TEMPERATURE AND *IN VIVO* HUMAN SKELETAL MUSCLE FUNCTION AND METABOLISM

By

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DECLARATION

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Published papers:

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Gray, S. R., Söderlund, K., & Ferguson, R. A. (2006). Greater PCr utilisation in single human type IIA muscle fibres during the development of maximal power output at higher muscle temperatures. *Proc Physiol Soc* **3**, C40.

ABSTRACT

Increasing the temperature of the exercising muscle, passively or actively, leads to alterations in the contractile properties of the muscle, importantly an increase in power output. There is limited information, however, regarding the metabolic changes, if any, occurring within the muscle at higher temperatures and how these are related to the contractile changes occurring within the muscle and how such changes may, or may not, affect the efficiency of the working muscles.

The greater power output produced during maximal sprint cycling, after a passive increase in T_m, was associated with an increase in the rate of anaerobic ATP turnover and muscle fibre conduction velocity. Further investigation revealed that this greater anaerobic ATP turnover within the muscle was the result of a greater activity of type IIA fibres in the cadence range of 160-180 revs.min^{-1}.

When the external power output of the muscle remains constant during more prolonged cycling exercise, performed at 60 revs.min^{-1}, there was also a greater rate of anaerobic ATP turnover in the first 2 min of exercise, with no differences in the remainder of exercise after passive elevation of T_m. There were no differences in the aerobic energy contribution or the kinetics of the $\dot{V}O_2$ response between T_m conditions. These changes led to a decrease in mechanical efficiency in the first 2 min of exercise, which was associated with a tendency for a greater PCr degradation in type I fibres. When T_m was elevated via prior intense exercise there was decrease in mechanical efficiency, during 6 min of heavy exercise, at both 60 and 120 revs.min^{-1}. There was also a greater "absolute" primary amplitude and decrease in the slow component after prior exercise, with the response being greater at 120 revs.min^{-1}.

The present research has demonstrated that whilst an increase in T_m leads to a greater power output, during maximal exercise, mechanical efficiency is reduced as exercise progresses beyond a few seconds. Furthermore, at faster pedal rates T_m affects type IIA fibres whilst at slower pedal rates (60 revs.min^{-1}) there appears to be a preferential effect on type I fibres, highlighting the velocity specific effect of T_m.

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ABBREVIATIONS

[ ]	concentration
a/Po	curvature of the force-velocity relationship
ADP	adenosine 5'-diphosphate
ADP.Na.2H_2O	adenosine 5'-diphosphate sodium dihydrate
AMP	adenosine 5'-monophosphate
APS	ammonium persulfate
ATP	adenosine 5'-triphosphate
BV	blood volume
Ca^{2+}	calcium ion
cAMP	cyclic AMP
cc	cubic centimetre
CK	creatine kinase
CO_2	carbon dioxide
CPK	creatine phosphokinase
Cr	creatine
CV	coefficient of variation
d.m.	dry mass
dB	decibel
DTT	Dithiothreitol
EDTA	Ethylenediaminetetraacetic acid
EDTA.Na.2H_2O	Ethylenediaminetetraacetic acid sodium dihydrate
$EE\dot{V}O_2$	end exercise pulmonary oxygen uptake
EMG	electromyography

$FADH_2$	flavin adenine nucleotide (reduced form)
G	gauge
g/dl	grams per decilitre
g/l	grams per litre
G6PDH	glucose 6 phosphate dehydrogenase
H^+	hydrogen ion (proton)
H_2O	water
Hb	haemoglobin
HCl	hydrochloric acid
HK	hexokinase
HR	heart rate
Hz	hertz
K^+	potassium
K^+EDTA	potassium Ethylenediaminetetraacetic acid
kcal	kilocalorie
kD	kilodalton
$KHCO_3$	potassium bicarbonate
kJ	kilojoule
KOH	potassium hydroxide
$l.s^{-1}$	litres per second
LDH	lactate dehydrogenase
LT	lactate threshold
mAMP	milliampere
MFCV	muscle fibre conduction velocity
$Mg(Ac)_2.4H_2O$	magnesium acetate tetrahydrate

MHC	myosin heavy chain
min	minute
ml	millilitre
MLC	myosin light chain
mmHg	millimetre of mercury
$mmol^{-1}$	millimole per litre
MRT	mean response time
NAD^+	nicotinamide adenine dinucleotide (oxidized form)
NADH	nicotinamide adenine dinucleotide (reduced form)
$NADP.Na_2.4H_2O$	nicotinamide adenine dinucleotide phosphate disodium
$\dot{V}O_2$	rate of pulmonary oxygen uptake
O_2	oxygen
O_2def	oxygen deficit
$\dot{V}O_{2peak}$	peak rate of pulmonary oxygen uptake
PAS	Periodic Acid Schiff
PCA	perchloric acid
PCr	phosphocreatine
PDH	pyruvate dehydrogenase
Pi	inorganic phosphate
P_o	isometric force
PPi	pyrophosphate
PV	plasma volume
Q_{10}	temperature co-efficient
Q_M	blood flow
RCV	red cell volume

RER	respiratory exchange ratio
$revs.min^{-1}$	revolutions per minute
RPE	rating of perceived exertion
s	seconds
SDS	sodium dodecyl sulphate
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
TCA	trichloroacetic acid
TD	time delay
TEA	Tetraethylammonium chloride
TEMED	*N,N,N',N'*-Tetramethylethylenediamine Tetrahydrate
T_m	muscle temperature
T_{rec}	rectal temperature
U/mg	units per milligram
V	volts
(v/v)	volume/volume percent
V_{max}	maximal shortening velocity
V_{opt}	optimal velocity
(w/v)	weight/volume percent
W	watts
W_{max}	Maximum power
τ	time constant (time to reach 63 % of primary $\dot{V}O_2$)

Chapter 1

INTRODUCTION

1.1 INTRODUCTION

The ability of human skeletal muscle to generate and maintain power is of great importance, both in the world of sport and exercise and also in everyday life. When considering the generation of power by skeletal muscle there are several factors that need to be considered. Firstly, the mechanical and biochemical mechanisms leading to muscular contraction, including the velocity of shortening, will exert an influence on both the level of force and power produced by the muscle. The energy turnover required for muscular contraction will also play an important role with respect to the generation of force and power and hence both these areas will be a main focus of this thesis. Moreover, the conversion of chemical into mechanical energy during muscular contraction has the consequence of heat production, which is dissipated around the muscle and leads to a rise in muscle temperature (T_m). Such a rise in T_m has long been known to have an important effect on muscle contraction and so will also be a major focus of this thesis.

Prior to the majority of athletic events a warm up, of some description, will be carried out by the participant in the hope of an enhanced performance of the event, whether a 100 m sprint at the Olympics or a 52 km time trial in the Tour de France. During exercise itself the temperature of the working muscle will increase well above resting levels to approximately 40 °C (Robinson *et al.*, 1965;Saltin *et al.*, 1968), highlighting the need for research into the effect of such an increase in T_m on the contractile performance of skeletal muscle. The beneficial effects of warm up were first shown by the work of Asmussen and Boje (1945) who investigated the effects of both active and passive warming of body temperature (rectal temperature (T_{rec}) and T_m) on the performance of cycling exercise. These authors found that muscular performance was

improved to a similar extent by both active and passive warming and therefore concluded that the enhancement of performance was achieved mainly through the increase in temperature and not through any other effects associated with the prior exercise.

Since the work of Asmussen and Boje there has been a great deal of research carried out during exercise of various durations and modes which has generally supported their findings (e.g. Asmussen *et al.*, 1976;Binkhorst *et al.*, 1977;Bergh & Ekblom, 1979;Davies & Young, 1983;Sargeant, 1987;Stewart *et al.*, 2003). Whilst these studies involving passive warming are crucial in uncovering the effect of T_m on the musculature it is likely that in a practical sense T_m will actually be elevated through prior exercise. With that end in mind the effect that actively increasing T_m has on subsequent exercise performance has also been well documented; with prior exercise below ~ 60 % $\dot{V}O_{2peak}$ resulting in an increase in maximal power output and prior exercise above ~ 60 % $\dot{V}O_{2peak}$ leading to a decrease in maximal power output (De Bruyn-Prevost & Lefebvre, 1980;Sargeant & Dolan, 1987;Beelen & Sargeant, 1993;Stewart *et al.*, 2003). However, whilst the effect of elevating T_m, either passively or actively, on mechanical performance has received a great deal of attention there has been very little work detailing the metabolic response within the muscle, and therefore any possible mechanisms for the observed changes in contractile activity, especially during short term maximal exercise.

In fact, no studies to date have investigated the metabolic response of the muscle, either at a homogenate or single fibre level, when T_m is raised during the development of maximal power output. Some research has suggested that the greater power output observed at higher T_m is due to an increase in the power contribution of type I fibres

(Sargeant & Rademaker, 1996) although, again, this has never been directly investigated. Advances in analytical techniques now allow the direct measurement of metabolism in single human muscle fibres, which can be characterised according to their myosin heavy chain (MHC) composition, and may help provide information regarding the metabolic and contractile changes observed at higher T_m. Although there is little research into the metabolic response to changes in T_m during short-term maximal exercise there has been some research during more sustained exercise.

During isometric contractions one study by Edwards *et al* (1972) has investigated the effects of passively elevating T_m on the metabolic response within the muscle, with the results revealing that passive warming of the legs increases anaerobic metabolism. During dynamic cycling exercise, which will be more relevant in the athletic world and in everyday life, similar results have also been observed (Febbraio *et al.*, 1996). This increase in anaerobic energy turnover observed during dynamic exercise may represent a decrease economy or efficiency of the muscle, although no measure of $\dot{V}O_2$ was made, with aerobic metabolism providing the majority of energy during sustained exercise. This means that mechanical efficiency, which will be of fundamental importance if exercise progresses beyond a few seconds, could not be accurately measured in these studies. There have been a few reports of the effect of T_m on the $\dot{V}O_2$ response to exercise with $\dot{V}O_2$ being found to increase during heavy exercise at 60 revs.min^{-1} and conversely decrease $\dot{V}O_2$ at 120 revs.min^{-1} (Ferguson *et al.*, 2002). Further work, however, has found that $\dot{V}O_2$ is unaltered after the passive elevation of T_m during heavy cycling exercise at 70-95 revs.min^{-1} (Burnley *et al.*, 2002b) meaning that the effect of T_m remains unknown. In the study of Ferguson *et al* (2002) estimates of efficiency, were, therefore lower at 60 revs.min^{-1} and increased at

120 revs.min^{-1} after the increase in T_m. One drawback with the efficiency calculations employed in that study is that the portion of energy from anaerobic sources was estimated through blood lactate concentration, which does not necessarily reflect the response within the muscle as lactate can be metabolised by other tissues (Gladden, 2000;Gladden, 2004) meaning that no study to date has accurately reported the effect, if any, of passively increasing T_m on mechanical efficiency during dynamic cycling exercise.

