EFFECT OF PEDALLING RATES AND MYOSIN HEAVY CHAIN COMPOSITION IN THE VASTUS LATERALIS MUSCLE ON THE POWER GENERATING CAPABILITY DURING INCREMENTAL CYCLING IN HUMANS

Joanna Majerczak[1], Zbigniew Szkutnik[2,1], Krzysztof Duda[3,1], Malgorzata Komorowska[4], Leszek Kolodziejski[3] Janusz Karasinski[5], Jerzy A. Zoladz[1]

[1]Department of Physiology and Biochemistry, Faculty of Rehabilitation, University School of Physical Education, Krakow, Poland [2]Faculty of Applied Mathematics, AGH-University of Science and Technology, Krakow, Poland, [3]Cancer Institute, Krakow, Poland, [4]Department of Clinical Biochemistry, University Children Hospital, Faculty of Medicine, Jagiellonian University, Krakow, Poland, [5]Department of Cytology and Histology, Institute of Zoology, Jagiellonian University, Krakow, Poland

Correspondence to: Joanna Majerczak, Department of Physiology and Biochemistry, Faculty of Rehabilitation, University School of Physical Education, Al. Jana Pawla II 78, 31 - 571 Krakow, Poland,
e-mail: joanna.majerczak@awf.krakow.pl

RUNNING TITLE:

MyHC COMPOSITION AND POWER GENERATING CAPABILITY IN HUMANS

SUMMARY

In this study, we have determined power output reached at maximal oxygen uptake during incremental cycling exercise ($P_{I,\ max}$) performed at low and at high pedalling rates in nineteen untrained men with various myosin heavy chain composition (MyHC) in the vastus lateralis muscle. On separate days, subjects performed two incremental exercise tests until exhaustion at 60 rev · min^{-1} and at 120 rev · min^{-1}. In the studied group of subjects $P_{I,\ max}$ reached during cycling at 60 rev · min^{-1} was significantly higher (p=0.0001) than that at 120 rev · min^{-1} (287 ± 29 *vs.* 215 ± 42 W, respectively for 60 and 120 rev · min^{-1}). For further comparisons, two groups of subjects (n=6, each) were selected according to MyHC composition in the vastus lateralis muscle: group H with higher MyHC II content (56.8 ± 2.79%) and group L with lower MyHC II content in this muscle (28.6 ± 5.8%). $P_{I,\ max}$ reached during cycling performed at 60 rev · min^{-1} in group H was significantly lower than in group L (p=0.03). However, during cycling at 120 rev · min^{-1}, there was no significant difference in $P_{I,\ max}$ reached by both groups of subjects (p=0.38). Moreover, oxygen uptake (VO_2), blood hydrogen ion [H^+], plasma lactate [La^-] and ammonia [NH_3] concentrations determined at the four highest power outputs completed during the incremental cycling performed at 60 as well as 120 rev · min^{-1}, in the group H were significantly higher than in group L. We have concluded that during an incremental exercise performed at low pedalling rates the subjects with lower content of MyHC II possess greater power generating capabilities than the subjects with higher content of MyHC II in the vastus lateralis muscle. Surprisingly, at high pedalling rate, power generating capabilities in the subjects with higher MyHC II content in the vastus lateralis muscle did not differ from those found in the subjects with lower content of MyHC II in this muscle, despite higher blood [H^+], [La^-] and [NH_3] concentrations. This indicates that at high pedalling rates the subjects with higher percentage of MyHC II in the vastus lateralis muscle perform relatively better than the subjects with lower percentage of MyHC II in this muscle.

Key words: cycling, myosin heavy chain isoforms, muscle fatigue, oxygen uptake

INTRODUCTION

During daily life activity human muscles generate broad range of power outputs and contract at various velocities (for review see *e.g.* Sargeant and Jones 1995, Sargeant and de Haan 2006). It is well established that the maximal short term muscle power output is strongly dependent upon the muscle contraction velocities (Sargeant *et al.* 1981, Sargeant and Beelen 1993). During cycling exercise the maximal power output is normally reached while cycling at about 120 rev · min^{-1} (Sargeant *et al.* 1981, Sargeant and Beelen 1993, Sargeant and de Haan 2006). However, the optimal shortening velocity at which the maximal power output can be reached varies between subjects, being highest in the subjects with high content of type II (fast) muscle fibers and lowest in those with high content of type I (slow) muscle fibers (Sargeant and Beelen 1993, Sargeant and Jones 1995, Aagaard and Andersen 1998).

Far less is known regarding the effect of muscle fibers composition on the power generating capabilities during maximal incremental cycling exercise performed at various pedalling rates. It should be mentioned that the incremental exercise protocols are the most frequently used procedures for assessment of maximal oxygen uptake (VO_{2max}) and the endurance capacity in humans (see *e.g.* Astrand and Rodahl 1986, Wilmore and Costill 1999). In view of the available data (for review see Sargeant and Jones 1995, Sargeant and Beelen 1993), cycling at the power output corresponding to maximal oxygen uptake requires recruitment of all available types of muscle fibers. However, during cycling at 120 rev · min^{-1} the recruitment of type II muscle fibers starts earlier (*i.e.* at lower external power outputs), when compared to cycling at 60 rev · min^{-1} (see *e.g.* Sargeant 1994). Recruitment of the fatigue sensitive type II muscle fibers, characterized by lower metabolic stability (see *e.g.* Matheson *et al.* 1991, Zoladz *et al.* 2006), causes greater muscle phosphocreatine and glycogen depletion as well as greater disturbances in muscle metabolites concentrations *i.e.* accumulation of [ADP_{free}], [P_i], [AMP], [NH_3], [IMP], [H^+] - the factors normally associated with fatigue (see *e.g.* Dawson *et al.* 1980, Fitts 1994, Allen *et al.* 1995). This could be one of the reasons for the earlier fatigue while cycling at the same external power output with pedalling rates of 120 rev · min^{-1}, when compared to the cycling at 60 rev · min^{-1} (see *e.g.* Zoladz *et al.* 2000, Beleen *et al.* 1993).

In the present study, we have hypothesized that the power generating capabilities during maximal incremental cycling exercise performed at the pedalling rate of 60 rev · min^{-1} and at 120 rev · min^{-1} (similar to sprinting - see Sargeant and Beelen 1993, Sargeant and Jones 1995) will be also related to the content of various types of myosin heavy chain isoforms (MyHC I and MyHC II) in the vastus lateralis muscle in humans, which corresponds to the proportion of type I and type II (slow and fast) muscle fibers (see *e.g.* Fry *et al.* 1994, Aagaard and Andersen 1998). To our best knowledge, no studies were conducted to examine such a relationship. Our assumption is based on the earlier findings showing that the pedalling rate of 60 rev · min^{-1} is closer to the optimal velocity of shortening for type I muscle fibers, whereas pedalling rate of 120 rev · min^{-1} is closer to the optimal velocity of shortening for type II muscle fibers (see *e.g.* Sargeant and Jones 1995). Therefore, in the present study, we have hypothesized that the subjects with higher content of MyHC II in the vastus lateralis muscle will perform relatively better at 120 rev · min^{-1} than at 60 rev · min^{-1}, when compared to the subjects with lower content of MyHC II in this muscle.

SUBJECTS AND METHODS

Subjects

Nineteen untrained, but physically active, non-smoking men (mean ± SD: aged 23.7 ± 2.6 years; body mass 72.4 ± 6.8 kg; height 178.9 ± 4.7 cm; BMI 22.61 ± 1.91 kg · m^{-2}; VO_{2max} 50.2 ± 5.1 ml · kg^{-1} · min^{-1}) participated in this study. Subjects gave informed written consent and were aware of the aims of the study. The study protocol was approved by the Local Ethical Committee and was performed in accordance with the Declaration of Helsinki. Since the subjects had only little experience with cycling (on recreational level) especially at high frequencies *i.e.* 120 rev · min^{-1}, one week before starting the main exercise protocols, the subjects reported to the laboratory in order to practice cycling at this frequency for about 6-10 minutes.

