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MYOSIN HEAVY CHAIN COMPOSITION IN THE *VASTUS LATERALIS* MUSCLE IN RELATION TO OXYGEN UPTAKE AND HEART RATE DURING CYCLING IN HUMANS

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In this study we examined the relationship between fast myosin heavy chain (MyHC2) content in the *vastus lateralis* and the rate of oxygen uptake (VO_2) and heart rate (HR) increase during an incremental exercise in 38, young, healthy men. Prior to the exercise test, muscle biopsies were taken in order to evaluate the MyHC composition. It was found that during cycling performed below the lactate threshold (LT), a positive relationship between MyHC2 and the intercept of the oxygen uptake and power output (VO_2-PO) relationship existed (r=0.49, *P*=0.002), despite no correlation between MyHC2 and the slope value of the VO_2-PO relationship (r= –0.18, *P*=0.29). During cycling performed above the LT, MyHC2 correlated positively with the magnitude of the nonlinearity in the VO_2-PO relationship; *i.e.* with the accumulated VO_2'excess' (r=0.44, *P*=0.006) and peak VO_2'excess' (r=0.44, *P*=0.006), as well as with the slope of the HR-PO relationship (r=0.49, *P*=0.002). We have concluded that a greater MyHC2 content in the *vastus lateralis* is accompanied by a higher oxygen cost of cycling during exercise performed below the LT. This seems to be related to the higher energy cost of the non-cross-bridge activities in the muscles possessing a greater proportion of MyHC2 content. In the case of heavy-intensity exercise, a higher MyHC2 content in the *vastus lateralis* is accompanied by greater non-linearity in the VO_2-PO relationship, as well as a steeper increase in HR in the function of an increase of PO. This relationship can be explained by greater disturbances in metabolic stability in type II muscle fibres during exercise, resulting in a decrease of muscle mechanical efficiency and greater increase of heart rate at a given power output. Therefore, MyHC composition has an impact on the oxygen cost of cycling both below and above the LT.

Key words: *excercise, cycling, myosin heavy chain isoforms, oxygen uptake, heart rate, lactate threshold*

INTRODUCTION

Maximal oxygen uptake (VO_{2max}) or peak oxygen uptake (peak VO_2) are frequently used as indicators of aerobic exercise capacity in humans (1-3). Indeed, those variables provide a good estimation of the cardio-pulmonary capacity (2). However, a precise prediction of the physical capacity of humans requires also additional data concerning the oxygen cost of work, which varies at different exercise intensities (4, 5), as well as among different subjects (5-7). It is well known that during moderate intensity exercise, *i.e.* when exercise is performed at intensities below the lactate threshold (LT), an increase of power output requires an increase in the VO_2 amounting to about of 9-10 ml of oxygen consumed per 1 minute per 1 Watt of the increase in generated power (5, 7-10). However, during exercise performed above the LT (*i.e.* in the heavy intensity domain), the oxygen cost of work is non-proportionally higher, as reflected by both the existence of the slow component of the VO_2 kinetics during constant power output exercise (4) and the presence of the non-linearity in the VO_2-power output relationship during incremental exercises in humans (5, 6, 9). In this intensity domain exercise, the slope of the VO_2-PO relationship during cycling can be as high as 12–13 ml O_2 min^{-1} $Watt^{-1}$ (11).

Taking into consideration the afore-mentioned non-linearity in the VO_2-PO relationship, the VO_2-PO relationship during incremental cycling exercise in the entire range of exercise intensities (from the lowest up to the PO corresponding to VO_{2max}) should be characterized by two separate linear dependencies; *i.e.* below and above the LT (7). Barstow et al. (7) have proposed that the slope of the VO_2 to power output relationship below the LT should be called S1 and above the LT up to power output reached at VO_{2max}-S_2. Moreover, the entire range of VO_2 to power output relationship approximated to linearity is called S_T (7). It should be stated, however, that the third approach, *i.e.* the calculation of the S_T is justified only in the ramp test procedures during which the VO_2 to PO relationship is close to linearity (1). Therefore, in the case of incremental exercise tests (especially involving steps >2 min), during which a clear non-linearity in the VO_2-PO relationship is present, estimations only concerning the S_1 and S_2 are justified.

The physiological background of the greater oxygen cost when performing exercise in the heavy intensity domain is not completely understood (12, 13), although, in the light of

experimental evidence (14), the factor(s) responsible for the non-proportional high oxygen cost of work during heavy intensity cycling is located in the locomotor muscles. The main determinant of the oxygen cost of cycling besides cycling efficiency (a ratio between energy input and power production) is considered to be the muscle fibre type composition (13, 15, 16). The contractile properties of the muscle fibre are dependent on the myosin heavy chain (MyHC) isoform, which governs maximum shortening velocity (17), maximal power output (18), the ATP consumption rate during isometric contraction, and the tension cost (19).

Little, however, is known concerning the effect of MyHC composition on the VO_2-power output relationship during moderate and heavy exercise intensities studied in the same subjects. Therefore, the aim of this study was to present the relationship between MyHC composition in the *vastus lateralis* and the parameters describing the VO_2-PO relationship, as well as the HR-PO relationship both below and above the LT in a large group (n=38) of young, healthy men. It was hypothesized that a higher content of the fast isoform of MyHC (MyHC2) in the *vastus lateralis* will be accompanied by a higher slope value of the VO_2 to PO relationship during cycling performed below the LT, and also by a higher magnitude of non-linearity in the VO_2-PO relationship above the LT. Moreover, we hypothesized that a greater content of fast MyHC isoforms in the working leg muscles would be accompanied by a higher rate of increase in HR during incremental exercise.

SUBJECTS AND METHODS

Subjects

The study was conducted with permission of the Ethical Committee, according to principles established in the Declaration of Helsinki regarding research of human subjects.

Thirty-eight untrained, but physically active, male volunteers took part in this study. The subjects were asked to avoid heavy exercise the day before and on the day of the test. Moreover, the subjects were asked to follow a normal mixed diet over the three days prior to the test that was composed of ~55% carbohydrates and ~25% of fat and ~20% of protein, plus to avoid caffeinated and alcoholic beverages 24 hours before exercise. The subjects provided written informed consent to participate in the study.

Exercise protocol

An incremental exercise was performed on the cycloergometer Ergo-Line GmbH & Co KG 800s (Bitz, Germany), following a 6-minute resting period that allowed for the establishing of basic cardio-respiratory parameters. The exercise test was performed at pedalling rates of 60 rev · min^{-1}, starting from a power output of 30 Watt (W) and followed by a gradual increase of 30 W every 3 minutes until exhaustion (5). The following criteria of reaching VO_{2max} during incremental exercise were applied: (a) a plateauing in VO_2 in relation to the increase in power output was evident, (b) the heart rate (HR) at the end of exercise was close to age-predicted HR_{max}, which has amounted to 192±0.3 beats·min^{-1} (*i.e.* above 95% of predicted HR_{max}), (c) the respiratory exchange ratio (RER) end-exercise was above 1.1.

Gas exchange variables

Gas exchange variables were recorded continuously breath by breath using the Oxycon Champion, Mijnhardt BV (Bunnik, The Netherlands) starting from the 6th minute before exercise until the end of the test. The gas analyzer was calibrated with certified calibration gases, as previously described (5).