As mentioned previously, whilst passively elevating T_m is crucial in providing insight into the effect of T_m, it will be an active warm up which is generally performed athletes prior to competition. Whilst Asmussen and Boje (1945) concluded that the benefits of active warm up were mainly due to the increase in temperature, associated with prior activity, this is now thought not to be the whole story (Gray, 2001). For this reason the effect of 'active warm-up' or prior exercise on the physiological response to exercise will also be investigated in this thesis. A bout of prior exercise has previously been shown to decrease gross efficiency during a subsequent bout of heavy cycling exercise at 80 revs.min^{-1} (Sahlin *et al.*, 2005), with this response yet to be investigated at higher contraction velocities where an elevated T_m has been shown to increase efficiency (Ferguson *et al.*, 2002).

The aim of this thesis is firstly to determine the response of an increase in T_m on the rate of anaerobic ATP turnover and single fibre metabolism during the development of maximal power output and during more sustained sub-maximal exercise in humans. Following this the effects of passive heating on mechanical efficiency will be accurately determined, for the first time, during these very different modes of exercise. Finally, the effect of actively increasing T_m on mechanical efficiency is

determined during submaximal exercise at both relatively slow and fast contraction frequencies.

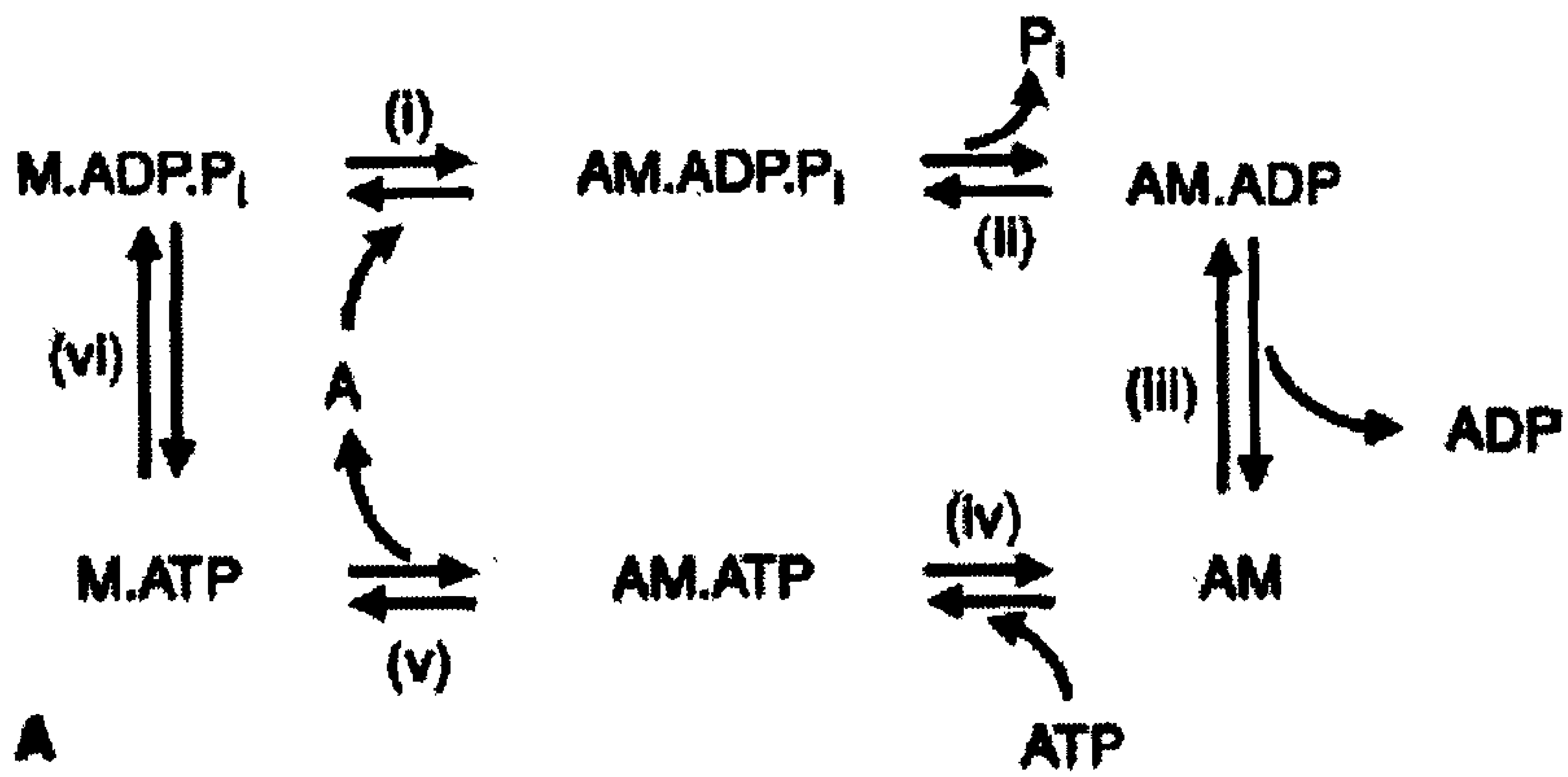
Chapter 2

LITERATURE REVIEW

2.1 MUSCLE CONTRACTION

In the past there have been a number of theories regarding the mechanisms behind muscular contraction, several of which have neither been confirmed or refuted (for review see Cooke, 1997;Huxley, 2000). The most plausible and widely accepted theory for the mechanism of skeletal muscle contraction is the cycling cross-bridge model within the framework of the sliding filament, originally proposed by A.F. Huxley (1957) and subsequently extended by Huxley and Simmons (1971). There is now a general agreement that the interaction of actin, myosin and ATP proceeds in a stepwise fashion resulting in force production. It is also probable that there are a number of intermediate steps in the cycle which can, or will in the future, be identified with different mechanical steps in the cross-bridge cycle, as shown in Fig 2.1. Simply put, the basic event underlying muscle contraction is an ATP-driven, cyclic, interaction of crossbridges (myosin heads) between the thick (myosin) and thin (actin) filaments in a sarcomere.

When skeletal muscle contracts the interaction of the globular portion of the myosin protein (the myosin head) with the actin filament gives stiffness to the muscle but it is the release of inorganic phosphate (Pi) which leads to a conformational change in the myosin head, known as the working stroke. It is this stroke that is responsible for the generation of the force that pulls the actin filament into the centre of the sarcomere (for review see Gordon *et al.*, 2000). Recent evidence has however suggested that the tail, rather than the head, of the myosin molecule may be the moving component resulting in the generation of force (Rayment *et al.*, 1993).



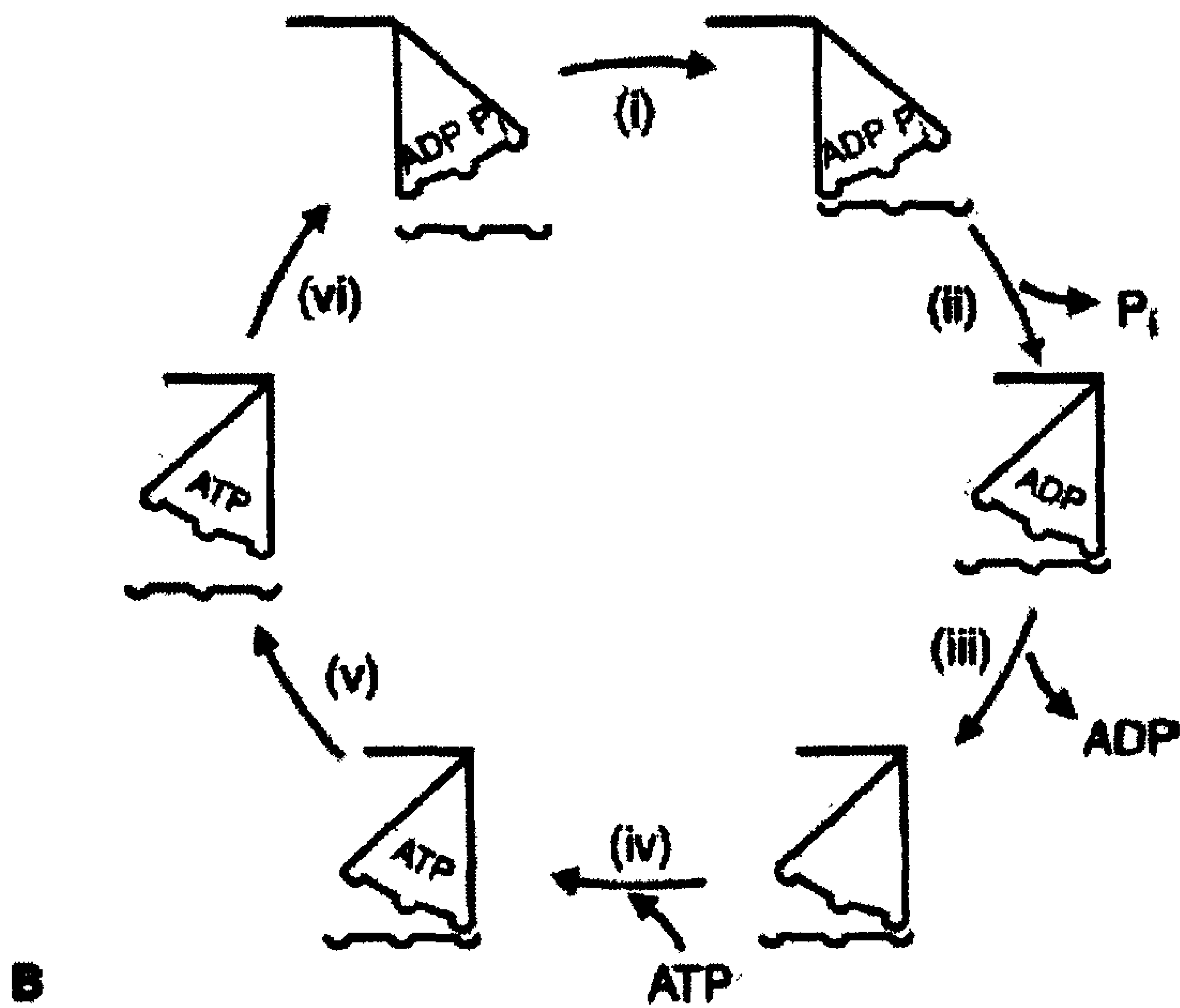


Fig 2.1. *Schematic of cross-bridge cycling showing the various biomechanical (A) and the likely mechanical changes associated with these steps (B). Force is generated as Pi is released in step (ii). Where AM is actomyosin; M is myosin; Pi is inorganic phosphate. (Taken from Jones et al., 2004).*

Regardless of the intricacies of cross bridge activity, an area under continuous research, work is essentially carried out by the muscle, powered by the free energy liberated from the hydrolysis of ATP (Cain & Davies, 1962), catalysed by myosin or myofibrillar ATPase (mATPase) located in the myosin head (Rayment *et al.*, 1993).