Exercise protocol

The incremental exercise test was performed on the cycloergometer Ergo-Line GmbH & Co KG 800s (Bitz, Germany). Before the test, a 6-min resting period was allowed to determine the resting stage of the cardio-respiratory parameters, as well as to withdraw the blood samples. The exercise test started at power output 30 W, followed by gradual increase amounting to 30 W every 3 min and it was continued until exhaustion. The incremental test was performed on separate days at two different pedalling rates: 60 and 120 rev · min^{-1} in the stable conditions, *i.e.* air temperature of about 22°C and relative humidity of about 50%.

Gas exchange variables

Gas exchange variables were measured continuously *breath by breath* using the Oxycon Champion, Mijnhardt BV (Bunnik, The Netherlands), starting from 6^{th} minute prior to exercise until the test was stopped. Before and after each test, gas analysers were calibrated with certificated calibration gases, as previously described by Zoladz *et al.* (1995).

Blood sampling

Blood samples were taken using an Abbot Int-Catheter, Ireland (18G/1.2 x 45 mm) inserted into the antecubital vein about 15 minutes prior to the onset of the exercise. The catheter was connected to an extension set using a "T" Adapter SL Abbot, Ireland (the tube 10 cm in length). Immediately before taking each blood samples, 1 ml of blood volume was taken in order to eliminate blood from the catheter and the T-set. Blood samples for blood gases and hydrogen ion concentration as well as plasma lactate and plasma ammonia concentrations were taken prior to the exercise test, at the end of each step of the incremental exercise (the last 15 seconds before increase of power output) and at the moment of ending the exercise protocol. Blood samples for plasma potassium concentration were taken prior and at the end of the exercise protocol.

Hydrogen ion concentration, PO_2 and PCO_2

Blood partial pressure of oxygen (PO_2) and carbon dioxide (PCO_2) as well as hydrogen ion concentration [H^+] were determined using a Ciba –Corning analyser 248 (England). Blood bicarbonate concentration [HCO_3^-] was calculated by this unit.

Plasma lactate measurements

The blood samples for plasma lactate concentration [La^-] (0.5 ml each) were placed in 1.8 ml Eppendorf tubes containing 1 mg ammonium oxalate and 5 mg sodium fluoride and mixed for about 20 seconds and then centrifuged at 4000 rev · min^{-1} for 4 min. The obtained samples of blood plasma (200 µl) were stored at minus 32°C for further analysis of lactate concentration using an automatic analyser Vitros 250 Dry Chemistry System, Kodak (Rochester, NY, USA). Detection limit was 0.5 mmol · l^{-1}. Lactate threshold (LT) in this study was defined as the highest power output above which plasma lactate concentration showed a sustained increase of more than 0.5 mmol · l^{-1} · $step^{-1}$ (see *e.g.* Zoladz *et al.* 1995). Lactate threshold was identified in the incremental exercise test performed at 60 rev · min^{-1}. During cycling at 120 rev · min^{-1} LT was not detected, because sharp increase in lactate concentration was present already from the first power output *i.e.* from 30 W.

Plasma ammonia measurements

The blood samples for plasma ammonia concentration [NH_3] measurements were placed in 1.3 ml tube with lithium heparin, collected on ice till the end of exercise and then centrifuged at 4000 rev · min^{-1} for 3 min. The obtained samples of blood plasma were stored in the temperature minus 32°C for further analysis of ammonia concentration using an automatic analyser Vitros 250 Dry Chemistry System, Kodak (Rochester, NY, USA) after conversion of ammonium ions [NH_4^+] into gaseous ammonia [NH_3]. Detection limit was 1.0 µmol · l^{-1}.

Plasma potassium measurements

The blood samples for plasma potassium concentration [K^+] measurements were placed in 1.3 ml tube with lithium heparin and after exercise protocol centrifuged at 4000 rev · min^{-1} for 3 min. Plasma venous potassium concentration [K^+] was determined using Chiron Diagnostic 644 $Na^+/K^+/Cl^-$ analyser, U.K.

Muscle biopsy

Muscle biopsy samples were taken from the *vastus lateralis m. quadricipitis femoris* 15 cm above the upper margin of *patella,* under local anaesthesia (1% lidocaine), using 5 mm Bergström needle. Specimens were frozen and stored in liquid nitrogen until further analyses.

Myosin extraction

Muscle biopsies were mounted in Shandon cryostat with Tissue-Tek and 30-50 cryosections, 30 µm thick, were cut from each biopsy. 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-mercaproethanol, 2.3% SDS, pH 6.8 (Andersen and Aagaard 2000). Samples were briefly vortexed and boiled for 3 min in water bath. Myosin extracts were clarified at 13000 × g for 5 min and supernatants were freezed at minus 20^{o}C until further use.

SDS-PAGE

SDS-polyacrylamide gel electrophoresis was carried out according to Carraro and Catani (1983) with 3% stacking gel and 6% separating gel containing 37.5% glycerol, in 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, 2.5% 2-mercaptoethanol and boiled for 3 min. Myosin 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 h. Densitometric analysis of protein bands was performed using a video camera Fotodyne Incorporated and computer software Gel Pro Analyzer. Relative amounts of MyHC protein were expressed in optical density units (OD).

Data analysis

In this study, the exercise induced changes (the difference between end exercise and rest value) in the gas exchange variables as well as in blood [H^+], [HCO_3^-], [La^-], [NH_3], [K^+] were analysed during incremental cycling at 60 and 120 rev · min^{-1} in the whole group of nineteen subjects as well as in two different subgroups of subjects (n=6, each): the group H with the higher content of MyHC II in the vastus lateralis muscle and the group L with the lower content of MyHC II in this muscle (see Results). Statistical significance was tested using Wilcoxon-signed-rank test (for paired samples; non-asymptotic, exact, two-sided *p*-values are presented) and Wilcoxon-Mann-Whitney test (for two independent samples; non-asymptotic exact, two-sided *p*-values are presented).

The oxygen uptake (VO_2) as well as $[H^+]$, $[HCO_3^-]$, $[La^-]$ and $[NH_3]$ were analysed in the group H and L in the range of the four highest power outputs completed during cycling at 60 rev · min^{-1} and during cycling at 120 rev · min^{-1} (*i.e.* 180-270 W and 90-180 W, respectively for pedalling rates 60 and 120 rev · min^{-1}). During an incremental cycling at 60 rev · min^{-1}, 270 W was the highest completed power output obtained by all subjects from group L and by four subjects from group H (for two of them the highest power output was 260 and 265 W, data included to the analysis). During an incremental cycling at 120 rev · min^{-1}, the last power output (*i.e.,* 180 W) was completed by five subjects from group H and by five subjects from group L. Since the changes in $[H^+]$, $[HCO_3^-]$, $[La^-]$ and $[NH_3]$ in the range of power outputs given above were non-linear (see Figures 2-5), we have transformed the original data to logarithmic scale, in order to be able to perform valid analysis of covariance (ANCOVA). In the first step of analysis, we tested equality of slopes in the group H and L (parallelism test) of the linear dependencies between power output (in the ranges 180-270 W and 90-180 W, respectively for pedalling rates 60 and 120 rev · min^{-1}) and the chosen variable (*i.e.* VO_2, $\log[H^+]$, $\log[HCO_3^-]$, $\log[La^-]$, $\log[NH_3]$), separately for pedalling rate 60 and 120 rev · min^{-1}. Since the hypotheses of identical slopes in the groups H and L have not been rejected, ANCOVA was then used with one factor only, *i.e.* the MyHC II content in the vastus lateralis muscle, to test the equality of the intercepts (see *e.g.* Seber 1977). This was done separately for 60 and 120 rev · min^{-1}.

The analysis was performed using the statistical packages STATISTICA 7.1 and StatXact 6.1.

RESULTS

MyHC composition in the vastus lateralis muscle

Densitometric analysis of MyHC I and MyHC II resolved in polyacrylamide gel showed that in the group of nineteen subjects mean content of MyHC I was 57.1 ± 12.4% and mean content of MyHC II was 42.9 ± 12.4%. From the group of nineteen subjects two extreme groups of subjects (n=6, each), according to the expression of MyHC II in the vastus lateralis muscle, were selected. The group H with the significantly (p=0.002) higher content of MyHC II (mean value of MyHC II 56.8 ± 2.8%) and the second group called L with the lower proportion of MyHC II (mean value of MyHC II 28.6 ± 5.8%).