Plasma lactate concentration

Venous blood samples were collected *via* an Abbot Int-Catheter(Abbot, Sligo, Ireland; 18 gauge, 1.2 mm×45 mm) inserted into an antecubital vein and connected to an extension set using a 'T' Adapter (SL Abbot, Ireland; tube 10 cm in length). Blood samples (0.5 ml each) were taken at rest before the test, at each step of the incremental exercise test and at the end of the exercise protocol. Samples were transferred to 1.8 ml Eppendorf tubes containing 1 mg ammonium oxalate and 5 mg sodium fluoride, mixed for about 20 s and centrifuged at 1055 × *g* for 4 min. The supernatants containing blood plasma (about 0.2 mL) were stored at –40°C until further analysis of $[La^-]_{pl}$ (Vitros 250 Dry Chemistry System; Kodak, Rochester, NY, USA). The detection limit was 0.5 mmol L^{-1}.

Heart rate (HR)

The heart rate in this study was determined from the ECG curve, registered continuously, using the Hellige SMS 181 unit, Germany.

Muscle biopsy

Muscle biopsies were obtained under local anesthesia (1% Lignocainum Hydrochloricum, Polfa S.A., Warszawa, Poland) from the right *vastus lateralis m. quadricipitis femoris*, approximately 15 cm above the upper margin of the *patella*, with a 5 mm ϕ Bergström needle (Stille AB, Solna, Sweden) or 2 mm ϕ biopsy needle (Pro-MagTM I 2.2; Angiotech, Vancouver, Canada). All biopsies were taken ~3.0 cm deep and slightly distal to the ventral midline of the muscle. The depth of the biopsy taken by a Bergström needle was gauged by markings engraved in the biopsy needle. In the case of the Pro-MagTM system, the depth was set automatically. The specimens were frozen immediately in liquid nitrogen and used for electrophoresis.

Myosin extraction

Muscle biopsies were mounted in Shandon cryostat with Tissue-Tek and 30-50 cryosections, 30 m thick, were cut from each biopsy. The sections were transferred to Eppendorf tubes and myosin was extracted with 200–300 µL of lysing buffer consisting of 62.5 mM Tris, 10% glycerol, 5% 2-mercaptoethanol, 2.3% SDS, and pH 6.8. The samples were briefly vortexed and boiled for 3 min in a water bath. Myosin extracts were clarified at 13,000 × g for 5 min and supernatants were stored at minus 20°C until further use.

SDS-PAGE

SDS-polyacrylamide gel electrophoresis was carried out according to Carraro&Catani (20), with 4% stacking and 6% separating gels containing 37.5% glycerol in a Mini-Protean II electrophoresis system (Bio-Rad Laboratories, Hercules, USA). Myosin extracts were diluted 1:1 with sample buffer containing 0.1 M Tris-HCl pH 6.8, 2.5% SDS, and 2.5% 2-mercaptoethanol before being boiled for 3 min. Denatured extracts, diluted 1:10–1:20 with lysing buffer, were loaded onto stacking gel and run at a constant voltage of 60 V for 30 min and then at 180 V for 3 hours. The gels were stained with 0.1% Coomassie brilliant blue R-250 in 25% methanol and 10% acetic acid, and destained with

several changes of methanol/acetic acid. Densitometric analysis of slow (type 1) and fast (type 2) myosin heavy chains was performed using a CCD camera (Fotodyne Incorporated) and Gel Pro Analyzer software. The proportions of MyHC1 and MyHC2 were expressed (see *e.g.* 21) as a relative percentage of the total amount of MyHC present in biopsies from the *vastus lateralis* muscle.

Data analysis

Lactate threshold (LT)

The lactate threshold (LT) in this study was defined as the highest power output above which the plasma lactate concentration $[La^-]_{pl}$ showed a sustained increase of more than 0.5 mmol · L^{-1} · $step^{-1}$ (5).

Oxygen uptake to power output ratio during incremental cycling exercise

Oxygen uptake obtained during the maximal incremental cycling exercise, in the range of power output beginning from 30 W - up to the power output corresponding to the LT, was described individually for each subject from the studied group as a linear function:

$$VO_{2,\ below\ the\ LT} = a_1 + S_1 \cdot PO \quad [1]$$

where: PO is the power output, a_1 - the intercept and S_1 - the slope of the VO_2-PO relationship below the LT.

VO_2 obtained during maximal incremental cycling, in the range of power output beginning from the PO at the LT up to the maximal power output reached during the incremental exercise (*Obs.* PO_{max}) was described as a linear function (7):

$$VO_{2,\ above\ the\ LT} = a_2 + S_2 \cdot PO \quad [2]$$

where: PO is the power output, a_2 - the intercept and S_2 - the slope of the VO_2-PO relationship above the LT.

Based on the individual linear regression for the VO_2 to power output relationship established below the LT (see equation 1), the quantification of the magnitude of the VO_2 deviation from linearity observed above the LT (the accumulated VO_2 'excess', see *Fig. 1*) was done. The calculation of the VO_2 in the function of power output includes two parts: the estimation of the VO_2 below the LT (for $PO \leq PO_{LT}$) and the estimation of the VO_2 above the LT (for $PO \geq PO_{LT}$) (see equation 3).

$$VO_2(PO) = \begin{cases} a_1 + S_1 \cdot PO; & \text{for } PO \leq PO_{LT} \\ a_1 + S_1 \cdot PO + A \cdot \left(e^{\frac{PO - PO_{LT}}{B}} - 1 \right); & \text{for } PO \geq PO_{LT} \end{cases} \quad [3]$$

where: A is an amplitude coefficient of the non-linear part of the VO_2-PO relationship, B is a coefficient of the rate of an increase of the VO_2 in the non-linear range of the VO_2-PO relationship.

The accumulated VO_2 'excess' was calculated as the area between the values of the VO_2 estimated from the non-linear part of the equation (3) and the VO_2 predicted from the extrapolation of the linear dependence between VO_2 and PO (see equation 1) above the LT - up to the *Obs.* PO_{max}. The accumulated VO_2 'excess' measured in mL in this paper for the purpose of further comparisons was expressed in the absolute values (mL), and as a percentage of the expected oxygen uptake, as predicted based on the linear relationship between VO_2 and the PO established as above; *i.e.* up to the LT. In other words, it was presented as a percentage (%) of the area of the total VO_2 measured below the linear extension of the VO_2-PO relationship to the range of 30 W - up to the *Obs.* PO_{max}. Based on the individual linear regression for the VO_2 to power output relationship below the LT (see equation 1), the expected oxygen uptake (*Exp.* VO_2) at the *Obs.* PO_{max} was calculated. The peak VO_2 'excess' was expressed in absolute values (mL · min^{-1}) and as a percentage of the *Exp.* VO_2 at *Obs.* PO_{max}. Moreover, based

