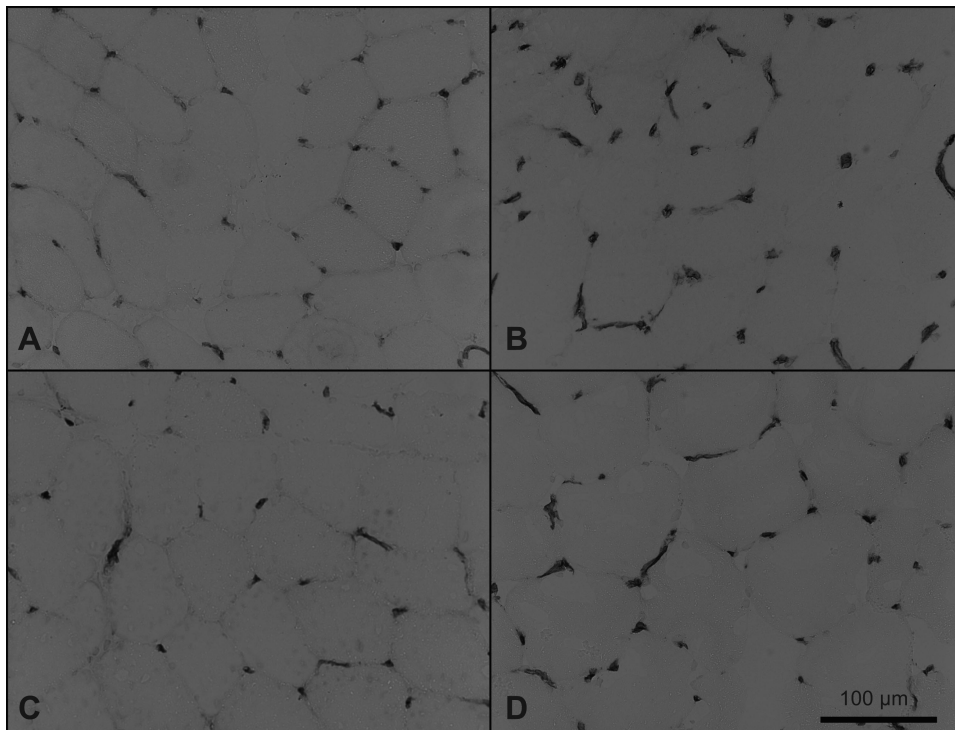


Fig. 2. Serial sections from vastus lateralis of a representative subject. The identification of capillaries was performed using monoclonal antibody CD 31. *A*: before CT. *B*: after CT. *C*: before IT. *D*: after IT. Scale bar = 100  $\mu\text{m}$ .

important lengthening of TTE with CT. We hypothesize, therefore, that capillary density improvement contributes toward lengthening TTE with CT, whereas the greater lengthening of TTE with IT is probably mediated by capillary density,  $\dot{Q}$ , and mitochondrial adaptations. Green (23) established that, with intense activity, ATP production rates are unable to match ATP utilization rates. This incapacity to produce a high level of ATP rates induces an accumulation of a range of metabolic by-products such as hydrogen ions, inorganic phosphates, or AMP. Selective by-products are believed to disturb  $\text{Na}^+/\text{K}^+$  balance,  $\text{Ca}^{2+}$  cy-

cling, and actomyosin interaction, resulting in performance impairment, which is often referred to as metabolic fatigue. Therefore, adaptations occurring with CT and IT may be implicated in the reduction of intracellular disturbance during exhaustive exercise to improve TTE.

#### Muscular Oxidative Capacity Adaptations

In situ respiration of skinned fibers is a means of assessing the function of the whole mitochondrial population within its