The body mass index of the subjects from group H was not significantly different from BMI of subjects from group L (21.6 ± 0.8 *vs.* 23.7 ± 2.6 kg $\cdot$ m^{-2}, respectively for the group H and L; p=0.18). There was a tendency to lower power output reached at lactate threshold during cycling at 60 rev $\cdot$ min^{-1} in group H when compared to group L (165 ± 16 *vs.* 140 ± 15 W, respectively for the group H and L, p=0.08).

Maximal oxygen uptake and power output reached at maximal oxygen uptake during cycling at 60 and 120 rev $\cdot$ min^{-1} in the group of nineteen subjects as well as in the groups H and L

Maximal oxygen uptake (VO_{2max}) in the group of nineteen subjects when cycling at 120 rev $\cdot$ min^{-1} was not significantly different (p=0.30) from VO_{2max} reached during cycling performed at 60 rev $\cdot$ min^{-1} (3663 ± 413 *vs.* 3622 ± 376 ml $\cdot$ min^{-1}, respectively for the 120 rev $\cdot$ min^{-1} and 60 rev $\cdot$ min^{-1}). Maximal oxygen uptake reached during cycling at 60 rev $\cdot$ min^{-1} in subjects from group H was not significantly different (p=0.60) from VO_{2max} of subjects from group L (3667 ± 187 *vs.* 3784 ± 257 ml $\cdot$ min^{-1}, respectively for the group H and L). Moreover, no significant difference (p=0.24) in VO_{2max} between subjects from both groups was found during cycling at 120 rev $\cdot$ min^{-1} (3565 ± 203 *vs.* 3774 ± 269 ml $\cdot$ min^{-1}, respectively for the group H and L).

In the group of nineteen subjects, the power output obtained at VO_{2max} ($P_{I,\ max}$) during cycling at 120 rev · min^{-1} was significantly lower (p=0.0001) than $P_{I,\ max}$ obtained during cycling at 60 rev · min^{-1} (215 ± 42 *vs.* 287 ± 29 W, respectively for 120 and 60 rev · min^{-1}). This reduction in power output obtained at VO_{2max} due to increase in pedalling rates from 60 to 120 rev · min^{-1} amounted to about 72 ± 39 W, *i.e.* $P_{I,\ max}$ at 120 rev · min^{-1} was about 25 percent lower when compared to 60 rev · min^{-1}. Moreover, in this group of subjects (n=19) the oxygen cost of generating $P_{I,\ max}$ ($VO_2/P_{I,\ max}$) during cycling at 120 rev · min^{-1} was significantly higher (p=0.009) than $VO_2/P_{I,\ max}$ during cycling at 60 rev · min^{-1} (15.8 ± 2.3 *vs.* 11.5 ± 0.6 ml · min^{-1} · W^{-1}, respectively for 120 and 60 rev · min^{-1}).

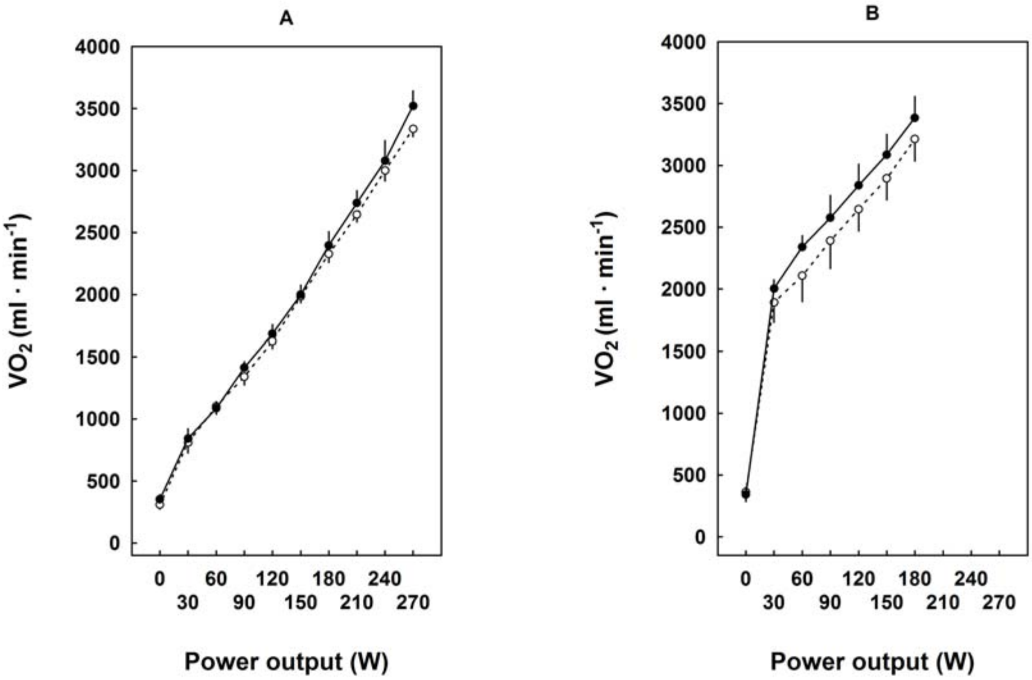
Power output obtained at VO_{2max} during cycling at 60 rev · min^{-1} for the subjects from group H was significantly lower (p=0.032) when compare to $P_{I,\ max}$ obtained for subjects from group L (279 ± 16 *vs.* 303 ± 17 W, respectively for the group H and L). However, during cycling at 120 rev · min^{-1}, $P_{I,\ max}$ obtained in the group H was not significantly different (p=0.38), from $P_{I,\ max}$ obtained in the group L (204 ± 31 *vs.* 224 ± 38 W, respectively for the group H and L). The reduction in $P_{I,\ max}$, due to increasing pedalling rates from 60 rev · min^{-1} to 120 rev · min^{-1} for the subjects from group H and L was not significantly different and amounted to about 25 percent (p=1.0).

Oxygen uptake, blood hydrogen ion and bicarbonate concentrations, plasma lactate and plasma ammonia concentrations during incremental cycling at 60 and 120 rev · min^{-1} in the group H and in the group L

Oxygen uptake (VO_2)

Oxygen uptake for the groups H (•) and L (◦) reached during an incremental cycling at 60 rev · min^{-1} is presented in Fig. 1A. Oxygen uptake in the range of power outputs 180-270 W was significantly higher in the group H than in the group L (ANCOVA, F=14.1; p=0.0005).

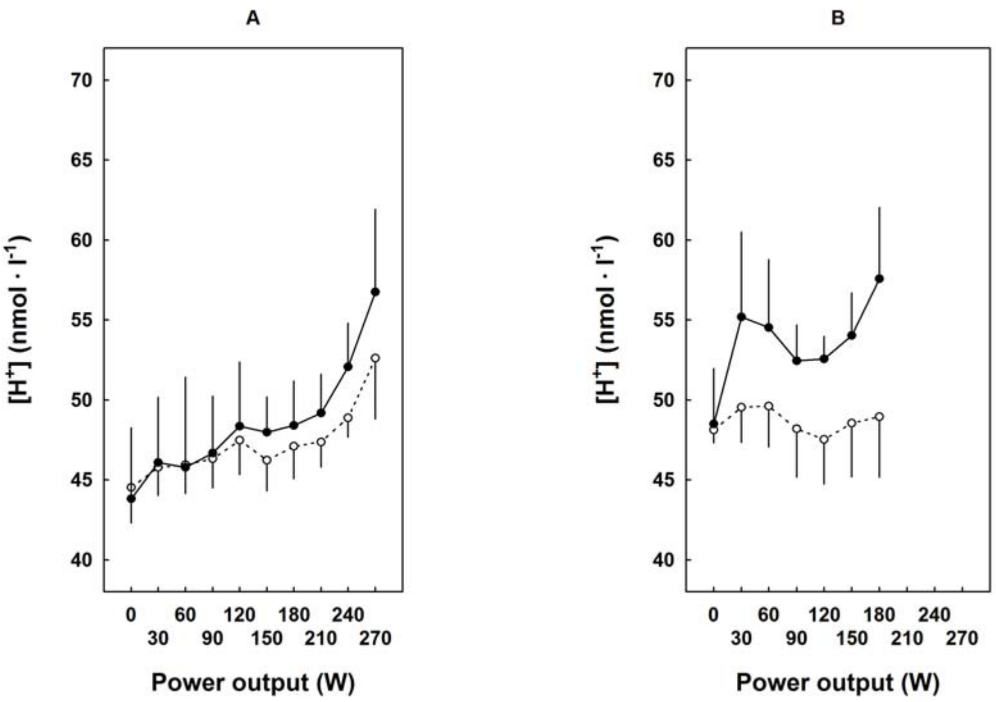
Oxygen uptake for the groups H (•) and L (◦) reached during an incremental cycling at 120 rev · min^{-1} is presented in Fig. 1B. The tendency to the higher oxygen uptake in the range of power outputs 90-180 W was observed in the group H, when compared the group L (ANCOVA, F=3.5; p=0.069).