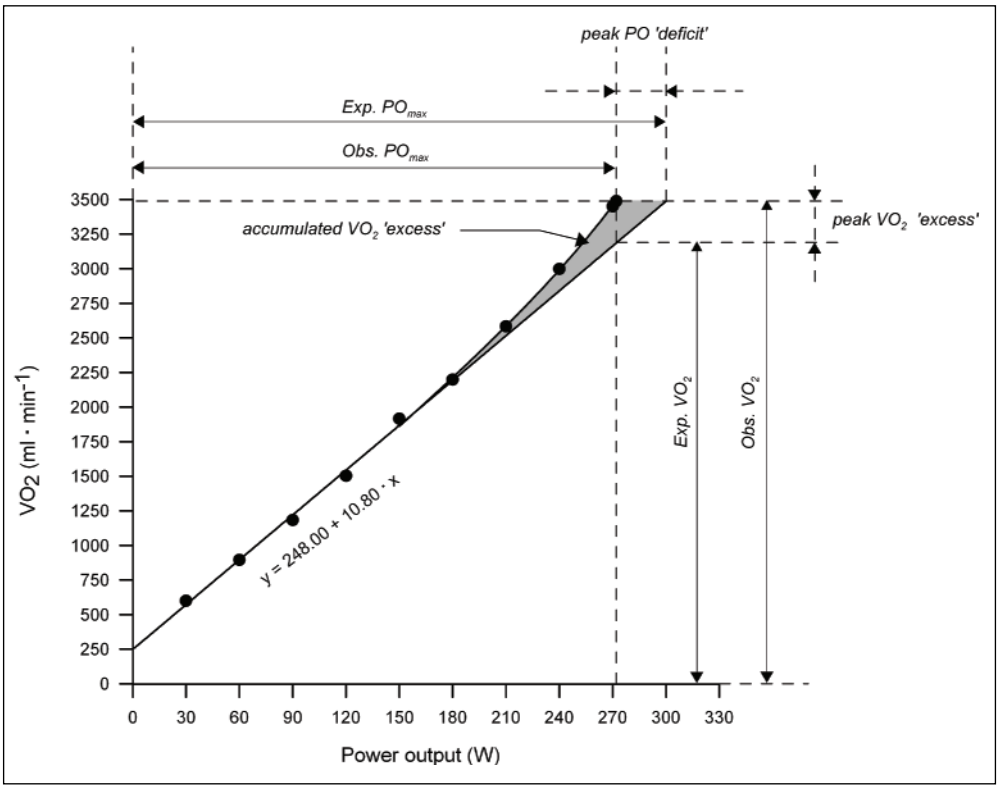

Fig. 1. Typical example of the oxygen uptake (VO_2) in function of the power output (PO) during incremental cycling exercise test starting from 30 W - up to maximal power output (*Obs.* PO_{max}). Power output at LT has amounted in this case 150 W.

on the individual linear regressions for the VO_2 to power output relationship below the LT, the expected power output (*Exp.* PO) at *Obs.* VO_{2max} has been calculated and the peak PO 'deficit' has been presented in absolute values (W) and as a percentage of the *Obs.* PO_{max} (22).

Moreover, according to Barstow *et al.* (7), oxygen uptake during cycling during the maximal incremental cycling exercise, in the range of power output beginning from 30 W - up to the *Obs.* PO_{max}, was described individually for each subject as a linear function:

$$VO_2, = a_T + S_T \cdot PO \quad [4]$$

where: PO is the power output, a_T - the intercept, and S_T - the slope of the VO_2-PO relationship in the range of power outputs from 30 W up to *Obs.* PO_{max}.

Heart rate during incremental cycling exercise

The heart rate (HR) obtained during the maximal incremental cycling exercise, in the range of power output beginning from 30 W - up to the power output corresponding to the LT, was described individually for each subject as a linear function, and the slope value of this relationship has been taken for the analysis (slope of the HR-PO relationship below the LT).

The heart rate obtained in the range of power output beginning from the PO at the LT up to the maximal power output reached during the incremental exercise (*Obs.* PO_{max}), was described as a linear function and the slope value of this relationship was taken for the correlation analysis (slope of the HR-PO relationship above the LT). We have also calculated the predicted HR_{max} using the formula: 208 – 0.7. age, developed by Tanaka *et al.* (23).

Statistics

The results presented in the tables and figures are expressed as means ±S.E.M. In addition, the minimum ÷ maximum (min ÷ max) and medians (Me) have been also presented. The normality of distribution has been examined using the Shapiro-Wilk's test. Due to the non-normal distribution of some variables (*i.e.* power output at LT, S_2, accumulated VO_2 'excess', and the $VO_{2net}/Obs.PO_{max}$, slope of the HR-PO relationship), the correlation analysis between two variables was tested using non-parametric Spearman's correlation analysis. The statistical significance of differences for paired samples was tested using the non-parametric Wilcoxon test and the non-asymptotic, exact, two-sided *P*-values, are presented. The significance was set at $P<0.05$. The statistics were created using the statistical packet STATISTICA 10.0 (StatSoft, Tulsa, OK, USA) and StatXact 9.0 (Cytel Software Corporation, Cambridge, MA, USA).

RESULTS

In the studied group of thirty-eight, physically active, healthy men (mean ±S.E.M.: age 23.3±0.4 years; BM 74.0±1.4 kg; BMI 23.0±0.3 kg · m^{-2}; VO_{2max} 49.0±0.9 mL · kg^{-1} · min^{-1}), the mean value of fast myosin heavy chain isoforms (MyHC2) in the *vastus lateralis* muscle amounted to 53.8±2.5%.

Oxygen uptake and heart rate during maximal incremental cycling exercise in the studied group (n=38)

Oxygen uptake (VO_2) and heart rate (HR) during the incremental cycling exercise, up to power output corresponding to 270 W, is presented in *Fig. 2*. The oxygen uptake followed a linear pattern of increase from the beginning of the test up to the LT (see *Table 1*), and above the LT a significant non-linearity in the VO_2-PO relationship was present (*Fig. 2A*). The heart rate studied in the range of power outputs 30 – 270 W was linear (*Fig. 2B*).

Oxygen uptake-power output relationship during maximal incremental cycling exercise

The slope of the VO_2 to PO relationship below the LT (S_1) was significantly lower ($P<10^{-4}$) than the slope value of the VO_2