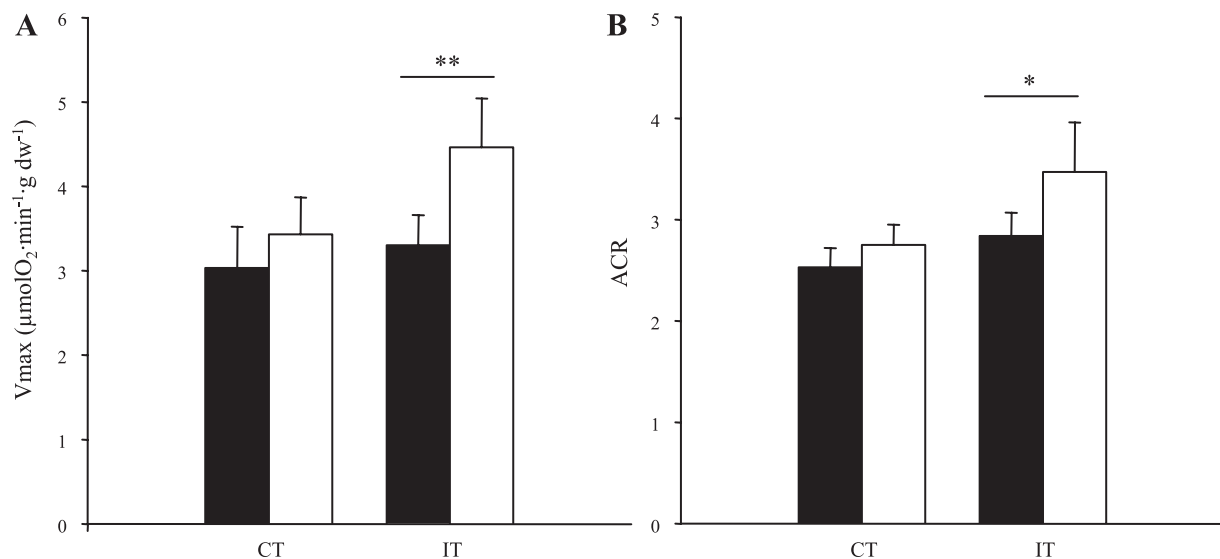
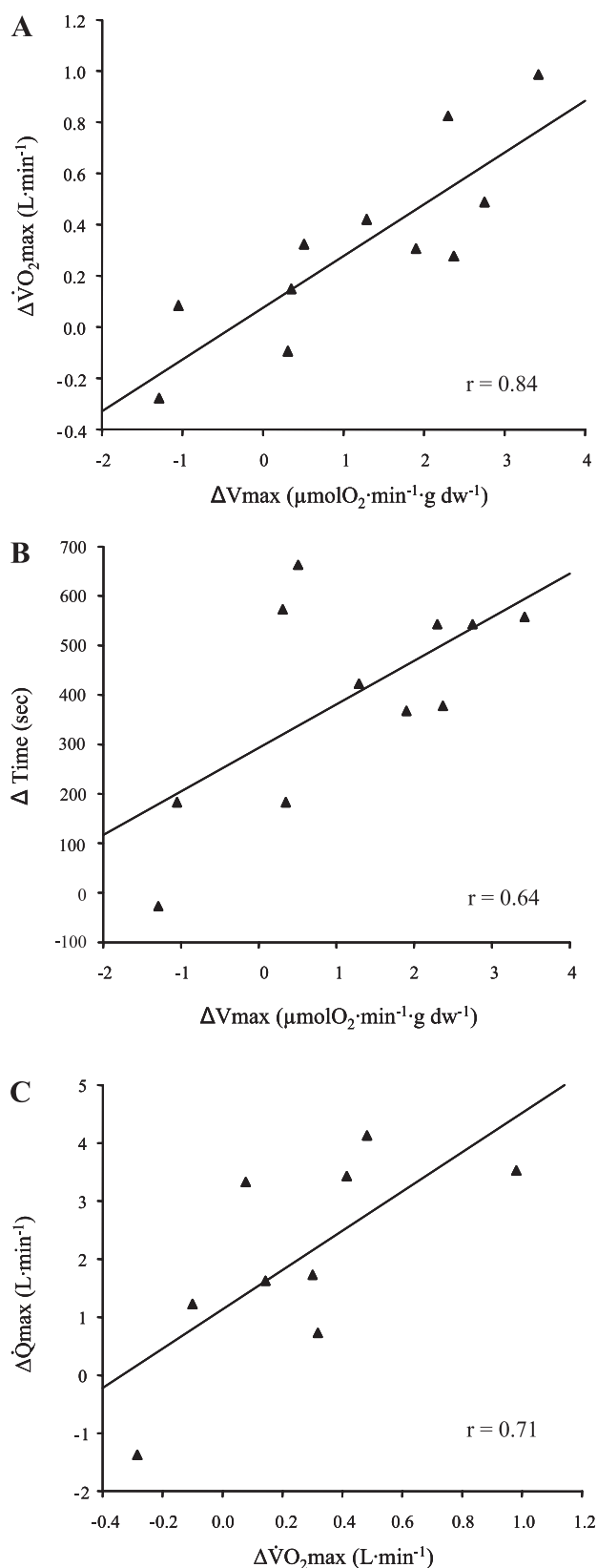