The force generated by this cross-bridge cycling (Fig 2.1) varies according to the degree by which myosin and actin overlap, as described by the length/tension relationship. This relationship was established by Gordon *et al* (1966) who, in isolated frog muscle, observed that over a certain range the developed tension was proportional to the degree of overlap between the actin and myosin filaments and so most likely the number of cross-bridge interactions between the myosin head and actin filament. The main feature of this relationship is that the force declines either side of an optimum sarcomere length, Fig 2.2.

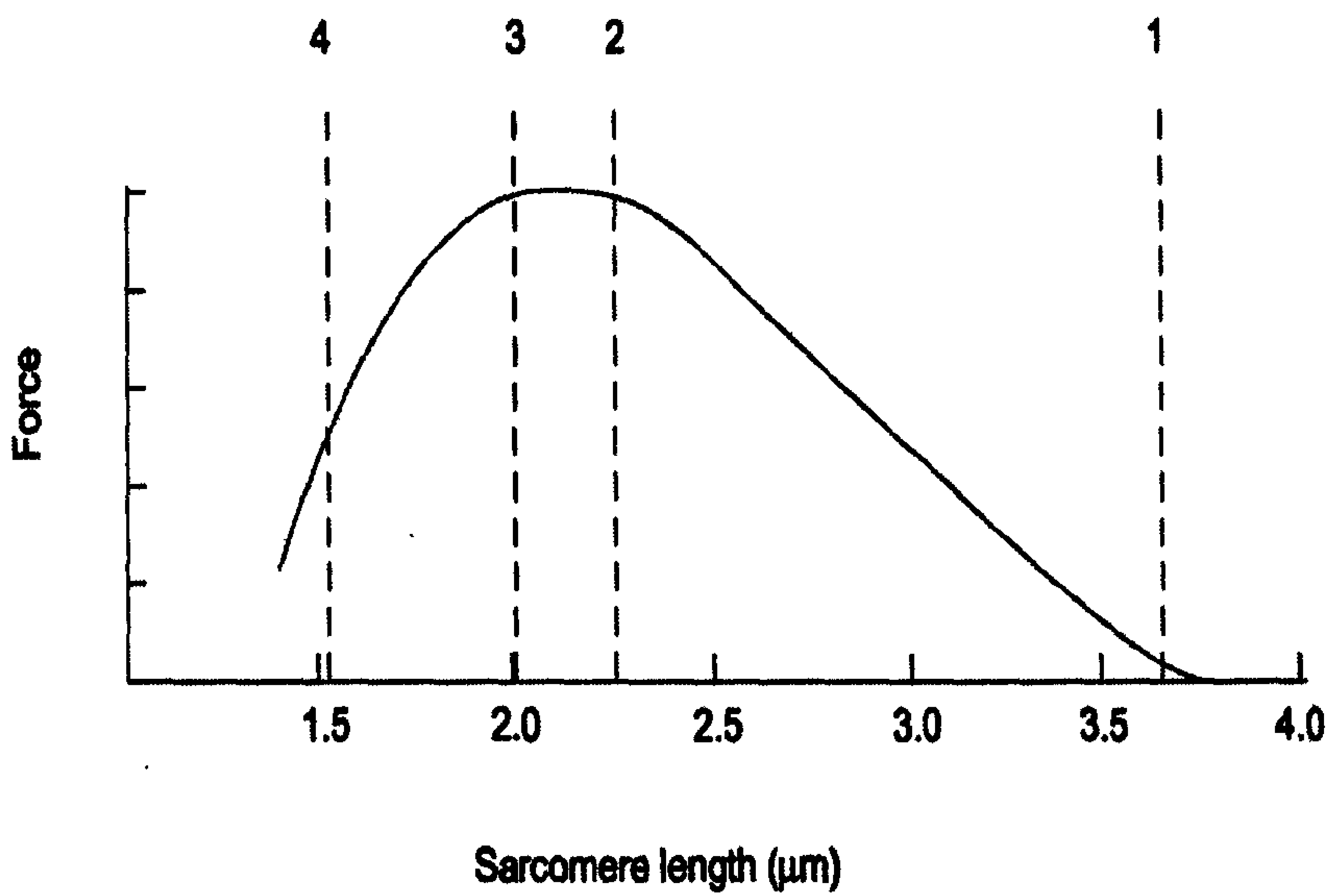
Another extremely important finding, furthering our understanding of muscular contraction, was that the force produced by the muscle will also vary with the velocity at which the muscle is shortening or lengthening. This relationship between force and velocity has been described by the formula of a rectangular hyperbola (Hill, 1938), represented by the solid line in Fig 2.3. This early 20th century description is still widely accepted in the present day and has more recently been demonstrated in whole human muscle preparations (De Ruiter & de Haan, 2000).

As the velocity of shortening increases the force developed by the muscle rapidly diminishes in a hyperbolic fashion, represented by a/Po, and eventually reaches zero at a velocity termed V_{max} which is dependent upon the rate of mATPase activity (Barany, 1967). Isometric force (P_o) is produced when the contraction velocity is zero

and this is dependent on the quantity of concurrent cross-bridge formations. Overall this relationship between the force and velocity of skeletal muscle reflects the interaction of the cross-bridges and the kinetics of their attachment and detachment (review Woledge *et al.*, 1985).

During locomotion the limb attached to the active muscle is moved through the combination of both force and velocity, in other words, power (power = force * velocity). It is this mechanical power, represented by the dashed line on Fig 2.3, which determines the contractile performance of the muscle during dynamic exercise and in everyday life. Power can be described as the rate of force development and is determined by the rate at which cross-bridge cycling can occur and the force generated by each cross bridge. The main determinant and thus the limiting factor in the determination of power output is primarily the rate of detachment of the actin-myosin cross bridges.

The power produced by the muscle during contraction has its own distinctive parabolic relationship with velocity. It can be seen from this parabola that this relationship reaches a maximum at an optimum velocity (Fig 2.3), which is ultimately determined by the relationship between force and velocity described previously. In isolated muscle preparations the optimum velocity is around 30 % of V_{max} (Sargeant, 1999;De Ruiter & de Haan, 2000). In human single muscle fibres He *et al* (2000) found that the ratio between optimal velocity and V_{max} is 0.22 for slow fibres, 0.26 for IIA fibres, and 0.28 for IIA-IIX fibres. Transferring this to whole-body exercise in humans Sargeant *et al* (1981) found that V_{opt} occurs at approximately 110 revs.min^{-1} during isokinetic cycling.



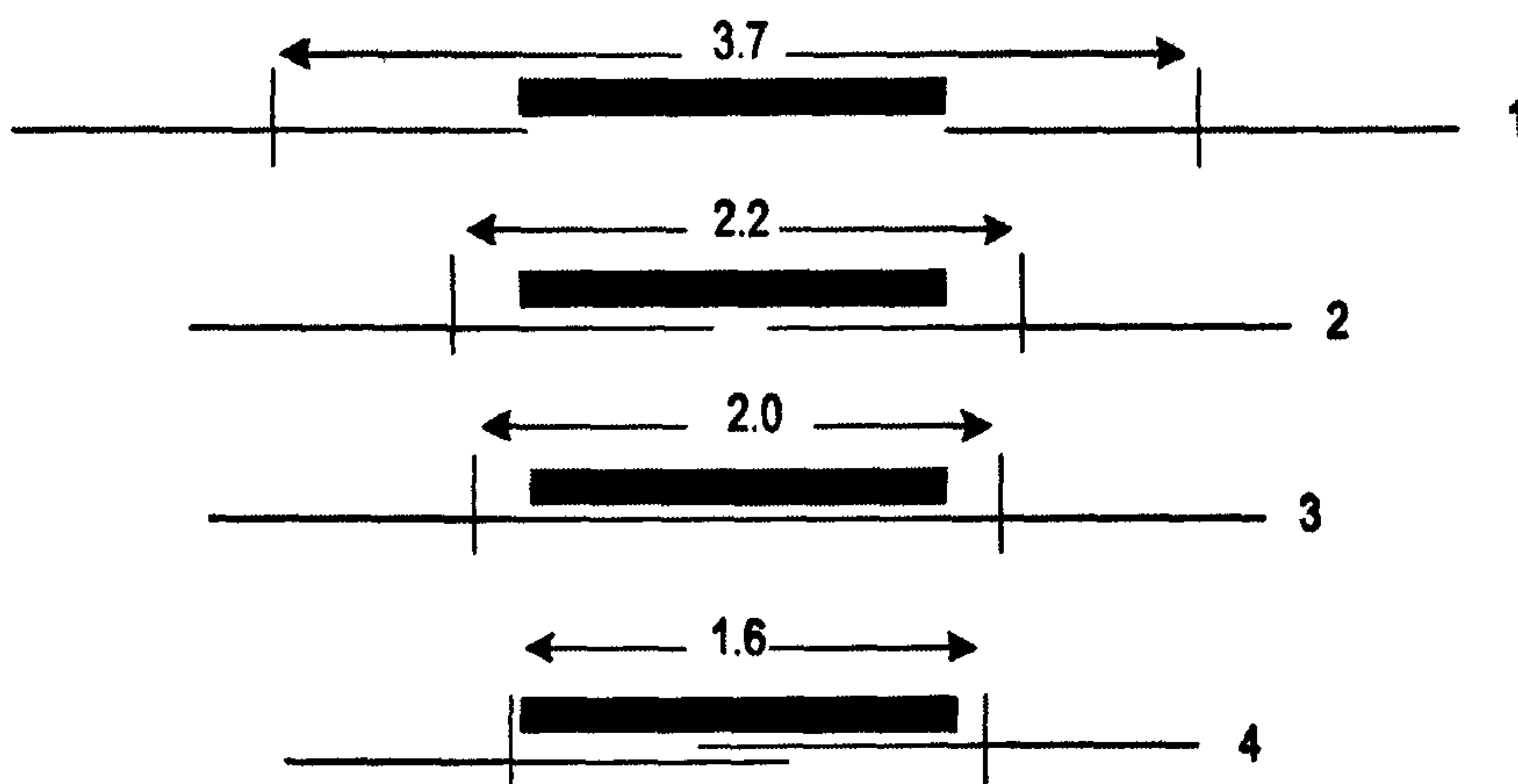


Fig 2.2. *Relationship between sarcomere length, myosin and actin filament overlap, and force. With the dark lines representing the myosin and the thin line the actin filament in the lower schematic. Taken from Maughan and Gleeson (2004).*