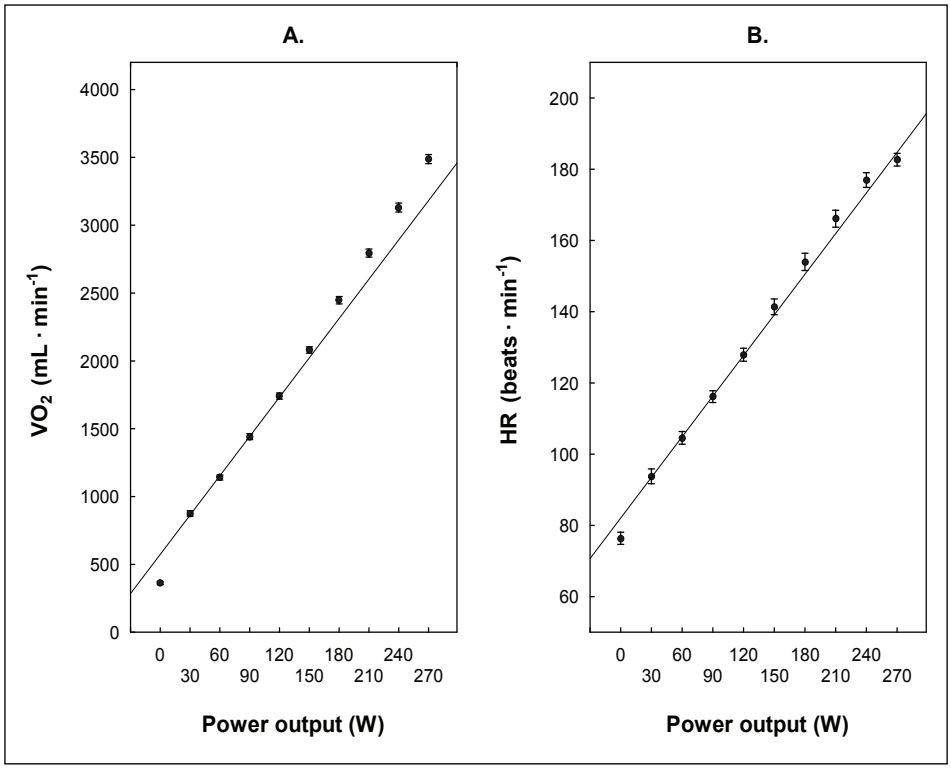

Fig. 2. The oxygen uptake (VO_2) (panel A) and heart rate (HR) (panel B) at rest and during an incremental cycling performed at 60 rev min^{-1} in the range of power outputs 30–270 W. Data presented as mean value ±S.E.M. at rest and at each power output for the group of the studied subjects. The solid line on the panel A represents the linear relationship between VO_2 and PO based on the data collected below the LT, *i.e.* from 30 to 140 W. Similarly, solid line on the panel B represents the linear relationship between HR and PO based on the data collected below the LT, *i.e.* from 30 to 140 W. In the range of exercise intensities from 30 up to 210 W data presented as mean ±S.E.M. for n=38 subjects, at 240 W for 37 subjects, and at 270 W for 28 subjects.

to PO relationship above the LT (S_2) (*Fig. 3A*). The slope value of the VO_2-PO relationship in the range of power outputs from 30 W up to *Obs.* PO_{max} (S_T) amounted to 11.3±0.13 mL $O_2 \cdot min^{-1} \cdot W^{-1}$, and was significantly higher than S_1 ($P<10^{-4}$) and significantly lower than S_2 ($P<10^{-4}$). The intercept value of the VO_2 to PO relationship below the LT (a_1) was significantly higher ($P<10^{-4}$) than the mean intercept value of the VO_2 to PO relationship above the LT (a_2). The intercept of the VO_2-PO relationship in the range of power outputs from 30 W up to *Obs.* PO_{max} (a_T) was significantly lower than a_1 ($P<10^{-4}$) and significantly higher than a_2 ($P<10^{-4}$).

The *Obs.* VO_{2max} (VO_2 reached at the end of the exercise protocol) was significantly higher (by ~12%, $P<10^{-4}$, *Table 1*) when compared to the *Exp.* VO_2 calculated at the *Obs.* PO_{max} (based on the individual linear regression between VO_2 and PO below the LT).

The magnitude of the deviation from linearity expressed as the accumulated VO_2 'excess' amounted to about 4.8±0.6%. The *Exp.* PO calculated at *Obs.* VO_2max has been found to be significantly higher (by ~14%, $P<10^{-4}$, *Table 1*) than the maximal power output reached at the end of the exercise protocol (*Obs.* PO_{max}). The net oxygen cost (VO_{2net}) of generating power output reached at VO_2max (*Obs.* PO_{max}) amounted to 11.8±0.12 mL $O_2 \cdot min^{-1} \cdot W^{-1}$.

Heart rate-power output relationship during maximal incremental cycling exercise

The slope coefficient of the HR-PO relationship in the range of power outputs from 30 W up to *Obs.* PO_{max} amounted to 0.40 beats $\cdot min^{-1} \cdot W^{-1}$, which means that during cycling exercise an increase of power output by 10 W required an increase of heart rate by ~4.0 beats $\cdot min^{-1}$. As found in this study, the slope value of the HR-PO relationship below the LT was not significantly different from the slope value of the HR-PO relationship above the LT (P=0.23, *Fig. 3B*).

Correlations

A significant positive correlation between MyHC2 content in the *vastus lateralis* and the pre-exercise VO_2 was observed (r=0.58, P=0.0001). A significant negative correlation between the MyHC2 content in the *vastus lateralis* and PO at LT was found (r= –0.61, $P<10^{-4}$). No significant correlation between MyHC2 content and

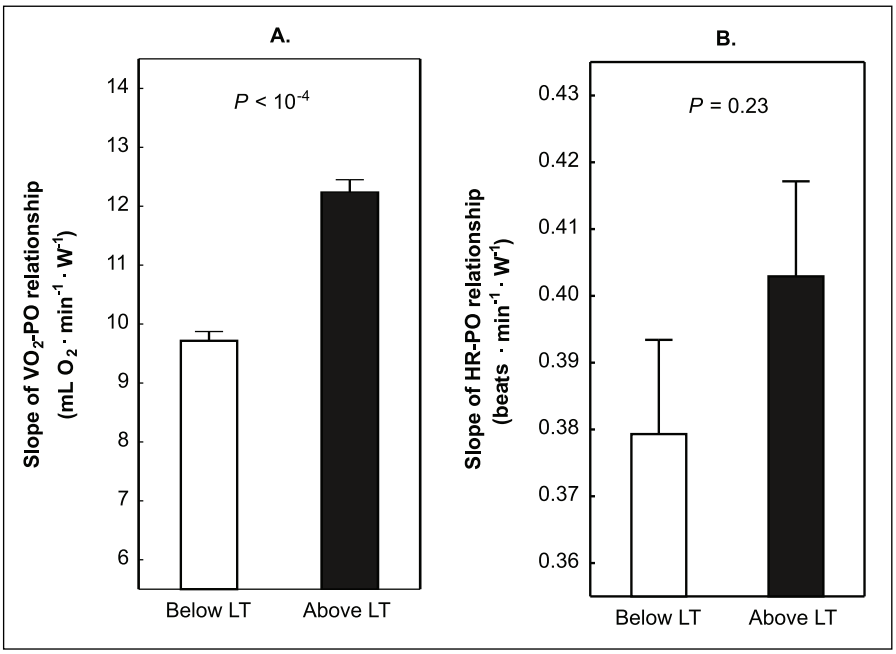

Fig. 3. The slope of the VO_2-PO relationship below (S_1) and above the LT (S_2) (panel A). The slope of the HR-PO relationship below and above the LT (panel B). Data presented as means ±S.E.M. for the 38 subjects.

Table 1. The parameters describing exercise tolerance (PO at LT, *Obs.* PO_{max}, *Obs.* VO_{2max}) and the parameters describing VO_2-PO relationship during maximal incremental cycling exercise (n=38).

