Journal of Sports Sciences, May 2008; 26(7): 701 – 707

Routledge
Taylor & Francis Group

ATP and phosphocreatine utilization in single human muscle fibres during the development of maximal power output at elevated muscle temperatures

STUART R. GRAY[1], KARIN SÖDERLUND[2], & RICHARD A. FERGUSON[1]

[1]*Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, Glasgow, UK and* [2]*The Swedish School of Sport and Health Sciences, Stockholm, Sweden*

(Accepted 12 October 2007)

Abstract

In this study, we examined the effect of muscle temperature (T_m) on adenosine triphosphate (ATP) and phosphocreatine utilization in single muscle fibres during the development of maximal power output in humans. Six male participants performed a 6-s maximal sprint on a friction-braked cycle ergometer under both normal ($T_m = 34.3^{\circ}C$, $s = 0.6$) and elevated ($T_m = 37.3^{\circ}C$, $s = 0.2$) muscle temperature conditions. During the elevated condition, muscle temperature of the legs was raised, passively, by hot water immersion followed by wrapping in electrically heated blankets. Muscle biopsies were taken from the vastus lateralis before and immediately after exercise. Freeze-dried single fibres were dissected, characterized according to myosin heavy chain composition, and analysed for ATP and phosphocreatine content. Single fibres were classified as: type I, IIA, IIAX25 (1 – 25% IIX isoform), IIAX50 (26 – 50% IIX), IIAX75 (51 – 75% IIX), or IIAX100 (76 – 100% IIX). Maximal power output and pedal rate were both greater ($P < 0.05$) during the elevated condition by 258 W ($s = 110$) and 22 rev · min^{-1} ($s = 6$), respectively. In both conditions, phosphocreatine content decreased significantly in all fibre types, with a greater decrease during the elevated condition in type IIA fibres ($P < 0.01$). Adenosine triphosphate content was also reduced to a greater ($P < 0.01$) extent in type IIA fibres during the elevated condition. The results of the present study indicate that after passive elevation of muscle temperature, there was a greater decrease in ATP and phosphocreatine content in type IIA fibres than in the normal trial, which contributed to the higher maximal power output.

Keywords: *Myosin heavy chain, energy metabolism, single fibres, cycling*

Introduction

Passive elevation of muscle temperature (T_m) leads to a greater power output in humans (Asmussen & Boje, 1945; Asmussen, Bonde-Petersen, & Jorgensen, 1976; De Ruiter & de Haan, 2000; Sargeant, 1987). We have recently demonstrated this is associated with a greater rate of skeletal muscle adenosine triphosphate (ATP) turnover, primarily through an increased rate of phosphocreatine utilization (Gray, De Vito, Nimmo, Farina, & Ferguson, 2006). Since all muscle fibre types are metabolically active during short-term maximal exercise (Karatzaferi, de Haan, van Mechelen, & Sargeant, 2001b), consideration must be given to the relative contribution of individual muscle fibres, which have diverse contractile and metabolic properties, to the generation of power output. To gain further insight into this area it is necessary to investigate the metabolic response of these differing fibre types through single fibre analysis. Phosphocreatine splits immediately on muscle contraction in amounts energetically sufficient to account for the external work performed (Infante, Klaupiks, & Davies, 1965). Consequently, methods to measure ATP and phosphocreatine concentrations in single fibre fragments have previously been developed (Wibom, Söderlund, Lundin, & Hultman, 1991) and used to provide insight into the metabolic responses of single fibres to various modes of exercise (Conjard & Pette, 1999; Karatzaferi, de Haan, Ferguson, van Mechelen, & Sargeant, 2001a; Karatzaferi *et al.*, 2001b; Sahlin, Söderlund, Tonkonogi, & Hirakoba, 1997; Söderlund & Hultman, 1991). However, most of

Correspondence: R. A. Ferguson, School of Sport and Exercise Sciences, Loughborough University, Loughborough, Leicestershire, LE11 3TU, UK.
E-mail: r.ferguson@lboro.ac.uk

ISSN 0264-0414 print/ISSN 1466-447X online © 2008 Taylor & Francis
DOI: 10.1080/02640410701744438

these studies have been limited to the characterization of the two discrete, type I and type II fibre populations.