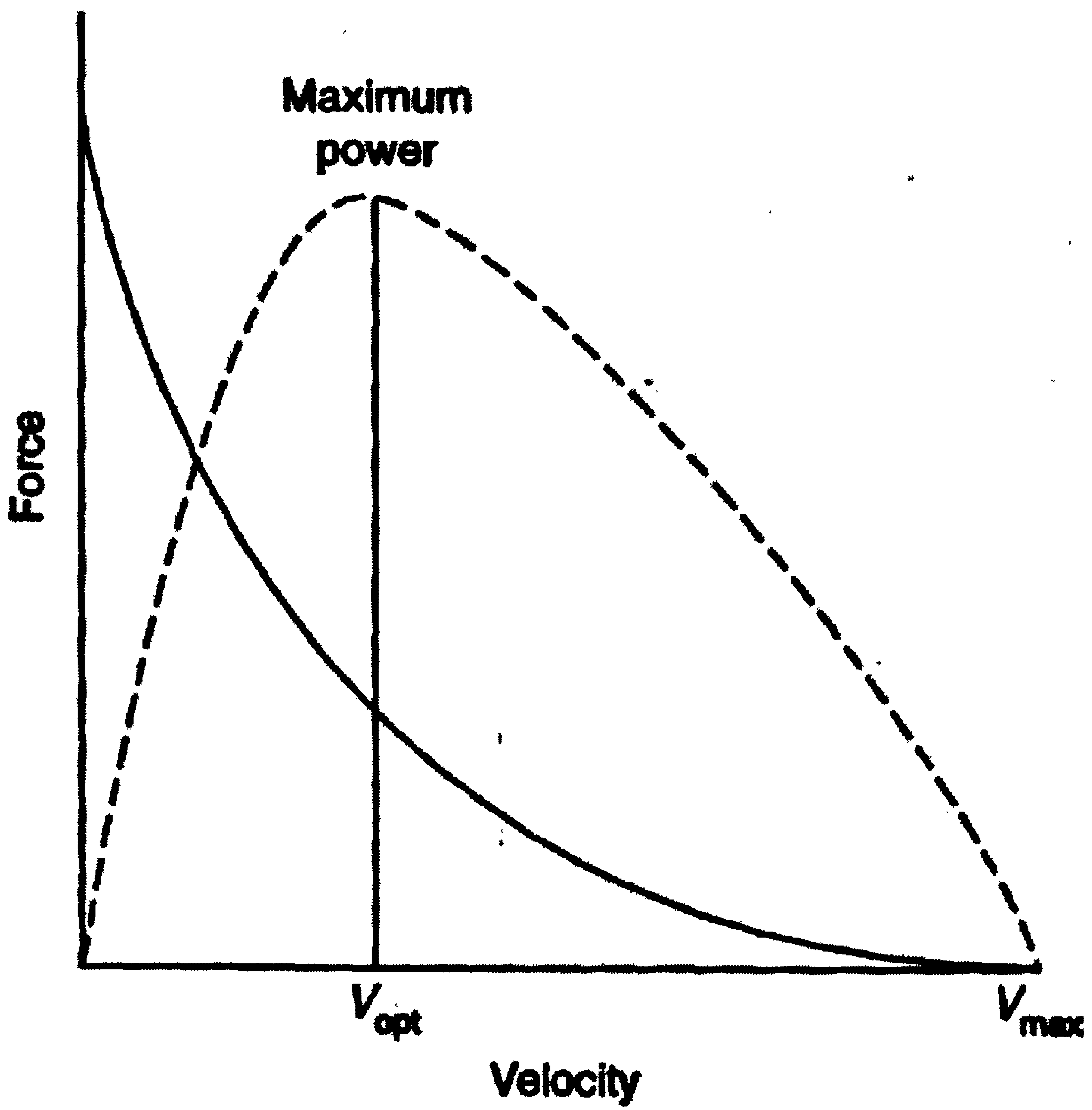


Fig 2.3. *General form of the force-velocity (solid line) and power-velocity (dashed line) relationships in human skeletal muscle. (Taken from Sargeant, 1999).*

2.2 SKELETAL MUSCLE METABOLISM

When discussing skeletal muscle energetics and metabolism, ATP is widely regarded as the universal currency of free energy in the majority of biological systems, including humans. An ATP molecule is a nucleotide which consists of an adenine, a ribose, and a triphosphate unit. As it is the energy carrier role of ATP that is of interest in metabolism, we can focus on its triphosphate moiety, which contains two phosphoanhydride bonds, giving ATP its energy-rich properties. When an ATP molecule is hydrolyzed to adenosine 5'-diphosphate (ADP) and Pi or when it is hydrolyzed to adenosine 5'-monophosphate (AMP) and pyrophosphate (PPi) a large amount of energy is liberated (-7.3 $kcal.mol^{-1}$ per ATP hydrolysed), which can subsequently be used, as mentioned previously, to fuel muscular contraction. It must be noted, however, that a quantity of this energy is converted into, and subsequently released as heat or another unknown form of energy.

Within human skeletal muscle the available store of ATP is extremely small, approximately 24 $mmol.kg^{-1}$ dm^{-1}, and is sufficient to fuel muscular contraction for only a few seconds at high workrates. The working muscles must, therefore, be supplied with ATP from some other source in order for contraction to continue. There are three metabolic pathways through which the human body can generate ATP: phosphocreatine breakdown, anaerobic glycolysis and oxidative phosphorylation.

2.2.1 Phosphocreatine hydrolysis

Due to the limited ATP stores, mentioned previously, skeletal muscle contains a reservoir, approximately 70-80 mmol kg dm^{-1}, of high potential phosphoryl groups in

the form of phosphocreatine (PCr), which is synthesised in the liver and taken in nutritionally, mainly in red meat. This high-energy phosphate compound can readily transfer its phosphoryl group to ADP in a reaction catalysed by the enzyme creatine kinase (CK), which has at least 4 sub-unit isoforms in vertebrate tissue (Wallimann *et al.*, 1992).

$$\text{PCr} + \text{ADP} + \text{H}^{+} \xrightarrow{\text{CK}} \text{ATP} + \text{Cr} \quad \text{[eq 1]}$$

It can be seen here that the buffering of the rapidly accumulating ADP during contractile activity re-synthesises ATP to help rapidly meet the energetic demands of exercise. In fact PCr has been shown to split immediately upon activation of a single muscle fibre (Infante *et al.*, 1965) and its immediate importance is further highlighted by the finding that [PCr] decreases, mono-exponentially, with no delay, in response to exercise and in proportion to the work rate (Rossiter *et al.*, 1999;Rossiter *et al.*, 2002). It is not surprising, therefore, that PCr hydrolysis has a dominant role in ATP production during short-term power output resulting in the PCr stores being completely depleted during intermittent electrical stimulation (Söderlund & Hultman, 1991).

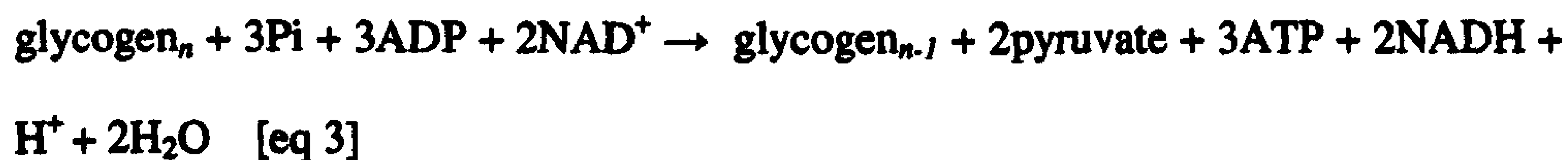
2.2.2 *Glycolysis*

The second pathway through which ATP can be generated is glycolysis, a nearly ubiquitous pathway in all biological systems. In simple terms, glycolysis is the chain of reactions which converts glucose 6-phosphate into pyruvate, with the concurrent production of a relatively small amount of ATP.

Glucose + 2Pi + 2ADP + $2NAD^+$ → 2pyruvate + 2ATP + 2NADH + $2H^+$ + $2H_2O$

[eq 2]

When blood glucose is used as substrate (eq 2), for each glucose molecule entering the glycolytic pathway two molecules of ATP and two molecules of pyruvate are produced, with a further four ATP molecules being produced from the subsequent oxidation of nicotinamide adenine dinucleotide (NADH). When glycogen is used (eq 3) as the primary substrate for glycolysis this overall reaction is slightly different with one more ATP and one less proton being produced.



$glycogen_n$ + 3Pi + 3ADP + $2NAD^+$ → $glycogen_{n-1}$ + 2pyruvate + 3ATP + 2NADH + H^+ + $2H_2O$ [eq 3]

In fact muscle glycogen is the major source of carbohydrate fuel in skeletal muscle, and its rate of degradation is primarily dependent upon exercise intensity (Vollestad & Blom, 1985;Spriet *et al.*, 1987a). These supplies are not believed to be a limiting factor during short-term maximal exercise (Hermansen & Vaage, 1977;Cheetham *et al.*, 1986) but are as exercise progresses beyond around 90 min (Hawley *et al.*, 1997). On top of that, liver glycogen also delivers glucose to the blood for transport to the active muscle, this process of converting stored glycogen into glucose is known as glycogenolysis. Glycogenolysis is regulated during exercise by the enzyme glycogen phosphorylase, which is present in two forms within skeletal muscle: (i) the less active *b* form; and (ii) the more active *a* form. The phosphorylase changes from the *b* to the *a* form as a result of increased sarcoplasmic [Ca^{2+}] at the onset of muscle

contractions and hormonal stimulation by adrenaline, a process mediated via the β-adrenoceptor and the intracellular second messenger cAMP (e.g. Richter, 1982).