Blood hydrogen ion concentration [H^+]

Blood hydrogen ion concentration for the groups H (•) and L (◦) reached during an incremental cycling at 60 rev · min^{-1} is presented in Fig. 2A. Blood hydrogen ion concentration in the range of power outputs 180-270 W was significantly higher in the group H than in the group L (ANCOVA, F=9.4; p=0.004).

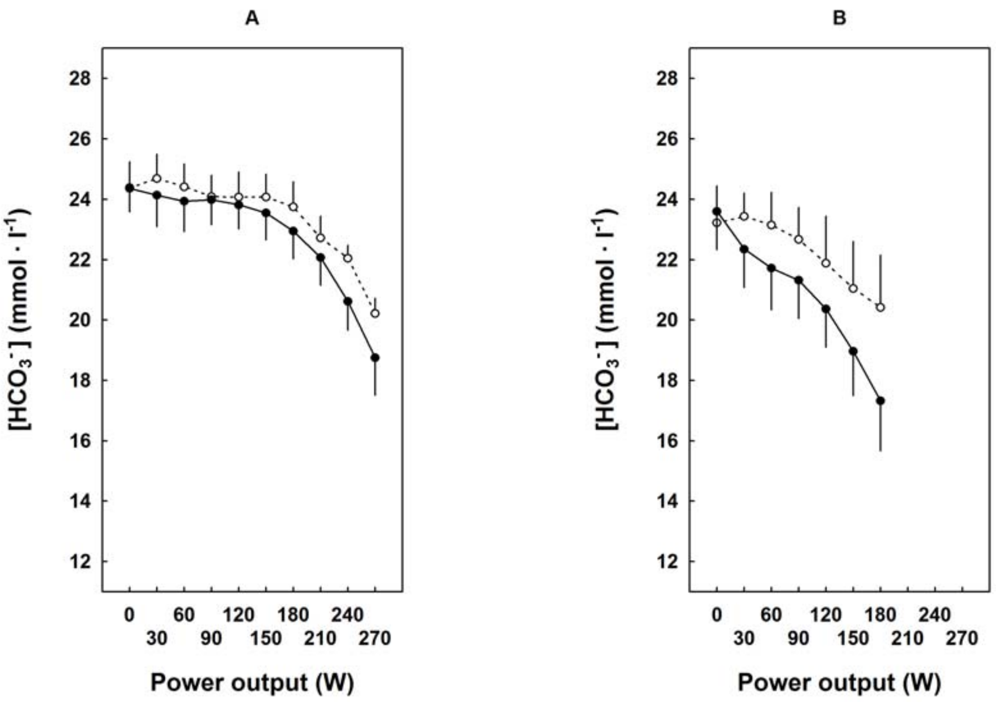
Blood hydrogen ion concentration for the groups H (•) and L (◦) reached during an incremental cycling at 120 rev · min^{-1} is presented in Fig. 2B. Blood hydrogen ion concentration in the range of power outputs 90-180 W was significantly higher in the group H than in the group L (ANCOVA, F=43.3; $p<10^{-4}$).



Blood bicarbonate concentration [HCO_3^-]

Blood bicarbonate concentration for the groups H (•) and L (◦) reached during an incremental cycling at 60 rev · min^{-1} is presented in Fig. 3A. Blood bicarbonate concentration in the range of power outputs 180-270 W was significantly lower in the group H than in the group L (ANCOVA, $F=18.1$; $p=0.0001$).

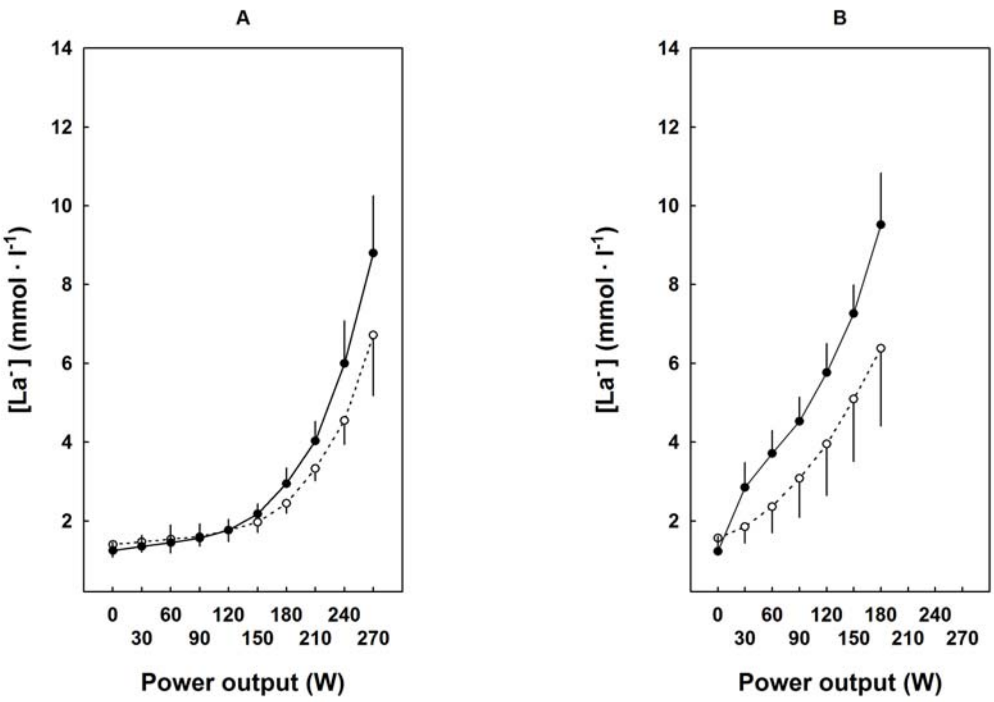
Blood bicarbonate concentration for the groups H (•) and L (◦) reached during an incremental cycling at 120 rev · min^{-1} is presented in Fig. 3B. Blood bicarbonate concentration in the range of power outputs 90-180 W was significantly lower in the group H than in the group L (ANCOVA, $F=21.5$; $p<10^{-4}$).



Plasma lactate concentration [La$^-$]

Plasma lactate concentration for the groups H (•) and L (◦) reached during an incremental cycling at 60 rev · min^{-1} is presented in Fig. 4A. Plasma lactate concentration in the range of power outputs 180-270 W was significantly higher in the group H than in the group L (ANCOVA, $F=33.3$; $p<10^{-4}$).