Skeletal muscle fibres can be characterized on the basis of their myosin heavy chain (MHC) isoform expression and it is now known that human muscle fibres may express two isoforms of MHC in varying amounts, leading to a continuum of fibre types and, as such, contractile and metabolic properties (Larsson & Moss, 1993; Schiaffino & Reggiani, 1996). Each fibre type has an individual force–velocity and power–velocity relationship dependent on its MHC expression, both of which are influenced by temperature (Bottinelli, Canepari, Pellegrino, & Reggiani, 1996; He, Bottinelli, Pellegrino, Ferenczi, & Reggiani, 2000) as a result of altered myofibrillar adenosine triphosphatase (mATPase) activity (Steinen, Kiers, Bottinelli, & Reggiani, 1996). During *in vivo* exercise in humans, it has been hypothesized that the greater power output generated at higher muscle temperatures, at pedal rates of $60-140\ \text{rev}\cdot\text{min}^{-1}$, is correlated with the percentage of type I fibres, suggesting an elevated power output of these fibres at higher temperatures (Sargeant & Rademaker, 1996). However, we have recently demonstrated that as pedal rate is increased to around $160-180\ \text{rev}\cdot\text{min}^{-1}$, participants with a greater proportion of MHC IIA appear to show greater increases in power output (Gray *et al.*, 2006). Both of these studies (Gray *et al.*, 2006; Sargeant & Rademaker, 1996) based their conclusions of a fibre type dependent effect of temperature on power output on correlations between temperature coefficients (Q_{10}) for power output and the relative proportions of fibre types, and not on direct measurement of single fibre metabolism.

The aim of the present study was to determine whether the greater power output observed at higher muscle temperatures is associated with a greater decrease in ATP and/or phosphocrteatine content in single muscle fibres of a specific type. We hypothesized that after elevation of muscle temperature, there would be a greater decrease in ATP and phosphocreatine content in fibres with a predominance of MHC IIA. This would indicate there is a greater metabolic rate in these fibres, suggesting a greater rate of cross-bridge cycling and contribution to the increased power output.

Methods

Participants

Six healthy males (mean age 25 years, $s=6$; height 1.82 m, $s=0.07$; body mass 77 kg, $s=11$), with no history of muscle or metabolic disorders, volunteered for the study. All participants were physically active but none were specifically trained. The participants were fully informed of the purposes, risks, and discomfort associated with the experiment before providing written, informed consent. This study conformed to current local guidelines and the Declaration of Helsinki and was approved by the local ethics committee.

Experimental protocol

Before the experimental trials began, participants were fully habituated to the exercise protocol on a minimum of three occasions. In the experimental sessions, participants performed a 6-s maximal sprint, on a friction braked cycle ergometer (Ergomedic 824E, Monark, Varberg, Sweden), under both normal and elevated temperature conditions, the order of which was counterbalanced and separated by at least 14 days. During the elevated temperature condition, muscle temperature was raised first by immersion of the legs, up to the gluteal fold, for 30 min in hot water (42.8°C, $s=1.4$). Participants then towelled dry, changed shorts, and rested on an examination couch. The biopsy site was then prepared and a flexible muscle temperature probe (Ellab, Copenhagen, Denmark) was inserted through a flexible venflon cannula (18 G) into the medial portion of the vastus lateralis to a depth of approximately 3 cm. The legs were then wrapped in electrically heated blankets until muscle temperature reached approximately 37–37.5°C. In the normal condition, participants rested at room temperature (resting muscle temperature of approximately ~34°C) with the same preparation to the leg as during the elevated condition. Rectal temperature was monitored throughout both trials, via a rectal thermistor probe (Grants Instruments Ltd., UK).

In both trials, after the appropriate muscle temperature was reached, a resting muscle sample was obtained. Participants then performed the sprint and a post-exercise biopsy was taken immediately (<10 s) after the cessation of exercise, with the participants remaining seated on the bike. The sprint exercise was performed on the cycle ergometer with the load set at 7.5% of the participants' body mass in both conditions and initiated from a stationary start. Power output was calculated every second from the known frictional load and the measurement of flywheel velocity and corrected for the acceleration of the flywheel (Lakomy, 1986). Flywheel velocity was monitored with a small DC generator, the output of which was fed into a computer (BBC Micro, Acorn Systems, UK) via an analog-to-digital converter at a sampling rate of 100 Hz.

Muscle sample collection and single fibre analyses

Muscle samples were taken from separate incisions, approximately 1 cm apart, made in the medial part of the vastus lateralis under local anaesthesia (1% xylocaine), using the needle biopsy technique (Bergstrom, 1962). Samples were immediately frozen (< 10 s) in liquid nitrogen and stored at −80°C for subsequent analysis. Contralateral legs were used for sampling in the normal and elevated conditions, the order of which was counterbalanced.