Looking at the full glycolytic pathway in more detail, Fig 2.4, it can be seen that either glucose or glycogen is converted into glucose-6-phosphate, the starting molecule for glycolysis. Flow then proceeds through the same reactions regardless of the initial substrate and results in the production of pyruvate, which is either transported into the mitochondria for oxidative phosphorylation or reduced by NADH to form lactate, a reaction catalysed by the enzyme lactate dehydrogenase (LDH). This production of lactate was originally associated with muscular fatigue (Westerblad & Allen, 1992) although this is now believed not to be the case (Westerblad *et al.*, 1997;Bruton *et al.*, 1998;Robergs *et al.*, 2004).

In fact, the nicotinamide adenine dinucleotide (oxidized form-NAD^+) produced from the LDH reaction actually feeds back into the glyceraldehyde-3-phosphate dehydrogenase reaction, allowing glycolysis to continue. Thus, the formation of lactate is said to "buy time" for the working muscle allowing ATP to be regenerated and exercise to proceed, until another ATP source, generally oxidative phosphorylation, is found or fatigue ensues. This lactate produced by skeletal muscle is then, during the recovery period or even during continuous prolonged exercise, taken up and metabolised by resting muscles and even into the working muscle, when performing low intensity exercise (Gladden, 2000). Lactate is also taken up into other highly oxidative tissues, such as cardiac muscle and liver tissue, where it is oxidised to pyruvate and ultimately to glucose via the Cori cycle (Gladden, 2000).

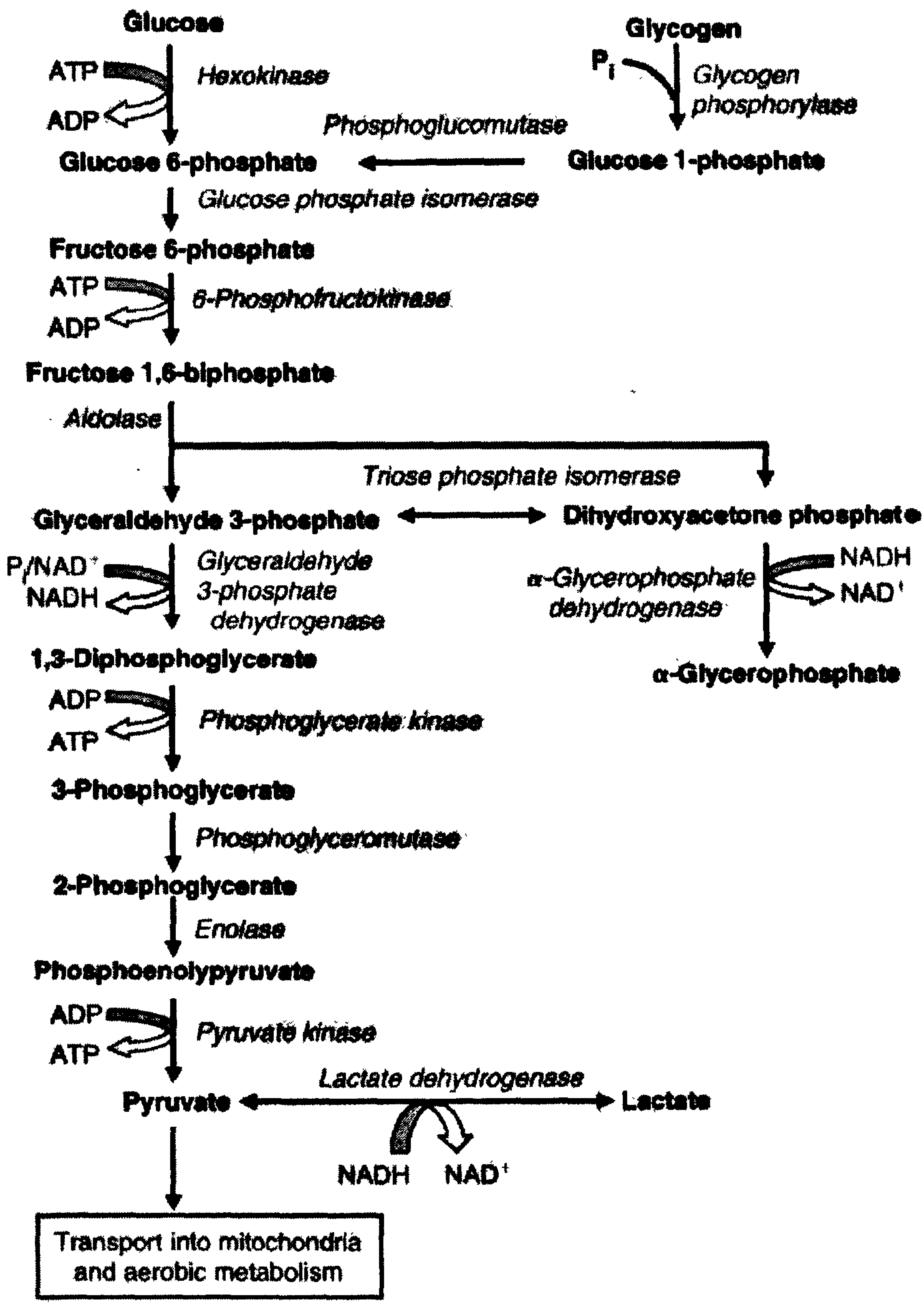


Fig 2.4. *Glycolytic pathway with both blood glucose and muscle glycogen as substrate. (Taken from Jones et al., 2004).*

In response to exercise the concentration of lactate appearing in the circulation increases in an intensity-dependent manner, with the precise nature of this relationship being the area of much study and some controversy. In the early 1970's Wasserman *et al* (1973) concluded that there was a critical work load (threshold) above which lactate accumulates within the blood and coined the term the anaerobic or lactate threshold to describe this phenomenon. A current description of the lactate threshold is that it is the point at which lactate production exceeds the removal/metabolism of lactate, resulting in an increase in lactate concentration within the circulation. At this point it is also worth noting that, while the majority of evidence supports the threshold behaviour, some authors dispute the existence of this threshold and suggest that blood lactate increase as a continuous function of exercise (Hughson *et al.*, 1987), although there is little work in support of such an assertion. The intensity of exercise at which this threshold occurs is dependent upon, amongst other things, the physical fitness of the participant. For example, endurance athletes having a threshold at a relatively high percentage of $\dot{V}O_{2peak}$ compared to a patient with chronic obstructive pulmonary disease, where lactate threshold occurs early on during exercise and can partly explain the earlier onset of fatigue in such a population (Whipp, 1987).

There have been a number of mechanisms hypothesised to cause the lactate threshold, including the original suggestion that there was a sudden onset of anaerobic glycolysis due to muscle cell hypoxia (Wasserman *et al.*, 1973). In more recent years the suggestion of a decrease in oxygen availability has been questioned with some investigators finding that the lactate threshold will occur when oxygen availability is not a limiting factor (Ward, 1999). This has led to a number of other suggestions to account for threshold behaviour of lactate which include an increase in type II fibre recruitment and a rise in muscle temperature (Ward, 1999). An increase in type II

fibre recruitment is proposed to lead to an increase in glycolysis, and thus lactate formation, due to the glycolytic nature of these fibres, which will be discussed in more detail at a later point. Similarly the rise in T_m as exercise progresses in intensity is also thought to increase anaerobic metabolism, a hypothesis which will be investigated in the current thesis. There is, however, little experimental evidence relating either fibre recruitment or T_m to lactate threshold and so further work is required to determine the precise mechanisms behind the lactate threshold.

The glycolytic pathway is regulated in several ways, with the key enzyme under regulatory control being phosphofructokinase (PFK). PFK is allosterically inhibited by high levels of ATP, lowering its affinity for fructose-6-phosphate, and action inhibited by AMP. A further control mechanism acting on this enzyme is the pH, with a high H^+ concentration inhibiting PFK activity. Hexokinase and glycogen phosphorylase, which are the first steps in glycolysis (Fig 2.4), are also regulated by glucose-6-phosphate and cAMP/Ca^{2+} respectively. Furthermore, the third irreversible step of glycolysis, the pyruvate kinase reaction, is also a sight of regulation with a high ATP concentration allosterically inhibiting pyruvate kinase activity.

2.2.3 Oxidative Phosphorylation

The magnitude of ATP produced glycolytically is small in comparison to the energy that can be liberated aerobically through oxidative phosphorylation, by means of the Krebs cycle and the electron transport chain. The point of entry to this oxidative pathway is acetyl CoA, which is formed inside the mitochondria by the oxidative decarboxylation of pyruvate, catalysed by the enzyme pyruvate dehydrogenase

(PDH).

$$\text{Pyruvate} + NAD^+ + \text{CoA} \xrightarrow{\text{PDH}} \text{acetyl CoA} + CO_2 + \text{NADH} \qquad \text{[eq 4]}$$

PDH also has two forms; the active form *a*, and the inactive form *b*, and must be converted to PDH*a* at the onset of exercise since resting levels are low (Hultman, 1996), a conversion stimulated by pyruvate and Ca^{2+}. After 5 and 15 s of intense exercise (110% $\dot{V}O_{2peak}$) PDH*a* has been shown to be only at quarter and a third of the 3 min values (Bangsbo *et al.*, 2002) and hence aerobic sources will contribute little during the initial period of exercise, but will be the major energy source in prolonged exercise (e.g. Parolin *et al.*, 1999). The phosphorylation of PDH and thus deactivation of the complex is increased when the ATP/ADP ratio increases. The acetyl CoA molecule, produced from the PDH reaction, combines with oxaloacetate to form citrate, a six carbon tricarboxylic acid, initiating the Krebs cycle. A number of reactions then lead to the release of protons and CO_2 and the regeneration of oxaloacetate, allowing the cycle to continue. The protons liberated from this cycle are carried by NADH and the reduced form of flavin adenine nucleotide ($FADH_2$) to the inner mitochondrial membrane where they enter the electron transport chain.

Briefly, the electron transport chain involves the protons liberated in the preceding Krebs cycle, along with electrons and are used in the generation of ATP (Whipp, 1994). The electrons are passed down the electron transport chain in a series of redox reactions, which results in a decrease in free energy. These electrons are finally accepted by O_2, the terminal electron acceptor, which is why this process is known as aerobic. The oxygen required by these systems to produce ATP will come from two methods of supply. The main mechanism by which oxygen is delivered to the

mitochondria is from the oxygen intake during respiration which passes from the lung into the circulation, the majority of which is bound to haemoglobin, and is ultimately delivered to the mitochondria. This has been termed the oxygen cascade, where the oxygen tension declines progressively from the atmosphere down to the mitochondria (Whipp, 1987).