Variable	mean ± SEM	Me	min ÷ max
Obs. VO_{2max}, mL $\cdot min^{-1}$	3570 ± 60	3528	2526 ÷ 4299
Exp. VO_2 at *Obs.* PO_{max}, mL $\cdot min^{-1}$	3227 ± 64	3230	2448 ÷ 4153
Peak VO_2 'excess', mL $\cdot min^{-1}$	362 ± 33	331	31 ÷ 728
Peak VO_2 'excess', %	11.6 ± 1.1	10.3	0.9 ÷ 25.8
Accumulated VO_2 'excess', %	4.8 ± 0.6	3.8	0.5 ÷ 14.5
PO at LT, W	141 ± 7	150	60 ÷ 270
Obs. PO_{max}, W	274 ± 5	273	210 ÷ 345
Exp. PO at *Obs.* VO_{2max}, W	313 ± 6	312	244 ÷ 408
Peak PO 'deficit', W	39 ± 4	33	3 ÷ 89
Peak PO 'deficit', %	14.4 ± 1.5	12.2	1.1 ÷ 34.1

Obs. VO_{2max} was noticed (r= –0.25, *P*=0.13), whereas a significant positive correlation between MyHC2 content and *Obs.* PO_{max} (r = –0.49, *P*=0.002) has been reported. Moreover, the MyHC2 content in the *vastus lateralis* correlated positively with the net oxygen cost of generating maximal power output (r=0.55, *P*=0.0003).

The MyHC2 content in the vastus lateralis and the oxygen cost of moderate-intensity cycling

A significant positive correlation between the MyHC2 content in the *vastus lateralis* and the intercept value of the VO_2 to PO relationship below LT (a_1), reflecting the oxygen cost of unloaded cycling, was found (r=0.49, *P*=0.002, *Fig. 4A*), whereas no significant correlation between MyHC and the slope value of this relationship (r= –0.18, *P*=0.29, *Fig. 4B*) has been observed.

The MyHC2 content in the vastus lateralis and the oxygen cost of heavy-intensity cycling

The correlation analysis between MyHC2 and the parameters describing the non-linearity of the VO_2-PO relationship is presented in *Fig. 5A* positive correlation (*P*<0.05)

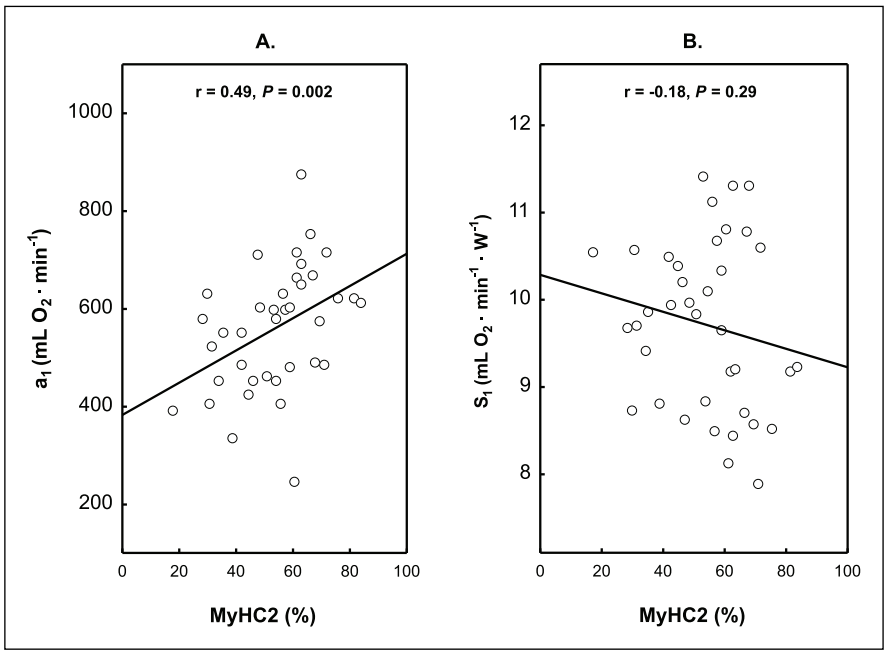

Fig. 4. The relationship between MyHC2 content and the intercept (a_1) (panel A) and slope value (S_1) (panel B) of the VO_2-PO relationship below the LT (n=38).

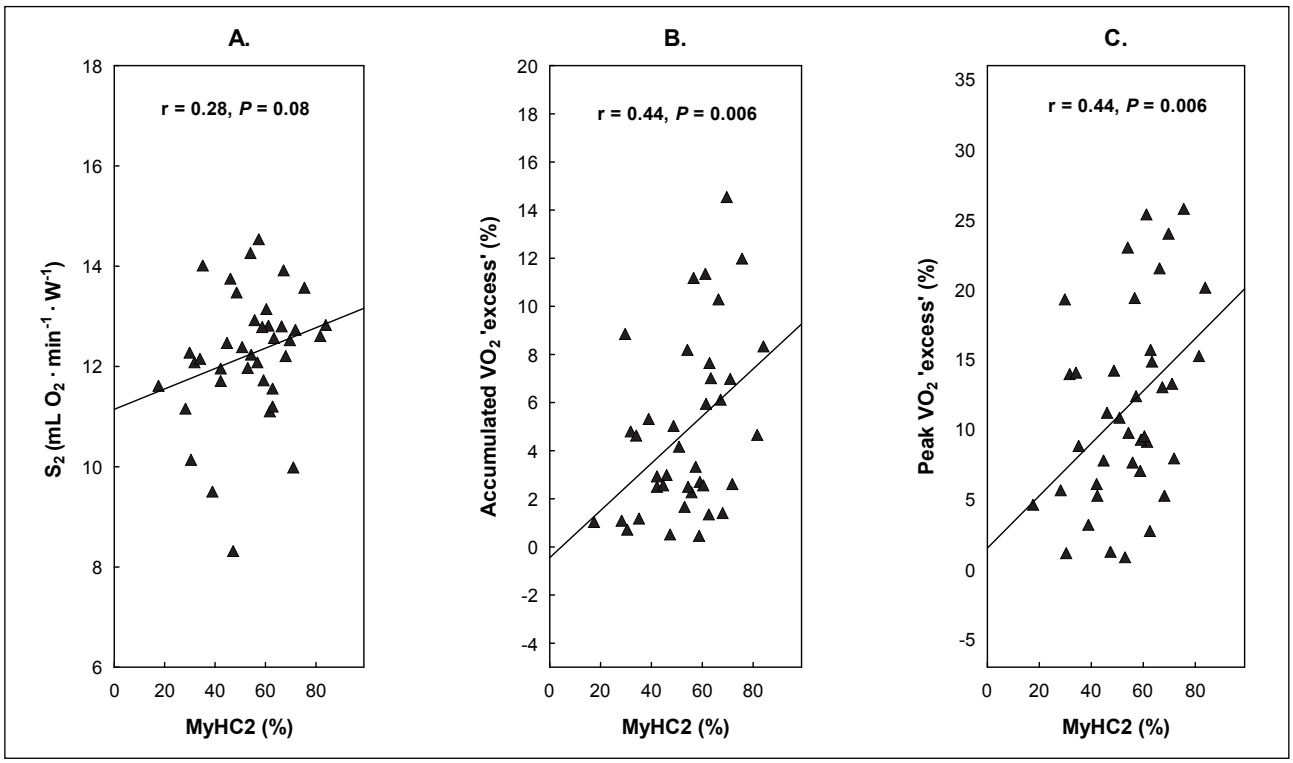

Fig. 5. The relationship between MyHC2 content and S_2 (panel A), MyHC2 content and accumulated VO_2 'excess' (panel B), MyHC2 content and peak VO_2 'excess' (panel C) (n=38).