Muscle samples were freeze-dried and approximately 50 fragments of single muscle fibres (3–4 mm in length), from each muscle sample, were manually dissected under a low-power light microscope (Leica Microsystems, Wetzlar, Germany). Each fibre was then cut approximately in half and the first fragment used for determination of MHC content and the second fragment for measurement of ATP and phosphocreatine content.

Myosin heavy chain content was determined by SDS-PAGE using a method derived from Fauteck and Kandarian (1995). For approximately each millimetre of fibre fragment, 10 μl of SDS buffer was added. Electrophoresis (Bio-Rad Mini-Protean 3) was then carried out on 6% (crosslinking 2.7%) polyacrylamide resolving gels with 4% (crosslinking 2.7%) stacking gels, which were electrophoresed at ~8°C for 18 h at a constant 100 V. Protein bands were visualized by silver staining, using a method modified from Oakley and colleagues (Oakley, Kirsch, & Morris, 1980), and quantified by densitometry (Bio-Rad GS8000 calibrated densitometer). Fibres were classified into one of six groups: type I, IIA, and one of four hybrid fibre groups, namely IIAX25 (1–25% MHC IIX), IIAX50 (26–50% MHC IIX), IIAX75 (51–75% MHC IIX), and IIAX100 (76–100% MHC IIX).

The second fibre fragment was weighed on a quartz fibre fish-pole balance (Lowry & Passonneau, 1972) calibrated by the spectrophotometric determination of weighed *p*-nitrophenol crystals. Fibre fragments, weighing 1–5 μg, were then analysed luminometrically for ATP and phosphocreatine content using the luciferase method described by Wibom *et al*. (1991). Briefly, each fibre was extracted in 200 μl of trichloroacetic acid (2.5%) followed by neutralization in 20 μl 2.2 M $KHCO_3$. Fifty microlitres of the extract was then added to a sucrose buffer containing D-luciferin and the assay carried out on a luminometer (1251, Bio Orbit Oy, Turku, Finland). The coefficient of variation (CV) for duplicate determination of ATP and phosphocreatine content in the same fibre extracts ($n = 38$) was 6.1 and 4.1% respectively.

Statistical analysis

Mean and peak pedal rate, power data, and rectal and muscle temperatures were compared between conditions using Student's *t*-tests. Within each fibre type, the effects of time and muscle temperature on ATP and phosphocreatine content were analysed by a two-way (temperature and time) analysis of variance (ANOVA) with repeated measures. Where a significant effect was detected, differences were located with *post-hoc* paired *t*-tests with Bonferroni correction. At each time point, both ATP and phosphocreatine content were compared between fibre types using Student's *t*-tests. Statistical significance was accepted at $P < 0.05$ and adjusted to $P < 0.01$ for analyses involving the Bonferroni correction. Data are presented as means and standard deviations (*s*).

Results

Temperature

Muscle temperature was higher ($P < 0.05$) in the elevated (37.3°C, $s = 0.2$) compared to the normal condition (34.3°C, $s = 0.6$). Rectal temperature was 0.1°C higher ($P < 0.05$) in the elevated (37.1°C, $s = 0.2$) compared to the normal condition (37.2°C, $s = 0.2$).

Power output and pedal rate

Maximal pedal rate during the sprint was higher ($P < 0.05$) in the elevated (179 rev·min^{-1}, $s = 30$) compared to the normal condition (157 rev·min^{-1}, $s = 24$) (Figure 1A). Consequently, maximal power output during the sprint was greater ($P < 0.05$) in the elevated (1427 W, $s = 493$) compared to the normal condition (1169 W, $s = 398$) (Figure 1B). Mean pedal rate during the sprint was higher ($P < 0.05$) in the elevated (141 rev·min^{-1}, $s = 30$) compared to the normal condition (122 rev·min^{-1}, $s = 24$). Consequently, mean power output during the sprint was greater ($P < 0.05$) in the elevated (971 W, $s = 247$) compared to the normal condition (850 W, $s = 230$).

Single fibre phosphocreatine and ATP content

Rest. In the normal condition, both ATP and phosphocreatine content were lower ($P < 0.05$) in type I fibres than in the five type II fibre groups (Tables I and II). Phosphocreatine and ATP content were the same within these type II fibre groups. In the elevated condition, phosphocreatine content was also lower in type I than in type II fibres (Table II). However, ATP content in the elevated condition was

the same in type I and II fibres (Table I). In each fibre type, there were no differences ($P > 0.05$) in resting ATP and phosphocreatine content between conditions.