Fig. 3. *A*: maximal ADP-stimulated respiration rates ( $V_{\text{max}}$ ) of in situ mitochondria from vastus lateralis. *B*: acceptor control ratio (ACR;  $V_{\text{max}}/V_0$ ) of in situ mitochondria from vastus lateralis. Oxygen consumption was measured in saponin permeabilized muscle fibers with glutamate and malate as substrates in an oxygraph cell equipped with a Clark electrode. Values are expressed in micromoles  $\text{O}_2$  per minute per gram of fiber dry weight. Values are expressed as means  $\pm$  SE. Significantly different from pretraining values, with  $*P < 0.05$  and  $**P < 0.01$ . Solid bars represent pretraining values, and open bars are posttraining values.



cellular environment, with saturating amounts of oxygen and substrates (39). This allows a direct measure of muscular oxidative capacities. The initial status of our subjects was similar to previous studies (51, 53).  $V_{\max}$  was not increased after CT, suggesting that this training stimulus was not sufficient to induce mitochondrial adaptations, even in sedentary subjects. This training modality involved training sessions of continuous metabolic stimulation, with ATP utilization rates corresponding to 61% of  $P_{\max}$ . These findings suggest that the number of sessions is not enough and/or that an  $O_2$  uptake threshold is necessary to activate the intracellular pathways responsible for the mitochondrial adaptations to occur. Conversely, IT was associated with improvement of  $V_{\max}$ . This enhancement attests a higher muscle oxidative potential after training and is in line with our data and previous data from others, using the skinned fiber technique (51, 53) or the isolated mitochondria technique (43, 46).

Because both training methods submitted the subjects to the same global mechanical work and training duration, the increase of muscular oxidative capacities observed after IT suggests that the fluctuations of  $O_2$  uptake, inducing repeated disturbances of cellular homeostasis, is a major parameter controlling the adaptations of muscular mitochondrial function. Mitochondrial activity and biogenesis in muscle cells begin with putative signals brought about by muscle contraction, and the magnitude of the signal is undoubtedly related to the magnitude of muscle energy expenditure, depending on the intensity and duration of the contractile effort (26). In our study, IT was characterized by repeated variations of intensity, associated with changes of redox potential and ATP/ADP ratio inducing variations of  $O_2$  uptake. Then, we can postulate that, in conditions in which global ATP consumption is similar, it seems that a fluctuation in ATP turnover and in high-energy phosphate flux during training sessions activates the signaling pathways, leading to mitochondrial biogenesis.

Several stimuli seem to influence mitochondrial biogenesis. Local hypoxia has been postulated to constitute a major signal for muscular adjustments to endurance exercise, given that there is a dramatic drop in muscle oxygen tension with the onset of exercise (28, 37, 38). Each block performed in an IT session is characterized by a drop in intensity, which may acutely decrease muscle oxygen tension and worsen local hypoxia. Accordingly, Grassi et al. (22) observed an accelerated decrease of muscle oxygenation from 60 to 65%  $\dot{V}O_{2\max}$ , suggesting a decrease of cellular  $P_{O_2}$ . The intensity of CT (below 60% of  $\dot{V}O_{2\max}$ ) is possibly too low to induce a significant fall of cellular  $P_{O_2}$  and, therefore, is unlikely to trigger the same signaling pathways.

#### *Importance of Peripheral and Central Adaptations in Improvement of Performance*

The factors controlling the  $\dot{V}O_{2\max}$  during whole body exercise in humans remain a matter of considerable debate (40, 45).

Fig. 4. After IT, A: relation between gain of oxidative capacities of skeletal muscle ( $\Delta V_{\max}$ ) and gain of maximal oxygen uptake ( $\Delta \dot{V}O_{2\max}$ ). B: relation between gain of oxidative capacities of skeletal muscle ( $\Delta V_{\max}$ ) and gain in time to exhaustion ( $\Delta TTE$ ). C: relation between gain of maximal oxygen uptake ( $\Delta \dot{V}O_{2\max}$ ) and gain of maximal cardiac output ( $\Delta Q_{\max}$ ). The relations are established after a period of 8 wk of interval training. The horizontal bars indicate values that are significantly different with \* $P < 0.05$  and \*\* $P < 0.01$ .