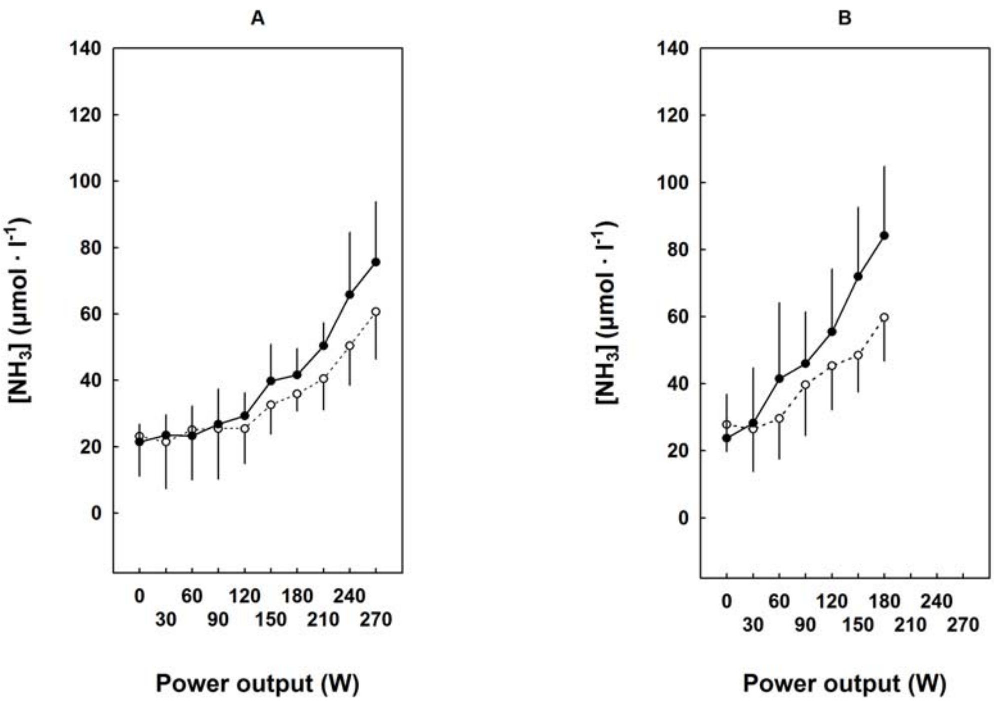
Plasma lactate concentration for the groups H (•) and L (◦) reached during an incremental cycling at 120 rev · min^{-1} is presented in Fig. 4B. Plasma lactate concentration in the range of power outputs 90-180 W was significantly higher in the group H than in the group L (ANCOVA, $F=38.6$; $p<10^{-4}$).



Plasma ammonia concentration [NH_3]

Plasma ammonia concentration for the groups H (•) and L (◦) reached during an incremental cycling at 60 rev · min^{-1} is presented in Fig. 5A. Plasma ammonia concentration in the range of power outputs 180-270 W was significantly higher in the group H than in the group L (ANCOVA, F=13.1; p=0.0007).

Plasma ammonia concentration for the groups H (•) and L (◦) reached during an incremental cycling at 120 rev · min^{-1} is presented in Fig. 5B. Plasma ammonia concentration in the range of power outputs 90-180 W was significantly higher in the group H than in the group L (ANCOVA, F=12.0; p=0.001).



Exercise induced changes (Δ: the difference between end exercise and rest value) in gas exchange variables and in blood [H^+], [HCO_3^-], [La^-], [NH_3], [K^+] concentrations during cycling at 60 and 120 rev · min^{-1} in the group of nineteen subjects

The results of the exercise induced changes (Δ) in gas exchange variables, *i.e.* oxygen uptake (VO_2), carbon dioxide production (VCO_2) and minute ventilation (V_E) as well as in blood [H^+], [La^-], [NH_3], [K^+] concentrations during cycling at 60 and 120 rev · min^{-1} in the group of nineteen subjects are presented in Table 1. In the group of nineteen subjects when cycling at 120 rev · min^{-1} significantly higher ΔV_E ($p=0.007$), higher Δ[H^+] ($p=0.008$) and the tendency to higher Δ[La^-] ($p=0.098$) were found, when compared to cycling at 60 rev · min^{-1}. No significant differences in ΔVO_2 ($p=0.42$), ΔVCO_2 ($p=0.77$), as well as in Δ[NH_3] ($p=0.72$) and Δ[K^+] ($p=0.55$) were found when cycling at 60 and 120 rev · min^{-1}.

Table 1

Table 1. The exercise induced changes (Δ: the difference between end exercise and rest value) in oxygen uptake (ΔVO_2), carbon dioxide production (ΔVCO_2), minute ventilation (ΔV_E), plasma lactate concentration $\Delta[La^-]$, plasma ammonia concentration $\Delta[NH_3]$, blood hydrogen ion concentration $\Delta[H^+]$, blood bicarbonate concentration $\Delta[HCO_3^-]$; data obtained during the incremental cycling performed at 60 rev · min^{-1} and at 120 rev · min^{-1} for 19 subjects (Wilcoxon-signed-rank test for paired data with non-asymptotic, exact *p*-value). In case of exercise induced changes in plasma potassium concentration data for 17 subjects were shown.

	60 rev· min^{-1}			120 rev· min^{-1}			*p - value*
	Me	**min : max**	$\bar{x} \pm SD$	**Me**	**min : max**	$\bar{x} \pm SD$	
ΔVO_2 (VO2net) (ml · min^{-1})	**3280**	**2349 : 3923**	**3294 ± 349**	**3321**	**2421 : 4357**	**3334 ± 383**	**0.42**
ΔVCO_2 (ml · min^{-1})	**3587**	**2896 : 4360**	**3671 ± 314**	**3703**	**2776 : 4467**	**3639 ± 379**	**0.77**
ΔV_E (l · min^{-1})	**98.8**	**82.1 : 135.9**	**115.1 ± 18.2**	**119.9**	**85.6 : 138.4**	**113.9 ± 17.3**	**0.007**
$\Delta[H^+]$ (nmol · l^{-1})	**12.9**	**0.0 : 32.6**	**14.1 ± 8.0**	**35.7**	**23.4 : 50.2**	**35.7 ± 6.6**	**0.008**
$\Delta[HCO_3^-]$ (mmol · l^{-1})	**-6.5**	**-9.5 : -2.2**	**-6.5 ± 1.7**	**-6.1**	**-10.4 : -1.9**	**-6.6 ± 2.0**	**0.96**
$\Delta[La^-]$ (mmol · l^{-1})	**8.4**	**3.5 : 13.2**	**8.5 ± 2.3**	**9.5**	**3.9 : 13.9**	**9.4 ± 2.6**	**0.098**
$\Delta[NH_3]$ (µmol · l^{-1})	**60.0**	**16.0 : 158.0**	**68.9 ± 31.7**	**58.0**	**5.0 : 165.0**	**71.7 ± 36.8**	**0.72**
$\Delta[K^+]$ (mmol · l^{-1})	**1.7**	**1.1 : 2.5**	**1.7 ± 0.4**	**1.7**	**1.1 : 2.2**	**1.7 ± 0.3**	**0.55**

Exercise induced changes (Δ: the difference between end exercise and rest value) in gas exchange variables and in blood $[H^+]$, $[HCO_3^-]$, $[La^-]$, $[NH_3]$, $[K^+]$ concentrations during cycling at 60 and 120 rev · min^{-1} in group H and in the group L

During cycling at 60 rev · min^{-1}, the exercise induced increases in VO_2, VCO_2, V_E, $[H^+]$, $[La^-]$, $[NH_3]$ and $[K^+]$ were not significantly different between subjects from group H and L. During cycling at 120 rev · min^{-1}, the exercise induced increases in VO_2, VCO_2, V_E, $[La^-]$, $[K^+]$ were not significantly different between subjects from group H and L. However, in the subjects from group H, a significantly higher $\Delta[H^+]$ (p=0.045) and a tendency to a higher $\Delta[NH_3]$ (p=0.13) were observed, when compared to subjects from group L during cycling at 120 rev · min^{-1}.

DISSCUSION

In the present study, the power output reached by the subjects (n=19) at the $\dot{V}O_{2max}$ ($P_{I, max}$) during incremental cycling performed at 120 rev · min^{-1} was by 25 percent lower (p=0.0001) than during cycling at 60 rev · min^{-1}. Moreover, during cycling performed at 120 rev · min^{-1}, higher oxygen cost of generating $P_{I, max}$ (p=0.009) was observed, when compared to the cycling at 60 rev · min^{-1} (see Results), which is in agreement with the previous study (Zoladz *et al.* 1995, Zoladz *et al.* 2000). It is well known that during cycling at high pedalling rates oxygen cost of cycling at a given power output is greater, when compared to cycling at low pedalling rates (see *e.g.* Gaesser and Brooks 1975, Sargeant and Beelen 1993, Zoladz *et al.* 1995). The reason for the lower mechanical efficiency of cycling at high pedalling rates remains unclear, however. The most often presented rational is higher contribution of internal work to the generated total power output (Francescato *et al.* 1995) and/or greater recruitment of less efficient fast muscle fibers (Leary *et al.* 2003) to the power generation (Sargeant and Beelen 1993, Beleen *et al.* 1993).