Post-exercise ATP. Two-way analysis of variance revealed that there was an effect of time ($P < 0.05$) on the ATP content of the type IIA, IIAX50, IIAX75, and IIAX100 fibre groups. *Post-hoc* analysis identified that ATP content decreased ($P < 0.01$) with exercise in each of these fibre groups in the normal condition, but only in the type IIA and IIAX50 fibres in the elevated condition. Within the type IIA fibre group, analysis of variance revealed a significant effect ($P < 0.05$) of temperature on ATP content, with *post-hoc* paired *t*-tests revealing a greater decrease ($P < 0.01$) after the elevation in muscle temperature. There was a decrease of 3.1 mmol · kg^{-1} (dm) (13%) in the elevated condition compared to a decrease of 1.6 mmol · kg^{-1} (dm) (6%) in the normal condition.

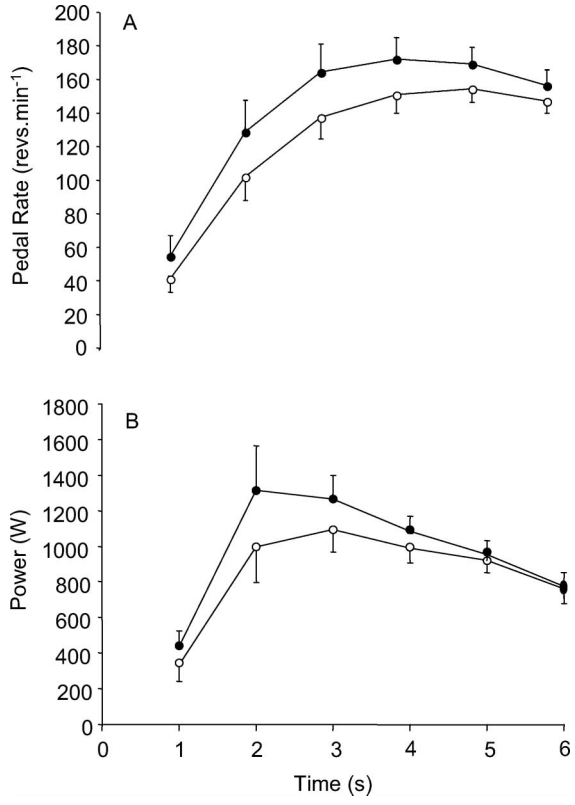

Figure 1. Pedal rate (A) and power output (B) during 6-s maximal sprint exercise under normal (open circles) and elevated (solid circles) muscle temperature conditions. Values are mean ± standard deviation; $n = 6$.

Post-exercise PCr. In all fibre groups, analysis of variance demonstrated that there was an effect ($P < 0.05$) of time on phosphocreatine content, with *post-hoc t*-tests showing that it decreased ($P < 0.01$) in all fibre groups in both conditions. Analysis of variance also showed an effect ($P < 0.05$) of temperature in both the IIA and IIAX25 fibre groups, with post-hoc *t-tests* showing that in the type IIA fibre group the decrease in phosphocreatine with exercise was greater ($P < 0.01$) in the normal (decrease of 57.1 mmol · kg^{-1} (dm), 71.2%) than in the elevated condition (48.7 mmol · kg^{-1} (dm), 59.3%). There was only a tendency for a greater decrease in the IIAX25 fibres ($P = 0.02$, not significant due to Bonferroni correction) after the elevation in muscle temperature.

Discussion

The present study has demonstrated that together with the increase in maximal power output, a passive elevation of muscle temperature resulted in a greater decrease in both ATP and phosphocreatine content in type IIA muscle fibres, with no differences among type I or IIAX hybrid fibres.

Resting phosphocreatine content was ~20% higher in type II fibre groups than in type I fibres, which is in line with several previous studies (Sahlin *et al.*, 1997; Söderlund, Greenhaff, & Hultman, 1992;

Table I. Adenosine triphosphate content in MHC-characterized single muscle fibres before and after a 6-s maximal sprint under normal and elevated muscle temperature conditions (values are mmol · kg^{-1} (dm)).