We showed that  $\Delta\dot{V}_{\max}$  was correlated with  $\Delta\dot{V}_{O_{2\max}}$  and  $\Delta\text{TTE}$  after IT. These data are consistent with previous studies (33, 41), demonstrating the importance of muscular metabolic adaptations in the improvements of exercise performance in the sedentary. During submaximal exercise, muscle  $O_2$  delivery is closely matched to mitochondrial  $O_2$  demand, which is itself driven by the cellular charge (i.e., ATP demand) provided by exercise (4). The correlation, therefore, suggests that the enhancement of  $\dot{V}_{O_{2\max}}$  and endurance capacity are dependent on the increase of muscular mitochondrial capacities, increasing the potential for oxidative production of ATP. TTE and even more interestingly,  $\dot{V}_{O_{2\max}}$ , are improved after CT without enhancement of  $V_{\max}$ , indicating that other, “nonmitochondrial” adaptations need to occur. Greater improvement of capillary density and vascular conductance with CT could participate to improve muscle perfusion and thus  $O_2$  supply.

It is well known that  $\dot{Q}$  is classically a major limiting factor of the  $\dot{V}_{O_{2\max}}$  (4, 40). We observed that  $\Delta\dot{Q}_{\max}$  was correlated with  $\Delta\dot{V}_{O_{2\max}}$  after IT. This was not the case after CT, whereas  $\dot{V}_{O_{2\max}}$  was significantly increased, suggesting that  $\dot{Q}$  was not the only mechanism involved in improving  $\dot{V}_{O_{2\max}}$ .

We found  $D_{a-\bar{v}O_2}$  kinetics to be faster than  $\dot{Q}$  kinetics, and we are thus in agreement with studies (11, 36), which found that tissue deoxygenation kinetics were faster than  $\dot{V}_{O_2}$  kinetics during constant high-intensity exercise. Moreover, we suggested earlier that the exercise-induced circulatory response is mainly under metabolic control (i.e., oxygen demand) (15). Because  $D_{a-\bar{v}O_2}$  was directly dependent on muscular oxidative capacities and, because  $\dot{Q}$  has to increase to maintain sufficient  $O_2$  supply, our results suggest that the increase of  $\dot{Q}$  response following IT was dependent on the increase of muscular oxidative capacities. We observed a lower  $\tau_1$  for  $\dot{V}_{O_2}$  with IT in the absence of any changes in  $\dot{Q}$  and  $D_{a-\bar{v}O_2}$  kinetics. However, we measured systemic values of  $\dot{Q}$ , and training may influence muscle perfusion and blood flow redistribution, as suggested by Beere et al. (6). Similar adaptations in our study, might have improved muscle  $O_2$  availability at the beginning of the exercise, thereby speeding  $\dot{V}_{O_2}$  on-kinetics, even in the absence of significant  $\dot{Q}$  changes.

### Study Limitations

We used an impedance device to determine  $\dot{Q}$  because  $\dot{Q}$  kinetics determination needs a beat-to-beat signal, which was impossible to obtain with other methods. We know that the impedance device is not considered to be a reference method compared with direct Fick measurement. Numerous problems occurred with the use of the impedance method originally proposed by Kubicek (30a) and, for these reasons, we currently use in our laboratory a modified method to calculate stroke volume. In this method, the  $Z_0$  evaluation is unnecessary, and the positions of the electrodes do not exert any confounding effects (10). Moreover, our impedance device was validated twice compared with the “gold standard” direct Fick method, and we obtained good accuracy and reproducibility both during incremental exercise test to exhaustion (35) and during continuous constant load exercise (10).

### Perspectives and Significance

The current investigation reveals that endurance training programs with similar exercise duration and similar total mechanical workload but different  $O_2$  fluctuations, induce specific peripheral and central adaptations. In particular, repeated fluctuations of  $O_2$  consumption during training sessions seem to be necessary to improve muscular oxidative capacities. Together, these results provide a mechanistic framework to explain the greater efficiency of interval type over continuous-type training on endurance performance enhancement. Moreover, our observations suggest that enhancements of muscular mitochondrial function are actively involved in the observed  $\dot{V}_{O_{2\max}}$  improvements. Furthermore, the implication of muscle oxidative capacities and  $\dot{Q}_{\max}$  in  $\dot{V}_{O_{2\max}}$  enhancement bring new evidence in relation to the factors controlling  $\dot{V}_{O_{2\max}}$  after experimental intervention such as exercise training, which remains a matter of debate (40, 45).

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