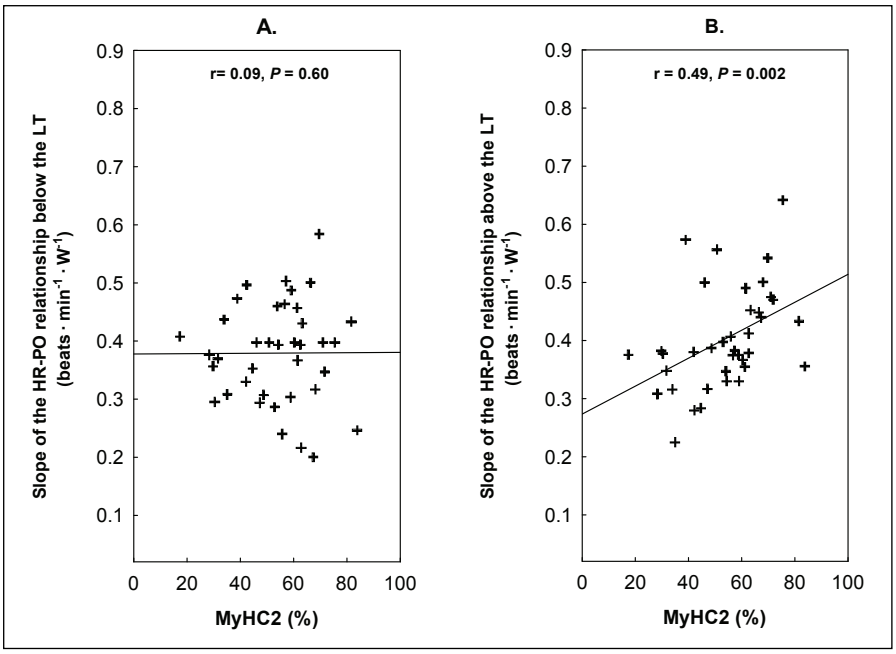

Fig. 6. The relationship between MyHC2 content and the slope of the HR-PO relationship below the LT (panel *A*). The relationship between MyHC2 content and the slope of the HR-PO relationship above the LT (panel *B*) (n=38).

between MyHC2 and the S_2 'excess' r=0.44 to S_1 ratio (r=0.36, *P*=0.03), the accumulated VO_2 'excess' (r=0.44, *P*=0.006, *Fig. 5B*), and the peak VO_2 'excess' (r=0.44, *P*=0.006, *Fig. 5C*) has been reported. Moreover, a tendency towards a positive relationship between the MyHC2 content and S_2 (r=0.28, *P*=0.08, *Fig. 5A*) was noticed. No significant correlation between the MyHC2 content and a_2 was found (r=0.23, *P*=0.17)). A significant positive relationship between the MyHC2 content in the *vastus lateralis* and the S_T (r=0.38, *P*=0.02), as well as between MyHC2 and a_T, has been found (r=0.40, *P*=0.01).

The MyHC2 content in the vastus lateralis and heart rate during maximal incremental cycling

A significant positive correlation between MyHC2 and the slope of the heart rate-power output relationship in the range of power output from the LT up to the *Obs.* PO_{max} (r=0.49, *P*=0.002, *Fig. 6B*) was present in the studied group of subjects (n=38), whereas no such relationship was noticed between the MyHC2 content and the slope of the HR-PO relationship below the LT (r=0.09, *P*=0.60, *Fig. 6A*). The MyHC2 content in the *vastus lateralis* correlated positively with the slope of the HR-PO relationship in the range of power output from 30 W up to *Obs.* PO_{max} (r=0.41, *P*=0.01).

DISCUSSION

In this study, in a large group of young, healthy men (n=38), we have shown as others before (5, 6, 9, 22), that during incremental cycling exercise the VO_2-PO relationship is non-linear, as judged by a significantly higher S_2 (12.2±0.2 mL $O_2 \cdot min^{-1} \cdot W^{-1}$) than the S_1 value (9.7±0.2 mL $O_2 \cdot min^{-1} \cdot W^{-1}$) ($P<10^{-4}$, *Fig. 3A*). Moreover, our results suggest that myosin heavy chain composition in the working skeletal muscle influences both the oxygen uptake (*Figs. 4A* and *5*) and heart rate (*Fig. 6B*) during incremental cycling exercise. Interestingly, there is no relationship between the MyHC2 content in the *vastus lateralis* and the slope of the VO_2-PO relationship (S_1) besides the slope of the HR-PO relationship during cycling performed in the moderate intensity domain. However, during exercise exceeding the LT, the rate of an increase in VO_2 and in HR in relation to power output is steeper in subjects with a greater content of MyHC2 in the *vastus lateralis* muscle (*Fig. 5A* and *Fig. 6B*).

MyHC composition in the vastus lateralis and the oxygen cost of cycling in the moderate intensity domain

It has been demonstrated by Barstow *et al*. (7) that the slope value of the VO_2-PO relationship, both during cycling below LT (S_1) as well as above the LT (S_2) during the ramp protocol, positively correlates with the content of slow contracting muscle fibres in the working skeletal muscles. In our group of young, healthy men (n=38), we have found that the MyHC composition in the *vastus lateralis* muscle does not correlate with the slope of the VO_2-PO relationship during cycling performed below the LT (S_1, *Fig. 4B*). Based on our results, during moderate-intensity cycling (below LT), there is no difference in the rate of the increase in the energy cost related directly to an increase in power output (cross-bridge activities) between subjects with a varied MyHC composition in the working muscle (*Fig. 4B*). It should be underlined, however, that in the study by Barstow *et al.* (7), the VO_2-PO relationship was determined using a ramp protocol, whereas in the present study we have used an incremental exercise test. Both protocols could give varied results of the VO_2-PO relationship, especially above the LT. Moreover, Barstow *et al*. (7) used myofibrillar ATPase histochemistry to determine muscle fibre types, whereas we have used gel electrophoresis to measure the proportion of MyHC2 and MyHC1. Therefore, a comparison of the outcome of these studies requires some caution.