	Normal				Elevated			
Fibre type	Rest	(*n*)	Post-exercise	(*n*)	Rest	(*n*)	Post-exercise	(*n*)
I	22.7 ± 2.8	(42)	23.7 ± 3.3	(48)	24.6 ± 4.1	(30)	22.9 ± 2.9	(68)
IIA	24.8 ± 3.7[a]	(78)	23.2 ± 4.3[b]	(84)	23.9 ± 3.9	(59)	20.8 ± 4.1[b,c]	(86)
IIAX25	25.1 ± 3.7[a]	(26)	22.6 ± 4.5	(24)	23.1 ± 2.6	(9)	22.8 ± 4.8	(26)
IIAX50	25.8 ± 3.0[a]	(18)	20.4 ± 4.4[b]	(42)	23.5 ± 4.4	(32)	22.3 ± 3.7[b]	(15)
IIAX75	25.8 ± 4.7[a]	(18)	19.8 ± 2.7[b]	(8)	22.7 ± 3.1	(8)	19.8 ± 1.4	(8)
IIAX100	26.0 ± 2.6[a]	(3)	17.4 ± 4.7[b]	(12)	26.3 ± 4.6	(9)	19.4 ± 4.8	(5)

Note: Values are expressed as mean ± standard deviation with the number of fibres analysed represented by (*n*). [a]Significant difference vs. type I fibres ($P < 0.05$). [b]Significant difference vs. rest ($P < 0.01$). [c]Significant difference vs. normal condition at the same time point ($P < 0.01$).

Table II. Phosphocreatine content in MHC-characterized single muscle fibres before and after a 6-s maximal sprint under normal and elevated muscle temperature conditions (values are $mmol \cdot kg^{-1}$ (dm)).

	Normal				Elevated			
Fibre type	Rest	(*n*)	Post-exercise	(*n*)	Fibre type		Rest	(*n*)
I	67.6 ± 12.1	(42)	34.1 ± 10.0^{b}	(48)	72.5 ± 16.8	(30)	30.7 ± 6.5^{b}	(68)
IIA	82.2 ± 14.5^{a}	(78)	33.5 ± 14.1^{b}	(84)	80.3 ± 14.4^{a}	(59)	$23.1 \pm 9.3^{b,c}$	(86)
IIAX25	87.6 ± 18.0^{a}	(26)	35.2 ± 14.2^{b}	(24)	88.5 ± 15.8^{a}	(9)	23.5 ± 12.4^{b}	(26)
IIAX50	86.0 ± 18.1^{a}	(24)	31.4 ± 12.8^{b}	(42)	84.1 ± 18.4^{a}	(32)	28.4 ± 14.5^{b}	(15)
IIAX75	84.3 ± 20.5^{a}	(18)	32.6 ± 8.5^{b}	(8)	83.2 ± 15.4^{a}	(8)	24.3 ± 4.0^{b}	(8)
IIAX100	93.1 ± 9.3^{a}	(3)	30.6 ± 15.0^{b}	(12)	82.7 ± 9.1^{a}	(9)	19.5 ± 13.4^{b}	(5)

Note: Values are expressed as mean $\pm$ standard deviation with the number of fibres analysed represented by (*n*). [a]Significant difference vs. type I fibres ($P < 0.05$). [b]Significant difference vs. rest ($P < 0.01$). [c]Significant difference vs. normal condition at the same time point ($P < 0.01$).

Söderlund & Hultman, 1991; Tesch, Thorsson, & Fujitsuka, 1989). However, within the type II hybrid groups phosphocreatine content was the same, which contrasts with previous observations of a progressive increase in phosphocreatine content from type IIA through to IIX fibres (Sant'ana Pereira, Sargeant, Rademaker, de Haan, & van Mechelen, 1996). There was a 10% greater resting ATP content in type II fibre groups than in type I fibres in the normal condition. This contrasts with previous work in which similar resting ATP content in type I and II fibres was reported (Greenhaff, Söderlund, Ren, & Hultman, 1993; Greenhaff *et al.*, 1994; Karatzaferi *et al.*, 2001b). In the elevated condition in the present study, we also found a similar ATP content in type I and II fibres, even though the resting concentrations of ATP were not different after the rise in muscle temperature. Furthermore, as previously observed, there was no difference among the resting ATP contents of the type II hybrid fibres in both conditions (Karatzaferi *et al.*, 2001b; Sant'ana Pereira *et al.*, 1996). Although the results of the present study are in line with those of several previous studies, the discrepancies highlight the natural variation in resting ATP and phosphocreatine content observed through single fibre analyses.