The second oxygen source available in humans is myoglobin, a single chain globular protein consisting of 153 amino acids, with a mass of approximately 18 kD, which resides within the muscle itself. Myoglobin is structurally related to the circulating oxygen carrier haemoglobin and forms pigments which give the red colour to the muscles where it is present. Myoglobin acts as an intracellular store of oxygen, a property which is dependent on the presence of a haem group, consisting of protoporphyrin and a central iron atom. It is this haem group which gives both myoglobin and haemoglobin their red colours and it is the iron atom within this haem group which is able to bind directly with oxygen. This direct binding of oxygen to the iron group is inhibited by carbon monoxide, which has around a 200 fold greater binding affinity than oxygen (Olson & Phillips, 1996).

As well as acting as an intracellular oxygen supply myoglobin also has another function, particularly during exercise, which is to facilitate the transfer of oxygen into the mitochondria. This role is of particular importance during the initial period of exercise and also during intense exercise, when the partial pressure of oxygen in the cell is low (e.g. Astrand *et al.*, 1960). Myoglobin maintains its function to a greater extent, compared to haemoglobin, at these low pressures as it retains oxygen more readily under such conditions. This occurs due to nature of its dissociation curve, which takes the form of a rectangular hyperbola as apposed to the s-shaped

dissociation curve of haemoglobin. This role of myoglobin in exercise is highlighted by the finding that at the onset of submaximal exercise myoglobin can be completely desaturated of oxygen within 20 s, recovering after a rest period of around 45 seconds (Richardson *et al.*, 1995).

This energy liberated from the aforementioned redox reactions is subsequently used to pump protons across the inner mitochondrial membrane, causing a backflow of protons through an ATP synthase complex (complex V), thus forming ATP, as seen in Fig 2.5. Each molecule of NADH entering the electron transport chain liberates 3 ATP molecules, with 2 being produced for every $FADH_2$ molecule entering.

2.2.4 Quantification of anaerobic ATP turnover

In order to determine the ATP turnover produced anaerobically the deficit in the aerobic energy turnover has regularly been used. This method states that the shortfall, or lag, in the $\dot{V}O_2$ response at the onset of exercise (Hill & Lupton, 1923) must be met by anaerobic energy sources and that this 'oxygen deficit', first introduced by Krogh and Lindhard in 1920, is a good estimation of anaerobic ATP turnover. This has advantages over the alternative direct measure of metabolites, through muscle biopsies, due to the less invasive, and thus, more subject friendly nature of the procedure.

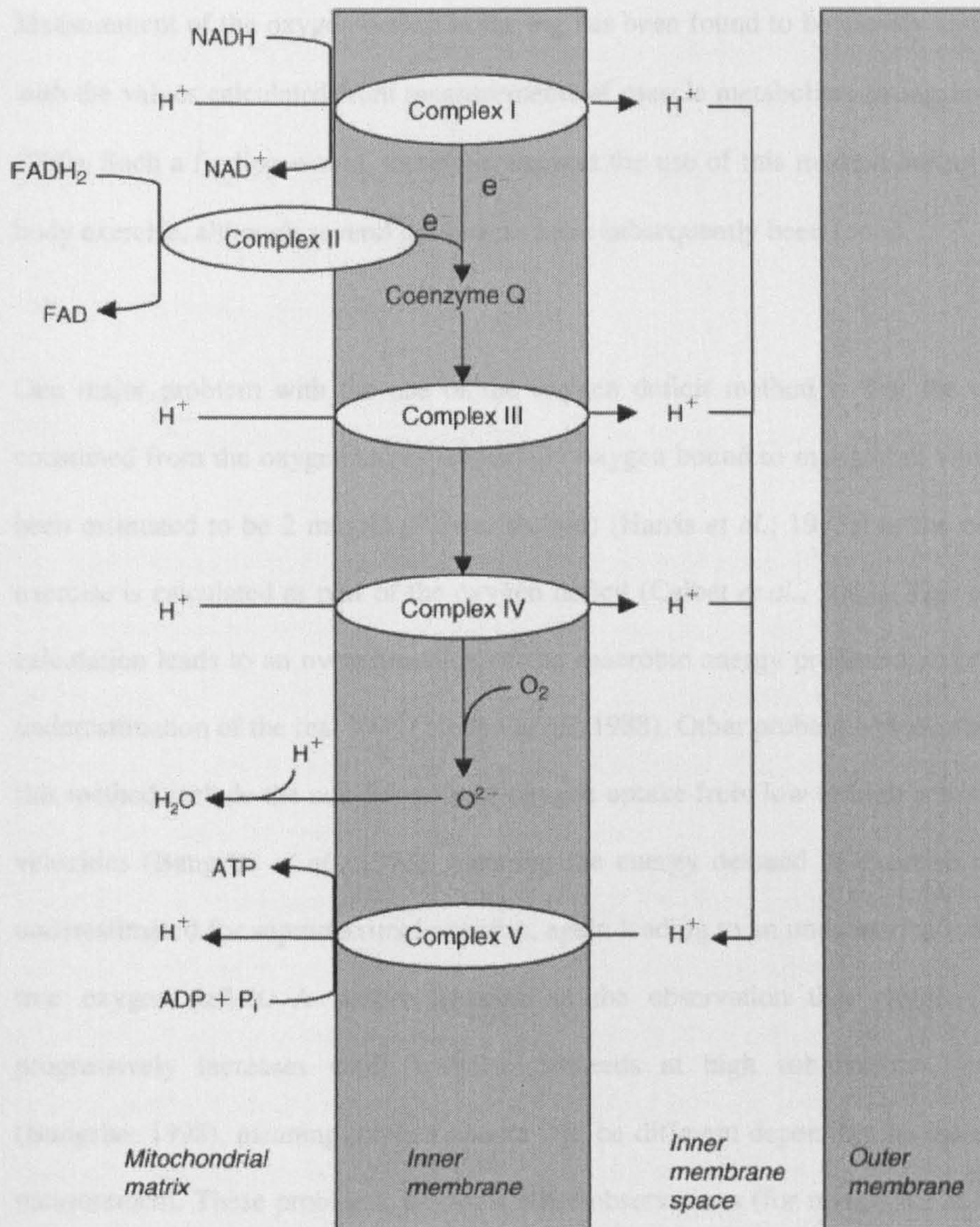


Fig 2.5. *The electron transport chain occurring in the mitochondrial matrix and inner and outer membranes. Oxygen is the terminal electron acceptor after the electrons pass through complex IV.*

Measurement of the oxygen deficit in the leg has been found to be closely associated with the values calculated from measurements of muscle metabolites (Bangsbo *et al.*, 1990). Such a finding would, therefore, support the use of this method during whole body exercise, although several drawbacks have subsequently been found.

One major problem with the use of the oxygen deficit method is that the oxygen consumed from the oxygen stores (especially oxygen bound to myoglobin which has been estimated to be 2 $mmol.kg^{-1}$ (wet weight) (Harris *et al.*, 1975)) at the onset of exercise is calculated as part of the oxygen deficit (Calbet *et al.*, 2003). This error in calculation leads to an overestimation of the anaerobic energy produced and thus an underestimation of the real $\dot{V}O_2$ (Medbo *et al.*, 1988). Other problems associated with this method include the non-linearity of oxygen uptake from low to high submaximal velocities (Bangsbo *et al.*, 1993), meaning the energy demand of exercise will be underestimated for supramaximal exercise, again leading to an underestimation of the true oxygen deficit. A further problem is the observation that oxygen uptake progressively increases while exercise proceeds at high submaximal velocities (Bangsbo, 1998), meaning oxygen uptake will be different depending on the time of measurement. These problems, amongst other observations (for review see Bangsbo, 1998) would suggest that the oxygen deficit will be inaccurate and unreliable in the determination of anaerobic energy production, particularly during high submaximal and supramaximal exercise where the energy demand is extrapolated from submaximal values.

Using a more direct method anaerobic ATP turnover can be calculated through utilisation of the muscle biopsy technique (Bergstrom, 1962) and is based on the

decrease in muscle PCr and ATP, as well as the accumulation of lactate.

$$\text{Anaerobic ATP production} = \Delta PCr + {}^{3}/_{2}\Delta Lactate + 2\Delta ATP \qquad \text{[eq 5]}$$

Taken from (Spriet, 1995)

Where Δ is the difference between pre/rest exercise and post exercise concentrations.

The main drawback with this method of quantification of anaerobic energy production is that this calculation (eq 5) does not take into account the lactate released into the blood from the exercising muscle. Therefore, the magnitude of anaerobic energy production for this method will also be underestimated. The obvious question that arises from these assertions is, by how much is this underestimated? Research by Bangsbo and colleagues has shown that energy release related to lactate ranged between 5 and 38 % of total anaerobic energy production (Bangsbo, 1998). Once more, even these calculations will be underestimated as they do not take into account that a quantity of lactate may have been metabolised by various tissues, including the heart and inactive muscles (for review see Gladden, 2000;Gladden, 2004). Another problem with the determination of the anaerobic ATP turnover of the exercising skeletal muscle is that it is difficult to quantify the mass of the muscles participating in the whole-body exercise and to determine to what extent these muscles are active (Richardson *et al.*, 1997).

In an attempt to solve these problems anaerobic energy production can be calculated using a single leg, dynamic knee-extensor exercise model, where exercise is confined to an isolated muscle. This novel technique was contrived by Andersen and Saltin

(1985) and several studies have now utilized this technique in order to investigate a number of physiological variables (e.g. Aagaard *et al.*, 1994;Ray & Dudley, 1998;Ferguson *et al.*, 2000;Ferguson *et al.*, 2001;Ferguson *et al.*, 2006). The problem of lactate release into the blood is minimized in this model as the blood flow from the lower leg is occluded. Furthermore, in this method the lactate uptake from the inactive hamstring and adductor muscles can be evaluated by measuring femoral venous blood from the contralateral resting leg. In the example given by Bangsbo (1998), this method reveals an underestimation of lactate release from the quadriceps muscle of about 3 %, and so is a reasonably accurate method of quantification of anaerobic energy production. It is known, however, that during knee-extensor exercise muscle blood flow is higher and arterial lactate levels are lower than those seen during whole body exercise. To alleviate this problem conditions similar to those taking place during whole body exercise can be estimated with contralateral leg and upper body exercise. This exercise model, therefore, allows a novel and accurate equation for the estimation of anaerobic energy turnover.