Despite no relationship between MyHC composition and the S_1 value, a positive relationship between the MyHC2 content in the *vastus lateralis* and the intercept value of the VO_2-PO relationship during cycling performed in the moderate-intensity domain has been reported (a_1, *Fig. 4A*). It is commonly accepted that the intercept value of the VO_2-PO relationship, reflecting the oxygen cost of unloaded cycling, represents the energy cost of non-cross bridge activities common to all power outputs, including resting energy expenditure, the energy cost of doing internal work, and energy cost of ion transport during active shortening (7). The positive relationship between the MyHC2

content and a_1, and the fact there is no relationship between MyHC2 and S_1, is in line with the data presented earlier (10) on a smaller group of subjects; *i.e.* there is a significantly higher intercept value of the VO_2-PO relationship during moderate intensity cycling (30–120 W) in subjects with a greater proportion of MyHC2 in their *vastus lateralis* (~61% of MyHC2) than in subjects with a lower MyHC2 content (~28% of MyHC2) in this muscle, with respect to there being no difference in the slope value of this relationship between those groups. The results of the present study (*Fig. 4A* and *Fig. 4B*) are also in line with data presented by Stuart *et al*. (24), in which a lower gross efficiency (PO/VO_2) has been demonstrated during a cycling exercise in sprinters (possessing a higher percentage of type II muscle fibres), when compared to distance runners (who possess a higher content of slow contracting type I muscle fibres), with no difference in delta efficiency between those two groups. If, as postulated before (7), S_1 ($\Delta VO_2/\Delta PO$), as a reciprocal of delta efficiency ($\Delta PO/\Delta VO_2$), represents the efficiency of contracting muscles (excluding the energy cost in non-cross bridge activities), and gross efficiency reflects the total oxygen cost of cycling (including the oxygen cost of non-cross bridge activities), it could be considered that the difference in the oxygen cost of moderate-intensity cycling between subjects with a varied proportion of MyHC in the working muscles is based on a difference in the energy cost of non-cross bridge activities (22).

It has been demonstrated that sarco(endo)plasmic (SR) calcium pumps (SERCA), together with myosin ATPase, consume most of the energy during muscle contractions (25). SERCA belongs to the system of cellular calcium homeostasis and are responsible for the rate of relaxation during active shortening (26). It was demonstrated that in both slow and fast skeletal muscle fibres during muscle contraction, the energy cost of Ca^{2+} sequestering into SR reaches about 40–50% of the total ATP used (25). It was reported that the SERCA content in fast muscle fibres (with an expression of MyHC2 isoform) is about ~5 to ~7 times higher than in slow muscle fibres (27, 28). Moreover, it was demonstrated that Ca^{2+} ATPase activity in the fast skeletal muscle fibres of humans is ~3 times higher than in slow muscle fibres (29).

The positive relationship between the MyHC2 content and the oxygen cost of unloaded cycling, as observed in our group of subjects ($r=0.49$, $P=0.002$, *Fig. 4A*), might be primarily explained by the higher content of SERCA pumps in the skeletal muscle of the subjects with a higher proportion of MyHC2 (27, 28), besides by higher ATP usage for calcium pumping to SR in this group of subjects (25, 29). The role of SERCA in the oxygen cost of moderate-intensity cycling was shown in our previous study (30). We have reported that endurance training in its early stage (5-weeks of the endurance training) is potent in down-regulating the SERCA content, and this was accompanied by a training-induced lowering of the oxygen cost of unloaded cycling during a moderate intensity exercise (30–120 W). Therefore, the greater oxygen cost of moderate intensity cycling in subjects possessing a higher amount of MyHC2 can be explained by higher energy usage by the SERCA pumps in fast skeletal muscle fibres. It should be noted that in the present study, the intercept value of the VO_2-PO relationship above the LT (a_2) was lower than below the LT (a_1) ($P<10^{-4}$). This suggests that the contribution of the non-cross bridge activities to the total energy cost of cycling at exercise intensities exceeding the LT is less than that in the case of a moderate-intensity exercise (*Fig. 3B*).

The MyHC composition in the vastus lateralis and the oxygen cost of cycling in the heavy intensity domain

In the present study, involving a large cohort of young, healthy men (n=38), the significantly higher (by ~27%) slope value of the VO_2 to PO relationship above the LT (S_2), when compared to the S_1 value (*Fig. 3A*, $P<10^{-4}$), clearly shows the presence of the non-linearity in the VO_2-PO relationship (*Fig. 1* and *Fig. 2A*). As a consequence of the non-linearity in the VO_2-power output relationship, the observed VO_{2max} was ~12% higher than the expected VO_2 at the end of incremental exercise (*Table 1*). Furthermore, the power output reached at VO_{2max} was 14% lower than the expected power output at VO_{2max} (*Exp.* PO at *Obs.* VO_{2max}) (*Table 1*). These results are close to those published previously (5, 6, 22).

The background of the non-linearity in the VO_2-PO relationship during incremental exercise tests and the slow component of the VO_2 kinetics during constant power output exercises is not completely understood (12, 13, 31). A progressive recruitment of the less-efficient fast type muscle fibres has been postulated to be a main factor responsible for VO_2 'excess' (32), since it is well known that more type II muscle fibres are recruited when exercise is performed in the heavy-intensity domain (33). However, data concerning the relationship between muscle fibre type distribution and the magnitude of the VO_2 'excess' during heavy-intensity exercise are inconclusive (9, 11, 34, 35). In the present study, we have shown, as others before, that higher a MyHC2 content in the *vastus lateralis* correlates negatively with the power output reached at LT (36), and with the PO reached at VO_{2max} (Results). We have found also that a higher content of fast type MyHC in the working skeletal muscle is accompanied by a greater magnitude of non-linearity in the VO_2-PO relationship during an incremental cycling exercise (*Fig. 5B-5D*). A tendency ($r=0.28$, $P=0.08$) towards a positive correlation between MyHC2 and S_2 was noticed (*Fig. 5A*). The lack of correlation between the MyHC2 content and S_1 ($r= -0.18$, $P=0.29$, *Fig. 4B*) and, on the other hand, a positive correlation between MyHC2 and the magnitude of the non-linearity in the VO_2-PO relationship at exercise intensities exceeding LT (*Fig. 5*), clearly shows that fast type muscle fibres are involved in the background of the lowering of muscle efficiency in that intensity domain. It seems to be very likely, as postulated previously (5), that the physiological background of the non-linear relationship in the VO_2-power output relationship and the slow component of the VO_2 on-kinetics is similar. According to recent studies (22, 37-39), the slow component of VO_2 kinetics is caused by muscle fatigue. Therefore, not the recruitment of type II muscle fibres *per se*, but rather their poorer metabolic stability during muscle work (*i.e.*, greater changes in ADP_{free}, P_i, H^+ and ΔG_{ATP} in relation to the pre-exercise level (40, 41) seems to be the cause of lower mechanical efficiency during exercise performed in the heavy-intensity domain (37). Indeed, during active shortening, type II muscle fibres, which possess lower metabolic stability than slow contracting type I muscle fibres (40, 41), produce a higher amount of factors related to fatigue, including hydrogen ions (42), ammonia (43) and reactive oxygen species (44). Using a model of the oxidative phosphorylation in skeletal muscle (45), it was demonstrated that during heavy-intensity exercise the time-dependent accumulation of metabolites related to muscle fatigue leads to the higher oxygen cost of generating power output, as reflected by the presence of VO_2 'excess' (46). We have demonstrated in our earlier study (47) that during whole body incremental cycling performed at exercise intensities exceeding LT, the subjects with a higher proportion of MyHC2 (~57%) in their *vastus lateralis* accumulate more hydrogen ions, lactate and ammonia in blood, which is in turn accompanied by a higher oxygen cost of cycling and lower power output reached at VO_{2max}, when compared to subjects with a lower content of MyHC2 (~27%). The opposite effect, *i.e.* an attenuation of the magnitude of the VO_2 'excess' above the LT accompanied by an attenuation of plasma lactate concentration and lowering of the plasma ammonia level after 5 weeks of moderate-intensity

training, was reported despite there being no changes in the MyHC content in the *vastus lateralis* (22). Therefore, the positive correlation between the MyHC2 content in the *vastus lateralis* and the magnitude of the VO_2 'excess' above the LT, as observed in the present study, seems to be attributable to poorer muscle metabolic stability in type II muscle fibres reflected by a higher increase of metabolites associated with fatigue, as discussed above (12, 22, 37).