The most interesting and original finding of this study was that the $P_{I, max}$ reached during incremental cycling performed at 60 rev · min^{-1} in the group of subjects with lower MyHC II content in the vastus lateralis muscle was significantly higher (p=0.03) (about 10%), than the $P_{I, max}$ reached in the group of subjects with the higher MyHC II content in this muscle. During cycling at 120 rev · min^{-1}, however, the difference in the $P_{I, max}$ between subjects from both groups was not significant (p=0.38). Moreover, we have shown that during incremental cycling at 60 as well as 120 rev · min^{-1}, in the range of the four highest power outputs completed higher oxygen uptake (VO_2) (Fig. 1A and Fig. 1B), blood hydrogen ion concentration [H^+] (Fig. 2A and Fig. 2B), plasma lactate concentration [La^-] (Fig. 4A and Fig. 4B) and plasma ammonia concentration [NH_3] (Fig. 5A and Fig. 5B) were found in subjects with higher MyHC II content in the vastus lateralis muscle, when compared to subjects with lower MyHC II content in this muscle.

These results show that the subjects with the lower content of MyHC II isoform in the vastus lateralis muscle perform relatively better, regarding the $P_{I, max}$, than the subjects with the higher MyHC II content in this muscle while cycling at 60 rev · min^{-1}. This difference, however, becomes less evident when cycling at 120 rev · min^{-1}. As mentioned above, during cycling at 60 as well as at 120 rev · min^{-1} the oxygen uptake and the concentrations of some metabolites in blood such as $[H^+]$, $[La^-]$ and $[NH_3]$ associated with muscle fatigue, measured at the four highest power outputs completed were higher in the subjects with the higher content of MyHC II isoforms in the vastus lateralis muscle. This is in accordance with previous studies, involving various experimental models, showing that during muscle contractions concentration of hydrogen ion (Westerblad and Lännergren 1988, Thorstensson and Karlsson 1976), lactate (Essen and Häggmark 1975) and ammonia (Meyer and Terjung 1979, Dudley *et al.* 1983) are higher in fast than in slow muscle fibers.

The early fatigue observed in the subjects with the higher content of MyHC II in the vastus lateralis muscle during cycling at 60 rev · min^{-1} could be due to higher energetic cost of exercise and faster accumulation in the muscles some metabolites such as $[H^+]$, $[K^+]$, [ADP], $[P_i]$, [IMP], $[NH_3]$ (for discussion of this point see Dawson *et al.* 1980; Fitts 1994, Allen *et al.* 1995, Sahlin *et al.* 1998, Woledge 1998). One may also consider an increased rate of production of the reactive oxygen species in the type II muscle fibres (Alessio *et al.* 1988, Anderson and Neufer 2006), which has been suggested as a potential factor contributing to muscle fatigue (Reid *et al.* 1992, Arbogast and Reid 2004, Medved *et al.* 2004, Juel 2006), especially at physiological temperatures (Moopanar and Allen 2005). However, surprisingly, in our study during cycling at 120 rev · min^{-1}, despite a higher concentrations of $[H^+]$, $[La^-]$ and $[NH_3]$ in blood, as observed at the four highest power outputs completed in the subjects with the higher MyHC II content in the vastus lateralis (see Fig. 2B, 4B, 5B), the $P_{I,\ max}$ was not significantly different (p=0.38) from that found in the subjects with the lower content of MyHC II in this muscle. This result suggests that, especially during cycling at high pedalling rates, the maximal power generating capabilities of the muscles with higher MyHC II isoforms percentage are less affected by the "fatiguing metabolites" than the muscles with lower MyHC II isoforms content. The reason for that is unknown but some explanations have been put forward.

It should be mentioned that recently some authors questioned the role of the hydrogen ion as a main factor responsible for muscle fatigue at physiological temperature (Pate *et al.* 1995, Westerblad *et al.* 1997). It is even suggested (Nielsen *et al.* 2001, Pedersen *et al.* 2004) that the intracellular hydrogen ion and lactate accumulation might have a protective function in muscle during activity, because they counteract the effects of the exercise induced increase in extracellular potassium concentration, considered as one of the candidates of muscle fatigue (for review, see Sjögaard 1990, Fitts 1994).

It should be noted that in our study during incremental cycling performed at 120 rev · min^{-1} we have observed a tendency (p=0.13) towards higher exercise induced increase in ammonia concentration in subjects with higher MyHC II content in the vastus lateralis when compared to subjects with lower MyHC II content in this muscle (see Results). It has been known for a long time that working muscles produce ammonia (Parnas 1929) and the main source of ammonia during intense exercise is AMP deamination (see *e.g.* Tullson and Terjung 1990). According to Korzeniewski (2006) the adenylate kinase and AMP deaminase reaction diminished the amount of ADP and lowers activation of anaerobic glycolysis therefore attenuates muscle acidification during high intensity exercise. Moreover, one could consider that production of ammonia during exercise may not necessary be harmful to the muscle, as suggested previously (see *e.g.* Banister and Cameron 1990), since at the muscle pH below 7.0, NH_3 may act as proton acceptor contributing to the attenuation of exercise induced acidosis. However, the capacity of buffering H^+ by ammonia is rather limited (for discussion of this point see Graham *et al.* 1995). Therefore, higher accumulation of ammonia in subjects with higher MyHC II content in the vastus lateralis muscle could play only a minor protective role in acid base status of the muscle during cycling at high muscle shortening velocity.

As presented in Fig. 1A, significantly higher VO_2 at a given power output during cycling at 60 rev · min^{-1} was found in the group of subjects with the higher MyHC II content in the vastus lateralis muscle when compared to the subjects with the lower MyHC II content in this muscle. A similar tendency was also observed during cycling at 120 rev · min^{-1} (see Fig. 1B). This observation is in agreement with previous study showing the relationship between oxygen cost of cycling and MyHC II content in the vastus lateralis muscle (Zoladz *et al.* 2002, Majerczak *et al.* 2006). The higher oxygen cost of generating power in subjects with higher MyHC II content in the vastus lateralis muscle in our study could be related to lower efficiency of type II muscle fibers mitochondria (Leary *et al.* 2003). However, it should be noted that data concerning efficiency of different muscle fibers type are inconsistent (Suzuki 1979, Medbø 1990, Coyle *et al.* 1992, Horowitz *et al.* 1994) and muscle fibers type efficiency is probably related to the intensity and muscle contraction velocity (see Sargeant and Beelen 1993, Sargeant and Jones 1995).

When discussing the effect of muscle fibers composition on the power generating capabilities and energy cost of work, one has to consider the effect of muscle shortening velocity on the muscle efficiency of various muscle fibers. According to the model presented by Sargeant (see *e.g.* Sargeant and Jones 1995), the optimal velocity of contractions of type I muscle fibers during cycling exercise is about 60 rev · min^{-1}, whereas the optimal shortening velocity of type II muscle fibers lies above 120 rev · min^{-1}. Therefore, the subjects who possess predominantly type I muscle fibers perform, during cycling at 60 rev · min^{-1}, closely to the optimal contraction velocity, hence they can generate a given external mechanical power output at a lower energy cost. However, cycling at high pedalling rates (120 rev · min^{-1}) that exceed the optimal contraction velocities of type I muscle fibers, is more preferential in terms of power generation capabilities and the mechanical efficiency for the subjects who possess high content of type II muscle fibers with much higher optimal shortening velocity (for discussion of this point see Sargeant and Beelen 1993, Sargeant and Jones 1995). This consideration is in agreement with the experimental data obtained in the present study.