Six seconds of maximal sprint exercise (in the normal condition) resulted in large reductions in phosphocreatine content (from 49% in type I fibres to 67% in IIAX100, respectively). This supports previous reports of a greater metabolic rate in type II fibres, and in particular those with a high proportion of the MHC IIX isoform, during short-term high-intensity exercise (Karatzaferi *et al.*, 2001b). The post-exercise ATP content was lower in the type II fibres, except the IIX fibres (Table I), compared with the type I fibres. This pattern and magnitude of the decline in ATP is consistent with that observed during a 10-s maximal sprint (Karatzaferi *et al.*, 2001b).

The effect of passively elevating muscle temperature was to increase ATP and phosphocreatine utilization in fibres expressing predominantly MHC IIA during the maximal sprint exercise, when power had increased. The greater power output when temperature was elevated is possibly due to a greater cross-bridge cycling between actin and myosin. Indeed, Karatzaferi *et al.* (2004) have clearly demonstrated the temperature dependency of cross-bridge cycling in relation to the force produced during the power stroke of the cycle, with a greater force being produced at higher temperatures. Moreover, mATPase activity has also been shown to increase at higher temperatures (Steinen *et al.*, 1996), which will also increase the rate of cross-bridge cycling, the velocity of shortening and, consequently, the power production of the muscle fibres. This will lead to a greater metabolic cost that is met by the elevated phosphocreatine hydrolysis, which itself may be achieved by an increased activity of creatine kinase, which approaches its optimum temperature at approximately 42°C (Wyss, Schlegel, James, Eppenberger, & Wallimann, 1990). Furthermore, previous work has demonstrated that phosphocreatine utilization in single fibres can account for the external work performed by the fibre (Infante *et al.*, 1965), making it likely that this increase in phosphocreatine hydrolysis in IIA fibres can account for the greater power output under these conditions.

Previous work has shown that passive elevation of muscle temperature also leads to an increase in anaerobic ATP turnover when force (Edwards *et al.*, 1972) and power output (Febbraio, Carey, Snow, Stathis, & Hargreaves, 1996) of the muscle remains constant between conditions. It is therefore difficult to ascertain whether the greater rate of anaerobic ATP turnover (Gray *et al.*, 2006) and the greater ATP and phosphocreatine utilization in type IIA fibres observed in the present study, at a higher muscle temperature, is due to the higher muscle temperature *per se* or a consequence of the greater work performed. In the present study, however, if the greater ATP and phosphocreatine utilization were

simply a consequence of the greater power output, and not temperature *per se*, then one would expect to observe greater ATP and phosphocreatine utilization in all fibre types and not specifically in the type IIA fibres. As the main purpose of the present study was to establish the mechanism behind the increase in maximal power output of skeletal muscle during *in vivo* exercise at higher muscle temperature, we have demonstrated that this increase in power is associated with an increased metabolism of type IIA fibres.

The greater metabolic rate in type IIA fibres may be explained by considering the individual power–velocity relationships of different fibre types. In the present study, participants reached pedal rates of 160–180 rev·min^{-1} within the first 3 s of starting the sprint. These pedal rates are close to the estimated maximal velocity (v_{max}) of ~165 rev·min^{-1} for type I fibres (Sargeant, 1994) and just beyond the estimated optimum velocity (v_{opt}) for MHC IIA fibres of ~130 rev·min^{-1} [estimated through a v_{max} ratio of 2.3 for type I:IIA fibres (Bottinelli *et al.*, 1996) and a v_{opt}/v_{max} ratio of 1/3 (Bottinelli *et al.*, 1996; He *et al.*, 2000)]. Any increase in temperature at around these pedal rates, while having an influence on cross-bridge cycling of type I fibres, will have little functional impact on the power-producing capability of these fibres at such high velocities, since v_{max} is almost exceeded. However, at these pedal rates type IIA fibres will be working on the descending right slope of the power–velocity relationship, where the temperature shift will have its greatest effect on power production. Muscle fibres containing the MHC IIX will still be affected by temperature, by increasing the rate of cross-bridge cycling and shifting the power–velocity relationship to the right. However, the functional impact of this will be limited, as there are so few predominantly MHC IIX fibres. From these estimations, therefore, it becomes clear why at the relatively high velocities reached in the present study we observed an increased metabolic rate in MHC IIA fibres which results in the increase in power output at higher temperatures, rather than type I fibres as suggested by Sargeant and Rademaker (1996) in which exercise was performed at the lower pedal rates of between 60 and 140 rev·min^{-1}.