It should be emphasized that, in the present study, we have examined the relationship between the oxygen cost of cycling below and above the LT, and the content of MyHC1and MyHC2. Using the method by Carraro&Catani (20) in this study we were unable to distinguish between MyHC2a and MyHC2x. Based on the recent experiment data showing that muscle fatigue is the key factor responsible for lowering muscle mechanical efficiency, and an increase of oxygen cost of work (22, 37-39), one could expect that the subjects with the highest proportion of the most fatigable muscle fibres with the highest content of MyHC2x would possess the highest oxygen cost of cycling, especially when cycling above the LT. Therefore, further studies are needed to establish the role of MyHC2x in the oxygen cost of cycling below and above the LT.

Another mechanism which can contribute to the non-liner increase in the VO_2-power output relation during high intensity exercise (48) is the formation of reactive oxygen species (ROS) during exercise. It is well established that intense physical exercise increases ROS formation (49-51). An increase of ROS production results in oxygen utilization not related to ATP synthesis that can then contribute to an increase of the oxygen cost of work during high intensity exercise. Moreover, the formation of free radicals could also affect muscle mitochondrial efficiency (52), resulting in a further increase of the oxygen cost of work. Additionally, reactive oxygen species can cause muscle fatigue by disturbing the mechanisms of power generation in skeletal muscles (49, 53), which could also contribute to the increase of the VO_2/PO ratio. Furthermore, enhanced oxidative stress can affect endothelial function (54, 55) and compromise oxygen delivery to the working muscles, resulting in early fatigue and a decrease of muscle efficiency. It seems likely that enhanced ROS production during exercise might indeed play a role in the observed increase of the VO_2/PO ratio during high intensity exercise, but the magnitude of their impact on the oxygen cost of work is yet to be established.

MyHC composition in the vastus lateralis and heart rate during incremental cycling exercise

In our group of subjects, an increase in heart rate in relation to the gradual increase of power output followed a linear pattern (see *Fig. 2B*); *i.e.*, no significant difference between the slope value of the HR-PO relationship below the LT versus that above the LT was found (P>0.05, *Fig. 3B*). We have found that an increase of power output by 10 W required an increase of HR by ~4.0 beats per minute. This value is similar to those reported previously (56). It should be mentioned that some authors reported a slower increase in HR in relation to an increase in exercise intensity above the LT, known as the heart rate deflection point (57). But others reported that this phenomenon is present only in about 50% of tested subjects (58). In the present study, we have found no difference in the slope value of the HR-PO relationship below the LT versus that above the LT (*Fig. 3*), which suggests that in the case of our subjects the effect of this phenomena (if occurring at all) on the rate of an increased HR bellow and above the LT was not significant.

The increased heart rate observed during exercise belongs to the cardiovascular response strategy, leading to an increase in blood flow to the working skeletal muscle (59, 60). During dynamic exercise, neural control of the cardiovascular system includes two mechanisms: central command, and a peripheral neural reflex originating in skeletal muscle; *i.e.*, exercise pressor reflex (59, 61). During a moderate-intensity exercise, an increase in the heart rate is mainly an effect of a decrease in the parasympathetic tone, whereas during heavy-intensity exercise a more gradual increase in heart rate occurs due to an increase in sympathetic stimulation, mediated *via* direct cardiac sympathetic innervation, circulating catecholamines and skeletal muscle-derived metabolites (59, 61). It is postulated that contraction-induced increases in lactate, H^+, and inorganic phosphate (61, 62), play an important role *via* both the arterial chemoreflex (63) and muscle metaboreflex (61) in the control of the heart rate. Activation of mechanoreceptors and chemoreceptors in muscle during exercise through the afferent neural fibres reflexively induces elevations in heart rate and blood pressure predominantly by increasing sympathetic nerve activity (64).

In the present study, we have found that the MyHC2 content in *vastus lateralis* correlated positively with the slope of the HR-PO relationship at exercise intensities exceeding the LT (r=0.49, *P*=0.002, *Fig. 6B*). This could be at least partly due to an enhanced demand for muscle blood flow caused by a greater oxygen cost of cycling in the subjects with a higher content of MyHC2 in their *vastus lateralis* muscle. Moreover, greater muscle metabolic disturbance above the LT, especially in subjects with a greater MyHC2 content in *vastus lateralis*, could contribute to the higher slope of the HR-PO relationship in those subjects. Accordingly, no correlation between the content of MyHC2 and the slope of the HR-PO relationship below the LT was found (r=0.09, *P*=0.60, *Fig. 6A*). Additionally, the positive correlation between the MyHC2 content and the slope of the HR-PO relationship in the entire range of exercise-intensities, *i.e.* from 30 up to *Obs.* PO_{max} (r=0.41, *P*=0.01), was found, which illustrates that the magnitude of an increase of HR in relation to an increase of power output is greater in subjects with a higher content of MyHC2 in their *vastus lateralis* muscle.

It should be noted that, in the present study, we have applied a rather low pedalling rate, *i.e.* 60 rev · min^{-1}, which is close to the optimum for the maximal mechanical efficiency of type I muscle fibres (65). Although this pedalling rate is the most frequently used when performing maximal incremental exercise tests when measuring VO_{2max} (2), the recent observations show that freely chosen pedalling rates during maximal incremental tests are by about 20 rev · min^{-1} higher; *i.e.* it amounts to about 80 revs per min (66). Nevertheless, our previous studies (5, 67, 68) showed that the oxygen cost of generating a given power output when cycling at varied pedalling rates in the range of 60–80 rev · min^{-1} was no different. Moreover, the VO_{2max} and power outputs reached at VO_{2max} were not significantly different when cycling at 60 or at 80 revs per min (5, 67, 68). Therefore, the pedalling rate of 60 rev · min^{-1}, which was applied in the present study, is in the range of appropriate pedalling rates for the measurement of VO_{2max} and the VO_2-power output relationship in untrained subjects. Nevertheless, further studies are needed to show the effect of higher pedalling rates, close to the optimal pedalling rates for the combined type I and type II muscle fibres (65), on the relationship between muscle MHC composition and the oxygen cost of cycling below and above the LT demonstrated in the present study.

In the present study, we have found that a greater MyHC2 content in the *vastus lateralis* is accompanied by a higher oxygen cost of cycling during exercise performed below the LT. This seems to be related to the higher energy cost of the non-cross-bridge activities in the muscles possessing a greater proportion of the MyHC2 content. In the case of heavy-intensity exercise, a higher MyHC2 content in *vastus lateralis* is accompanied by greater non-linearity in the VO_2-PO relationship, as well as a steeper increase in HR in the function of an increase of power

output. This relationship can be explained by greater disturbances in metabolic stability in type II muscle fibres during exercise, resulting in a decrease of muscle mechanical efficiency and a greater increase of heart rate at a given power output.

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