We have concluded that during maximal incremental exercise performed at low pedalling rates the subjects with the lower content of MyHC II possess greater power generating capabilities than the subjects with the higher content of MyHC II in the vastus lateralis muscle. Surprisingly, at high pedalling rate, power generating capabilities in the subjects with higher MyHC II content in the vastus lateralis muscle did not differ from those found in the subjects with the lower content of MyHC II in this muscle, despite higher blood [H^+], [La^-] and [NH_3] concentrations. This indicates that at high pedalling rates the subjects with higher percentage of MyHC II in the vastus lateralis muscle perform relatively better than the subjects with lower percentage of MyHC II in this muscle.

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REFERENCES

AAGAARD P, ANDERSEN JL: Correlation between contractile strength and myosin heavy chain isoform composition in human skeletal muscle. *Med Sci Sports Exerc* **30**: 1217-1222, 1998.

ALESSIO HM, GOLDFARB AH, CUTLER RG: MDA content increases in fast- and slow-twitch skeletal muscle with intensity of exercise in a rat. *Am J Physiol* **255**: C874-877, 1988.

ALLEN DG, LÄNNERGREN J, WESTERBLAD H: Muscle cell function during prolonged activity: cellular mechanisms of fatigue. *Exp Physiol* **80**: 497-527, 1995.

ANDERSEN JL, AAGAARD P: Myosin heavy chain IIX overshoot in human skeletal muscle. *Muscle Nerve* **23**: 1095-1104, 2000.

ANDERSON EJ, NEUFER PD: Type II skeletal myofibers possess unique properties that potentiate mitochondrial H_2O_2 generation. *Am J Physiol Cell Physiol* **290**: C844-851, 2006.

ARBOGAST S, REID MB: Oxidant activity in skeletal muscle fibers is influenced by temperature, CO_2 level, and muscle-derived nitric oxide. *Am J Physiol Regul Integr Comp Physiol* **287**: R698-705, 2004.

ASTRAND P-O, RODAHL K: Evaluation of physical performance on the basis of tests. In: *Textbook of work physiology*. *Physiological basis of exercise.* MD PROVENZANO (ed), McGraw-Hill, New York, 1986, pp 354-390.

BANISTER EW, CAMERON BJ: Exercise-induced hyperammonemia: peripheral and central effects. *Int J Sports Med* **11** Suppl 2: S129-142, 1990.

BEELEN A, SARGEANT AJ, LIND A, de HAAN A, KERNELL D, van MECHELEN W: Effect of contraction velocity on the pattern of glycogen depletion in human muscle fibre types. In: *Neuromuscular fatigue*. AJ SARGEANT, D KERNELL (eds), North Holland, Amsterdam, 1993, pp 93-95.

CARRARO U, CATANI C: A sensitive SDS-PAGE method separating myosin heavy chain isoforms of rat skeletal muscles reveals the heterogeneous nature of the embryonic myosin. *Biochem Biophys Res Commun* **11**: 793-802, 1983.

COYLE EF, SIDOSSIS LS, HOROWITZ JF, BELTZ JD: Cycling efficiency is related to the percentage of type I muscle fibers. *Med Sci Sports Exerc* **24**: 782-788, 1992.

DAWSON MJ, GADIAN DG, WILKIE DR: Mechanical relaxation rate and metabolism studied in fatiguing muscle by phosphorus nuclear magnetic resonance. *J Physiol* **299**: 465-484, 1980.

DUDLEY GA, STARON RS, MURRAY TF, HAGERMAN FC, LUGINBUHL A: Muscle fiber composition and blood ammonia levels after intense exercise in humans. *J Appl Physiol* **54**: 582-586, 1983.

ESSEN B, HÄGGMARK T: Lactate concentration in type I and II muscle fibres during muscular contraction in man. *Acta Physiol Scand* **95**: 344-346, 1975.

FITTS RH: Cellular mechanisms of muscle fatigue: *Physiol Rev* **74**: 49-94, 1994.

FRANCESCATO MP, GIRARDIS M, DI PRAMPERO PE: Oxygen cost of internal work during cycling. *Eur J Appl Physiol Occup Physiol* **72**: 51-57, 1995.

FRY AC, ALLEMEIER CA, STARON RS: Correlation between percentage fiber type area and myosin heavy chain content in human skeletal muscle. *Eur J Appl Physiol Occup Physiol* **68**: 246-251, 1994.

HOROWITZ JF, SIDOSSIS LS, COYLE EF: High efficiency of type I muscle fibers improves performance. *Int J Sports Med* **15**: 152-157, 1994.

GAESSER GA, BROOKS GA: Muscular efficiency during steady-rate exercise: effects of speed and work rate. *J Appl Physiol* **38**: 1132-1139, 1975.

GRAHAM TE, RUSH JWE, MCLEAN DA: Skeletal muscle amino acids metabolism and ammonia production during exercise. In: *Exercise metabolism*. M HARGREAVES (ed), Human Kinetics Publishers, 1995, pp 131-175.

JUEL C: Muscle fatigue and reactive oxygen species. *J Physiol* **576**: 1, 2006.

KORZENIEWSKI B: AMP deamination delays muscle acidification during heavy exercise and hypoxia. *J Biol Chem* **281**: 3057-3066, 2006.

LEARY SC, LYONS CN, ROSENBERGER AG, BALLANTYNE JS, STILLMAN J, MOYES CD: Fiber-type differences in muscle mitochondrial profiles. *Am J Physiol* **285**: R817-826, 2003.

MAJERCZAK J, SZKUTNIK Z, KARASINSKI J, DUDA K, KOLODZIEJSKI L, ZOLADZ JA: High content of MyHC II in vastus lateralis is accompanied by higher VO_2/power output ratio during moderate intensity cycling performed both at low and at high pedalling rates. *J Physiol Pharmacol* **57**: 199-215, 2006.

MATHESON GO, ALLEN PS, ELLINGER DC, HANSTOCK CC, GHEORGHIU D, MCKENZIE DC, STANLEY C, PARKHOUSE WS, HOCHACHKA PW: Skeletal muscle metabolism and work capacity: a 31P-NMR study of Andean natives and lowlanders. *J Appl Physiol* **70**: 1963-1976, 1991.

MEDBØ JI: Type I and type II fibres work with the same mechanical efficiency during bicycling. In: *Muscle and Motility.* G MARECHAL, U CARRARO (eds), Intercept, Andover, Hampshire, UK, 1990, pp 303-308.

MEDVED I, BROWN MJ, BJORKSTEN AR, MURPHY KT, PETERSEN AC, SOSTARIC S, GONG X, MCKENNA MJ: N-acetylcysteine enhances muscle cysteine and glutathione availability and attenuates fatigue during prolonged exercise in endurance-trained individuals. *J Appl Physiol* **97**: 1477-1485, 2004.

MEYER RA, TERJUNG RL: Differences in ammonia and adenylate metabolism in contracting fast and slow muscle. *Am J Physiol* **237**: C111-118, 1979.

MOOPANAR TR, ALLEN DG: Reactive oxygen species reduce myofibrillar Ca^{2+} sensitivity in fatiguing mouse skeletal muscle at 37 degrees C. *J Physiol* **564**: 189-199, 2005.

NIELSEN OB, DE PAOLI F, OVERGAARD K: Protective effects of lactic acid on force production in rat skeletal muscle. *J Physiol* **536**: 161-166, 2001.

PARNAS JK: Über die Ammoniakbildung im Muskel und ihren Zusammenhang mit Funktion und Zustandsänderung. Der Zusammenhang der Ammoniakbildung mit der Umwandlung des Adeninnucleotids zu Inosinsäure. *Bioch Zeitschr* **206**: 16-38, 1929.

PATE E, BHIMANI M, FRANKS-SKIBA K, COOKE R: Reduced effect of pH on skinned rabbit psoas muscle mechanics at high temperatures: implications for fatigue. *J Physiol* **486**: 689-694, 1995.

PEDERSEN TH, NIELSEN OB, LAMB GD, STEPHENSON DG: Intracellular acidosis enhances the excitability of working muscle. *Science* **305**: 1144-1147, 2004.