Anaerobic ATP production = $-\Delta$ATP $-\Delta$PCr + $^{3}/_{2}\Delta$muscle lactate + $^{3}/_{2}$ lactate + release + others [eq 6]

Taken from (Bangsbo *et al.*, 2000)

'Others' represents ATP production related to accumulation of pyruvate assumed to be 1/30th of accumulated muscle lactate (Spriet *et al.*, 1987a), lactate uptake by inactive tissues of the exercising leg (Bangsbo *et al.*, 1993) and accumulation of glycolytic intermediates (Spriet *et al.*, 1987b).

The use of knee extensor exercise, whilst allowing accurate quantification of the active muscle mass and thus a precise measure of anaerobic energy turnover, is not necessarily a good representation of the response seen during whole body exercise (Koga *et al.*, 2005b). During whole body exercise, as used in the athletic world and in every day life, like cycling and running the analytical techniques employed when using the knee-extensor model are somewhat problematic. The knee extensor model allows a direct measure of the arterio-venous differences in the concentrations of O_2 and lactate, meaning a measure of muscle oxygen uptake and lactate release from the muscle can be attained. In this method mixture with blood from the lower, non-active, limbs is avoided by the placement of an occlusion cuff inflated to 220 mmHg below the knee. In order to achieve these measurements it is necessary to insert cannulae in both the femoral artery, proximal to the inguinal region, and in the femoral vein, also allowing the muscle blood flow to be measured using the thermodilution technique (Andersen & Saltin, 1985). One of the key factors making this technique effective is that the exercise, and the measurements made, are confined to the quadriceps muscle group, the mass of which can be determined by anthropometric measurements (Andersen *et al.*, 1985).

During cycling, as used in the present thesis, isolation of the exercising muscle is not possible, and leads to limitations in quantifying anaerobic ATP turnover. Due to the greater active muscle mass during cycling exercise measuring the arterio-venous difference and blood flow across the 'active musculature' becomes impossible. Therefore, whilst muscle biopsies can still be acquired and metabolite levels measured, it is not possible to measure the amount of lactate released from the active muscles. These difficulties in quantifying anaerobic ATP turnover during whole body

exercise result in a number of assumptions being made in these studies, including the present thesis, and ultimately lead to an underestimation of anaerobic ATP turnover during such exercise.

During exercise of less than 6 s, such as that carried out in chapters 4 and 5 of the present thesis, there is a negligible release of lactate from the active musculature. When the exercise duration is short the underestimation of anaerobic ATP turnover will be small (Bangsbo, 1996). On the other hand, when the exercise duration is extended, as in chapters 6 and 7 of the current thesis, the underestimation of anaerobic ATP turnover will be more substantial (Bangsbo, 1998). Recent work has demonstrated that the lactate release from the contracting muscle the same under normal and elevated T_m conditions (Ferguson *et al.*, 2006). Therefore, when T_m is elevated prior to cycling exercise the underestimation of anaerobic ATP turnover will be similar under both control and heated conditions, making such comparisons reasonable,.

It can be seen, therefore, that there are two main methods of calculating the rate of anaerobic ATP turnover, both of which have their drawbacks particularly during whole body exercise (for review see Bangsbo, 1998). As detailed previously a large proportion of energy will, however, be provided via oxidative phosphorylation or the aerobic ATP turnover during exercise, and quantification of this ATP supply is necessary.

2.2.5 Quantification of aerobic ATP turnover

As exercise progresses beyond approximately 30 s the majority of the overall ATP

turnover will be from aerobic sources (Parolin *et al.*, 1999), with its contribution increasing as exercise progresses further (Medbo & Tabata, 1993). In humans the amount of ATP supplied through aerobic sources is generally measured at the level of the lung through the $\dot{V}O_2$, measured breath-by-breath, via an online gas analyser. Measuring oxygen uptake at the lung can provide us with a similar measurement to the oxygen uptake across the leg since these methods have previously been shown to be within 10 % of each other (Barstow *et al.*, 1994).

A common approach allowing quantitative analysis of $\dot{V}O_2$ is to mathematically model the response of the aerobic system to a bout of exercise. Such analyses can provide valuable information regarding the speed of rise in $\dot{V}O_2$ (tau(τ)), the amplitude of the response and, during exercise above the lactate threshold (LT), the magnitude of the $\dot{V}O_2$ slow component (Whipp and Wasserman, 1972). Whilst this analysis provides important information regarding the response of $\dot{V}O_2$ to exercise further calculations are required to estimate the aerobic ATP turnover. In order to carry out this analysis the respiratory exchange ratio (RER) can be used alongside the $\dot{V}O_2$ to calculate aerobic ATP turnover using the method derived by Glasgow physiologist J.B. Weir in 1949 a method employed in several investigations (e.g. Ferguson *et al*, 2000;Sahlin *et al*, 2005). The major limitation in such a quantification of the aerobic ATP turnover is accounting for contribution from oxygen bound to myoglobin within the muscle. As mentioned previously the amount of oxygen stored in this manner has been estimated to be approximately 2 $mmol.kg^{-1}$ (Harris *et al.*, 1975) and while this is a small amount when compared to the levels of oxygen supplied through respiration, it must be noted that any calculation of aerobic ATP turnover using respiratory gas exchange will be an underestimation. In many

investigations, and also in the current thesis, oxygen uptake is indeed measured at the lung and so such estimations of aerobic ATP turnover will be an underestimated. When taken alongside the aforementioned underestimation of anaerobic ATP turnover it is clear that using the available techniques during whole body exercise the total ATP turnover will be underestimated, although this may be to a similar extent at both temperatures.

Previous work has demonstrated that a 3 °C rise in T_m, similar to the exercise induced temperature rise and the increase seen after passive warming, is known to lead to a small rightward shift in the myoglobin-O_2 dissociation curve (Schenkman *et al.*, 1997). It is not clear, however, whether this small change in dissociation will have an appreciable effect on the aerobic ATP turnover after such a rise in T_m. The majority of experimental work supports the notion that at the onset of exercise the supply of oxygen is not limited and that the rate limiting factor in aerobic metabolism lies within the metabolic pathways themselves, possibly at the PDH (Stephens *et al.*, 2007) or CK (Rossiter *et al.*, 2002) reactions. The small increase in oxygen availability afforded by the increase in myoglobin-O_2 dissociation at higher T_m is likely to have a minimal effect on the comparison of aerobic ATP turnover, although underestimated, between conditions.

2.2.6 Rates of ATP turnover

The methods through which anaerobic and aerobic ATP turnover rates can be calculated have been detailed and it is, therefore, prudent to illustrate some of the rates previously observed during various durations, intensities and modes of exercise. Hultman and Sjöholm (1983) used electrical stimulation of the human quadriceps

muscle at 50 Hz, with occluded circulation, to study ATP turnover rates and demonstrated that after 1.28 s of contraction the total ATP turnover rate was 11 mmol ATP kg^{-1} (dry mass (d.m.))s^{-1} with 80 % of this total being produced by PCr degradation (9 mmol ATP kg^{-1} (d.m.)s^{-1}). During this period there was also a small accumulation of lactate, indicating a rapid onset of glycolysis during this period.

During 6 s of maximal sprint cycling Boobis *et al* (1982) found that the rate of anaerobic ATP turnover was also ~ 11 mmol ATP kg^{-1} (d.m.)s^{-1}, with 56 % of this ATP coming from PCr degradation and 44 % from glycolysis. Increasing the exercise duration further to 30 s Jones *et al* (1985) found that the glycolytic contribution increased to 61 % of the total anaerobic ATP turnover with the contribution from PCr decreasing to 39 %. None of the aforementioned studies accounted for the ATP contribution from oxidative phosphorylation which although likely to be small may still be significant.

Further work, where the aerobic contribution was accounted for has shown that from 6-15 s of sprint cycling glycolysis provides 57 % (~6 mmol ATP kg^{-1} (d.m.)s^{-1}) of the total ATP turnover, PCr provides 14 % (~1.5 mmol ATP kg^{-1} (d.m.)s^{-1}) and oxidative phosphorylation was estimated to contribute 29 % (~3 mmol ATP kg^{-1} (d.m.)s^{-1}) (Parolin *et al.*, 1999). When the sprint was allowed to continue from 15-30 s the glycolytic contribution had fallen to 23 % (~1.5 mmol ATP kg^{-1} (d.m.)s^{-1}), the PCr contribution was 15 % (~1 mmol ATP kg^{-1} (d.m.)s^{-1}) and the oxidative contribution increased to 62 % (~4 mmol ATP kg^{-1} (d.m.)s^{-1}).

Therefore, in the first couple of seconds, as demonstrated in Fig 2.6, PCr hydrolysis is the major energy source, with glycolysis providing a smaller percentage of the

energy. At around 5-10 s glycolytic energy production equals PCr energy production and becomes the major anaerobic energy source as the exercise proceeds. Furthermore, as mentioned briefly aerobic pathways provide little energy during the first few seconds of exercise but will contribute greatly during more sustained exercise (Medbo & Tabata, 1993;Parolin *et al.*, 1999).

It can be seen therefore that while anaerobic energy sources dominate in the initial period of exercise, and during exercise of a high intensity, aerobic sources are become dominant during more prolonged (<30 s) exercise, and particularly during low intensity exercise. Using these values of anaerobic and ATP turnover we can gain an insight into the economy or efficiency of muscular contraction, with any change in ATP turnover likely to represent a change in efficiency.