It has previously been reported that it is extremely difficult to reliably weigh freeze-dried single muscle fibres, hence some researchers use the phosphocreatine/creatine ratio (Beltman, Sargeant, Haan, van Mechelen, & de Haan, 2004), where fibres do not have to be weighed. The methods employed in the present study, however, through an accurate calibration of the balance, gave relatively low coefficients of variations of 6.1 and 4.1% and thus accurate measures of ATP and phosphocreatine content, respectively. It has also proved useful to investigate hybrid fibres in greater depth in the present study; however, because we were limited by the number of hybrid fibres found in each sample, to differentiate more hybrid groups would have been problematic. This also led, unavoidably, to differences in the number of fibres of each type analysed, especially within these hybrid fibres. Nevertheless, the present results support the need to recognize the metabolic differences along the continuum of metabolically diverse fibre types, especially within type II hybrid fibres.

In conclusion, we have demonstrated that together with a greater power output when muscle temperature is elevated there is a greater decrease in ATP and phosphocreatine content in type IIA fibres, but not in type I or IIAX hybrid fibres, after passive elevation of muscle temperature.

Acknowledgement

This work was supported by a Royal Society Research Grant.

References

Asmussen, E., & Boje, O. (1945). Body temperature and the capacity for work. *Acta Physiologica Scandinavica*, *10*, 1–22.

Asmussen, E., Bonde-Petersen, F., & Jorgensen, K. (1976). Mechano-elastic properties of human muscles at different temperatures. *Acta Physiologica Scandinavica*, *96*, 83–93.

Beltman, J. G., Sargeant, A. J., Haan, H., van Mechelen, W., & de Haan, A. (2004). Changes in PCr/Cr ratio on single characterized muscle fibre fragments after only a few maximal voluntary contractions in humans. *Acta Physiologica Scandinavica*, *180*, 187–193.

Bergstrom, J. (1962). Muscle electrolytes in man. *Scandinavian Journal of Clinical Laboratory Investigation Supplement*, *68*, 1–101.

Bottinelli, R., Canepari, M., Pellegrino, M. A., & Reggiani, C. (1996). Force–velocity properties of human skeletal muscle fibres: Myosin heavy chain isoform and temperature dependence. *Journal of Physiology*, *495*, 573–586.

Conjard, A., & Pette, D. (1999). Phosphocreatine as a marker of contractile activity in single muscle fibres. *Pflugers Archive*, *438*, 278–282.

De Ruiter, C. J., & de Haan, A. (2000). Temperature effect on the force/velocity relationship of the fresh and fatigued human adductor pollicis muscle. *Pflugers Archive*, *440*, 163–170.

Edwards, R. H. T., Harris, R. C., Hultman, E., Kaijser, L., Koh, D., & Nordesjo, L.-O. (1972). Effect of temperature on muscle energy metabolism and endurance during successive isometric contractions, sustained to fatigue, of the quadriceps muscle in man. *Journal of Physiology*, *220*, 335–352.

Fauteck, S. P., & Kandarian, S. C. (1995). Sensitive detection of myosin heavy chain composition in skeletal muscle under different loading conditions. *American Journal of Physiology*, *268*, C419–C424.

Febbraio, M. A., Carey, M. F., Snow, R. J., Stathis, C. G., & Hargreaves, M. (1996). Influence of elevated muscle temperature on metabolism during intense, dynamic exercise. *American Journal of Physiology*, *271*, 1251–1255.

Gray, S. R., De Vito, G., Nimmo, M. A., Farina, D., & Ferguson, R. A. (2006). Skeletal muscle ATP turnover and muscle fiber conduction velocity are elevated at higher muscle temperatures during maximal power output development in humans. *American Journal of Physiology*, *290*, R376 – R382.

Greenhaff, P. L., Nevill, A. M., Söderlund, K., Bodin, K., Boobis, L. H., Williams, C. *et al*. (1994). The metabolic responses of human type I and II muscle fibres during maximal treadmill sprinting. *Journal of Physiology*, *478*, 149 – 155.

Greenhaff, P. L., Söderlund, K., Ren, J. M., & Hultman, E. (1993). Energy metabolism in single human muscle fibres during intermittent contraction with occluded circulation. *Journal of Physiology*, *460*, 443 – 453.

He, Z.-H., Bottinelli, R., Pellegrino, M. A., Ferenczi, M. A., & Reggiani, C. (2000). ATP consumption and efficiency of human single muscle fibres with different myosin isoform composition. *Biophysical Journal*, *79*, 945 – 961.