REID MB, HAACK KE, FRANCHEK KM, VALBERG PA, KOBZIK L, WEST MS: Reactive oxygen in skeletal muscle. I. Intracellular oxidant kinetics and fatigue in vitro. *J Appl Physiol* **73**: 1797-1804, 1992.

SAHLIN K, TONKONOGI M, SODERLUND K: Energy supply and muscle fatigue in humans. *Acta Physiol Scand* **162**: 261-266, 1998.

SARGEANT AJ, HOINVILLE E, YOUNG A: Maximum leg force and power output during short-term dynamic exercise. *J Appl Physiol* **51**: 1175-1182, 1981.

SARGEANT AJ, BEELEN A: Human muscle fatigue in dynamic exercise. In: *Neuromuscular fatigue*. AJ SARGEANT, D KERNELL (eds), North Holland, Amsterdam, 1993, pp 81-92.

SARGEANT AJ: Human power output and muscle fatigue. *Int J Sports Med* **15**: 116-121, 1994.

SARGEANT AJ, JONES DA: The significance of motor unit variability in sustaining mechanical output of muscle. In: *Fatigue*. *Neural and muscular mechanism.* SC GANDEVIA, RM ENOKA, AJ McCOMAS, DG STUART, CHK THOMAS (eds), Plenum Press, New York and London, 1995, pp 323-338.

SARGEANT AJ, DE HAAN A: Human muscle fatigue: the significance of muscle fibre type variability studied using a micro-dissection approach. *J Physiol Pharmacol* **57** Suppl 10: 5-16, 2006.

SJÖGAARD G: Exercise-induced muscle fatigue: the significance of potassium. *Acta Physiol Scand Suppl* **593**: 1-63, 1990.

SEBER GAF: Linear Regression Analysis, John-Wiley & Sons, New York, 1977.

SUZUKI Y: Mechanical efficiency of fast and slow – twitch muscle fibers in man during cycling. *J Appl Physiol* **47**: 263-267, 1979.

THORSTENSSON A, KARLSSON J: Fatiguability and fibre composition of human skeletal muscle. *Acta Physiol Scand* **98**: 318-322, 1976.

TULLSON PC, TERJUNG RL: Adenine nucleotide degradation in striated muscle. *Int J Sports Med* **11** Suppl 2: S47-55, 1990.

WESTERBLAD H, LÄNNERGREN J: The relation between force and intracellular pH in fatigued, single *Xenopus* muscle fibres. *Acta Physiol Scand* **133**: 83-89, 1988.

WESTERBLAD H, BRUTON JD, LÄNNERGREN J: The effect of intracellular pH on contractile function of intact, single fibres of mouse muscle declines with increasing temperature. *J Physiol* **500**: 193-204, 1997.

WILMORE JH, COSTILL DL: Energy for movement. In: *Physiology of Sport and Exercise*. H GILLY, J RHODA, S MERZ BOTT, K BOJDA (eds), Human Kinetics, 1999, pp 113-154.

WOLEDGE RC: Possible effects of fatigue on muscle efficiency. *Acta Physiol Scand* **162**: 267-273, 1998.

ZOLADZ JA, RADEMAKER AC, SARGEANT AJ: Non-linear relationship between O_2 uptake and power output at high intensities of exercise in humans. *J Physiol* **488**: 211-217, 1995.

ZOLADZ JA, RADEMAKER AC, SARGEANT AJ: Human muscle power generating capability during cycling at different pedalling rates. *Exp Physiol* **85**: 117-124, 2000.

ZOLADZ JA, DUDA K, KARASINSKI J, MAJERCZAK J, KOLODZIEJSKI L, KORZENIEWSKI B: MyHC II content in the vastus lateralis m. quadricipitis femoris is positively correlated with the magnitude of the non-linear increase in the VO_2 / power output relationship in humans. *J Physiol Pharmacol* **53**: 805-821, 2002.

ZOLADZ JA, KORZENIEWSKI B, GRASSI B: Training-induced acceleration of oxygen uptake kinetics in skeletal muscle: the underlying mechanisms. *J Physiol Pharmacol* **57** Suppl 10: 67-84, 2006.

FIGURE LEGENDS

Fig. 1. Panel A. The oxygen uptake / power output relationship during incremental cycling in the range 30-270 W performed at 60 rev · min^{-1} for the group H (•) and for the group L (◦). Data presented as mean value ± SD at each power output, for n=6 subjects in both groups (H and L). A significantly higher oxygen uptake in the range of power outputs 180-270 W in the group H was found when compared to the group L (ANCOVA, p=0.0005). **Panel B.** The oxygen uptake / power output relationship during incremental cycling in the range 30-180 W performed at 120 rev · min^{-1} for the group H (•) and for the group L (◦). Data presented as mean value ± SD at each power output, for n=6 subjects in both groups (H and L). A tendency to higher oxygen uptake in the range of power outputs 90-180 W in the group H was found when compared to the group L (ANCOVA, p=0.069).

Fig. 2. Panel A. The hydrogen ion concentration / power output relationship during incremental cycling in the range 30-270 W performed at 60 rev · min^{-1} for the group H (•) and for the group L (◦). Data presented as mean value ± SD at each power output, for n=6 subjects in both groups (H and L). A significantly higher blood hydrogen ion concentration in the range of power outputs 180-270 W in the group H was found when compared to the group L (ANCOVA, p=0.004). **Panel B.** The blood hydrogen ion concentration / power output relationship during incremental cycling in the range 30-180 W performed at 120 rev · min^{-1} for the group H (•) and for the group L (◦). Data presented as mean value ± SD at each power output, for n=6 subjects in both groups (H and L). A significantly higher blood hydrogen ion concentration in the range of power outputs 90-180 W in the group H was found when compared to the group L (ANCOVA, $p<10^{-4}$).

Fig. 3. Panel A. The blood bicarbonate concentration / power output relationship during incremental cycling in the range 30-270 W performed at 60 rev · min^{-1} for the group H (•) and for the group L. Data presented as mean value ± SD at each power output, for n=6 subjects in both groups (H and L). A significantly lower blood bicarbonate concentration in the range of power outputs 180-270 W in the group H was found when compared to the group L (ANCOVA, p=0.0001). **Panel B.** The blood bicarbonate concentration / power output relationship during incremental cycling in the range 30-180 W performed at 120 rev · min^{-1} for the group H (•) and for the group L (◦). Data presented as mean value ± SD at each power output, for n=6 subjects in both groups (H and L). A significantly lower blood bicarbonate concentration in the range of power outputs 90-180 W in the group H was found when compared to the group L (ANCOVA, $p<10^{-4}$).

Fig. 4. Panel A. The plasma lactate concentration / power output relationship during incremental cycling in the range 30-270 W performed at 60 rev · min^{-1} for the group H (•) and for the group L (◦). Data presented as mean value ± SD at each power output, for n=6 subjects in both groups (H and L). A significantly higher plasma lactate concentration in the range of power outputs 180-270 W in the group H was found when compared to the group L (ANCOVA, $p<10^{-4}$). **Panel B.** The plasma lactate concentration / power output relationship during incremental cycling in the range 30-180 W performed at 120 rev · min^{-1} for the group H (•) and for the group L (◦). Data presented as mean value ± SD at each power output, for n=6 subjects in both groups (H and L). A significantly higher plasma lactate concentration in the range of power outputs 90-180 W in the group H was found when compared to the group L (ANCOVA, $p<10^{-4}$).

Fig. 5. Panel A. The plasma ammonia concentration / power output relationship during incremental cycling in the range 30-270 W performed at 60 rev · min^{-1} for the group H (•) and for the group L (◦). Data presented as mean value ± SD at each power output, for n=6 subjects in both groups (H and L). A significantly higher plasma ammonia concentration in the range of power outputs 180-270 W in the group H was found when compared to the group L (ANCOVA, p=0.0007). **Panel B.** The plasma ammonia concentration / power output relationship during incremental cycling in the range 30-180 W performed at 120 rev · min^{-1} for the group H (•) and for the group L (◦). Data presented as mean value ± SD at each power output, for n=6 subjects in both groups (H and L). A significantly higher plasma ammonia concentration in the range of power outputs 90-180 W in the group H was found when compared to the group L (ANCOVA, p=0.001).