Infante, A. A., Klaupiks, D., & Davies, R. E. (1965). Phosphorylcreatine consumption during single-working contractions of isolated muscle. *Biochimica Biophysica Acta*, *94*, 504 – 515.

Karatzaferi, C., Chinn, M. K., & Cooke, R. (2004). The force exerted by a muscle cross-bridge depends directly on the strength of the actomyosin bond. *Biophysical Journal*, *87*, 2532 – 2544.

Karatzaferi, C., de Haan, A., Ferguson, R. A., van Mechelen, W., & Sargeant, A. J. (2001a). Phosphocreatine and ATP content in human single muscle fibres before and after maximum dynamic exercise. *Pflugers Archive*, *442*, 467 – 474.

Karatzaferi, C., de Haan, A., van Mechelen, W., & Sargeant, A. J. (2001b). Metabolic changes in single human muscle fibres during brief maximal exercise. *Experimental Physiology*, *86*, 411 – 415.

Lakomy, H. K. A. (1986). Measurement of work and power output using friction-loaded cycle ergometers. *Ergonomics*, *29*, 509 – 517.

Larsson, L., & Moss, R. L. (1993). Maximum velocity of shortening in relation to myosin isoform composition in single fibres from human skeletal muscles. *Journal of Physiology*, *472*, 595 – 614.

Lowry, O. H., & Passonneau, J. V. (1972). *A flexible system of enzymatic analysis.* New York: Academic Press.

Oakley, B. R., Kirsch, D. R., & Morris, N. R. (1980). A simplified ultrasensitive silver stain for detecting proteins in polyacrylamide gels. *Analytical Biochemistry*, *105*, 361 – 363.

Sahlin, K., Söderlund, K., Tonkonogi, M., & Hirakoba, K. (1997). Phosphocreatine content in single fibers of human muscle after sustained submaximal exercise. *American Journal of Physiology*, *273*, C172 – C178.

Sant'ana Pereira, J. A., Sargeant, A. J., Rademaker, A. C., de Haan, A., & van Mechelen, W. (1996). Myosin heavy chain isoform expression and high energy phosphate content in human muscle fibres at rest and post-exercise. *Journal of Physiology*, *496*, 583 – 588.

Sargeant, A. J. (1987). Effect of muscle temperature on leg extension force and short-term power output in humans. *European Journal of Applied Physiology*, *56*, 693 – 698.

Sargeant, A. J. (1994). Human power output and muscle fatigue. *International Journal of Sports Medicine*, *15*, 116 – 121.

Sargeant, A. J., & Rademaker, A. (1996). Human muscle power in the locomotory range of contraction velocities increases with temperature due to an increase in power generated by type I fibres. *Journal of Physiology*, *491*, 128P.

Schiaffino, S., & Reggiani, C. (1996). Molecular diversity of myofibrillar proteins: Gene regulation and functional significance. *Physiological Reviews*, *76*, 371 – 423.

Söderlund, K., Greenhaff, P. L., & Hultman, E. (1992). Energy metabolism in type I and type II human muscle fibres during short term electrical stimulation at different frequencies. *Acta Physiologica Scandinavica*, *144*, 15 – 22.

Söderlund, K., & Hultman, E. (1991). ATP and phosphocreatine changes in single human muscle fibres after intense electrical stimulation. *American Journal of Physiology*, *261*, E737 – E741.

Steinen, G. J. M., Kiers, J. L., Bottinelli, R., & Reggiani, C. (1996). Myofibrillar ATPase activity in skinned human skeletal muscle fibres: Fibre types and temperature dependence. *Journal of Physiology*, *493*, 299 – 307.

Tesch, P. A., Thorsson, A., & Fujitsuka, N. (1989). Creatine phosphate in fiber types of skeletal muscle before and after exhaustive exercise. *Journal of Applied Physiology*, *66*, 1756 – 1759.

Wibom, R., Söderlund, K., Lundin, A., & Hultman, E. (1991). A luminometric method for the determination of ATP and phosphocreatine in single human skeletal muscle fibres. *Journal of Bioluminescence and Chemiluminescence*, *6*, 123 – 129.

Wyss, M., Schlegel, J., James, P., Eppenberger, H. M., & Wallimann, T. (1990). Mitochondrial creatine kinase from chicken brain: Purification, biophysical characterization, and generation of heterodimeric and heterooctameric molecules with subunits of other creatine kinase isoenzymes. *Journal of Biological Chemistry*, *265*, 15900 – 15908.