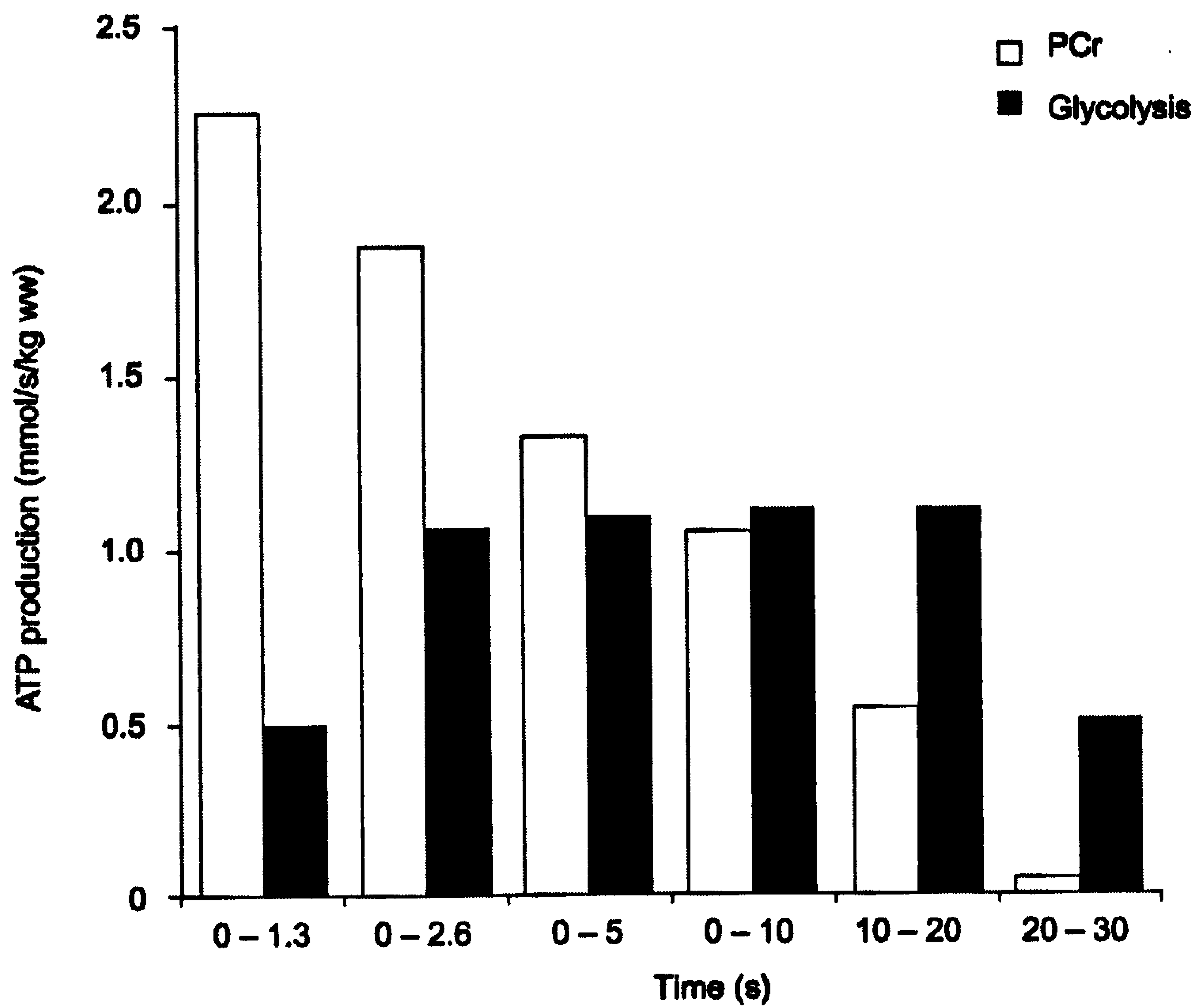


Fig 2.6. *Rates of anaerobic ATP turnover from PCr hydrolysis and glycolysis calculated from metabolite changes during 30 s of near maximal isometric exercise. (Taken from Maughan & Gleeson, 2004).*

2.2.7 *Mechanical efficiency*

'Mechanical efficiency' can be defined as the ratio between total mechanical power output and total rate of energy turnover. This is in contrast to the term economy which is related to the rate of energy turnover during isometric contractions when no work is carried out by the muscle (e.g. Barclay & Weber, 2004). In other words, efficiency represents the muscle's ability to convert chemical energy (ATP) into kinetic energy (force), and the energetic cost of this conversion, and is of great importance with regards to skeletal muscle function and thus athletic performance and daily life. At this point it is important to consider the elementary concepts of energy conversion.

The term enthalpy (H) describes the total heat or energy content of a substance, thus ΔH is the total change in heat during a reaction. Free energy (G) is the portion of H available to fuel work within the body and so if the change in this free energy (ΔG) is negative free energy is liberated and the reaction occurs spontaneously. On the other hand, a positive ΔG means that the reaction must be driven by another reaction that releases free energy. In human muscle, contraction is an endogenic process (ΔG is positive) and thus requires an exergonic process (ΔG is negative) to drive the reaction, which in this case is the hydrolysis of ATP. The portion of enthalpy unavailable for the performance of work is known as Entropy (S) and is considered to be a measure of the randomness or disorder of a system. The relationship that exists between these three concepts is given by:

$$\Delta H = \Delta G + T\Delta S \qquad \text{[eq 7]}$$

Where T is the absolute temperature in °K

During a chemical reaction only the free energy of the system can be converted into work.

The total energy cost of muscular contraction reflects the summed ATPase activity of muscle including mATPase as well as Ca^{2+} and membrane pump (ion) ATPases, with 20 – 50 % of ATP utilized with the latter two ATPases (e.g. Crow & Kushmerick, 1983). More recent work by Zhang *et al* (2006), carried out at more physiological temperatures than previous work, has found that during submaximal isometric contractions the amount of ATP consumed by the mATPase was only 20 %. Therefore, the 'thermodynamic efficiency' of muscular contraction is defined as the proportion of the free energy from ATP hydrolysis emerging as mechanical work, with the remainder being degraded to heat (Wilkie, 1974). The measurement of this heat produced is problematical (Kushmerick & Davies, 1969) and hence there is considerable perplexity when attempting to associate 'thermodynamic efficiency' with 'mechanical efficiency' (Woledge *et al.*, 1985). Nevertheless, heat production is regularly used as a quantitative measure of energy flux.

It has been known since the 1920's that when a muscle shortens and 'works' the energy (heat) liberated is approximately equal to the work done above that which is liberated during an isometric contraction (known as the Fenn effect (Fenn, 1923)). The sum of heat output (h) and work output (w) represent the enthalpy output (ΔH) which in turn can be related to the work output as a measurement of 'mechanical efficiency', thus:

$$\text{Mechanical efficiency} = w / (h + w) \qquad \text{[eq 8]}$$

It is important to note that all of the aforementioned metabolic processes, and therefore the mechanical efficiency, may differ between individual muscle fibres as there are different types of muscle fibre. A clear appreciation of the metabolic and mechanical properties of these different fibre types is important to our understanding of the precise muscular response to exercise.

2.3 SKELETAL MUSCLE FIBRE TYPES

2.3.1 Myosin heavy chain and fibre types

It has long been known that muscles differ in their appearance but it was Ranvier in 1873 that first recognised that the red and white muscle fibres, classically known as type I and type II fibres, also vary in their contractile properties. Since these classic investigations of Ranvier further discoveries have revealed that in human skeletal muscles there are more than two different types of fibres. These fibres are the type I (slow/oxidative/fatigue resistant), type IIA (fast/oxidative-glycolytic/moderately fatigable) and type IIX fibres (the fastest/glycolytic/easily fatigable), with IIA and IIX fibres being subdivided from the original type II classification fibre group (Schiaffino & Reggiani, 1996).

It is now widely accepted that these variations in the mechanical, and also the metabolic properties, between fibre types, noted by Ranvier, are determined to a large extent by the myosin protein. Myosin is composed of two MHC's two regulatory MLC's and two alkali or essential MLC's, each of which exists in several isoforms, and it is the MHC isoforms which determine the majority of fibres properties (for review see Schiaffino & Reggiani, 1996). For this reason, skeletal muscles are now

regularly characterised through MHC analysis, which is in good agreement with the traditional mATPase histochemical method (Ennion *et al.*, 1995). In other words, type I fibres contain type I MHC, type IIA fibres the type IIA MHC and type IIX fibres the type IIX MHC, with the MHC isoform present now regarded as synonymous with the fibre type. Unfortunately, and somewhat confusingly, the last fibre type (IIX) has, on the basis of conventional histochemistry, previously been referred to throughout the early literature as a human type IIB fibre type. In fact, these fibres were described as IIB fibres until Ennion *et al.*, (1995) demonstrated that these fibres contained the human equivalent of the rat IIX and not the IIB MHC gene. In spite of these findings type IIX fibres are, in some cases, still referred to as IIB fibres in the literature. The IIX nomenclature will be used throughout the current thesis.

An important observation regarding muscle fibres is that although type I and type IIA MHC isoforms are occasionally found in the same fibres, it has been observed that a the IIA and the faster IIX isoforms are present in variable amounts in the a large number of fibres (Larsson & Moss, 1993;Bottinelli *et al.*, 1994;Sant'ana Pereira *et al.*, 1995;Sant'ana Pereira *et al.*, 1996). Pure IIX fibres are very rare in humans, with one study finding only 0.3 % of the total fibre population of a needle biopsy sample, from the vastus lateralis, expressed solely MHC IIX (Andersen *et al.*, 1999). These fibres expressing MHC I/IIA and MHC IIA/IIX are known as hybrid fibres, with, as mentioned, the latter being the most common (Andersen *et al.*, 1999). This a continuum of variable co-expression of MHC is now widely detailed and also results in a concomitant continuum of contractile properties (Larsson & Moss, 1993).

The variation observed in the contractile properties has been shown to be primarily determined by the MHC isoform or isoforms present. For example, MHC has been

demonstrated to be the main determinant of the muscle fibres V_{max} (Reiser *et al.*, 1985;Bottinelli *et al.*, 1991;Bottinelli *et al.*, 1996) W_{max} (Aagaard & Andersen, 1998) and V_{opt} (Bottinelli *et al.*, 1991;Bottinelli *et al.*, 1996) as illustrated in Fig 2.7. Furthermore, the ATP consumption rate during isometric contraction, tension cost (Bottinelli *et al.*, 1994;Steinen *et al.*, 1996) and the maximally Ca^{2+} activated mATPase activity are also closely related to MHC isoform expression (Staron & Pette, 1986;Steinen *et al.*, 1996). MHC isoforms, therefore, largely determine the contractile and metabolic properties of muscle fibres, allowing them to adapt to the requirements of muscular performance *in vivo* according to functional demand.

Different fibre types have also been found to have varying resting metabolite levels, and also utilisation of these metabolites during exercise. The resting ATP content of type I and II fibres is similar, with some suggestion of a higher content in type II fibres (Söderlund *et al.*, 1992). Resting glycogen content of type I fibres is around 10-25 % lower than the levels found in type II fibres (Greenhaff *et al.*, 1991;Söderlund *et al.*, 1992;Greenhaff *et al.*, 1993). Similarly, the PCr content of type I fibres is around 5-15 % lower than in type II fibres (Tesch *et al.*, 1989;Söderlund & Hultman, 1991;Söderlund *et al.*, 1992) and has been shown to increase with IIX isoform percentage in the type IIAX hybrid fibres (Sant'ana Pereira *et al.*, 1995), although not always (Karatzaferi *et al.*, 2001a).

These variations in metabolite concentration can be partly explained by the difference in metabolism between these fibre types. The type I fibres are inherently oxidative, which is characterised by a high content of mitochondria, myoglobin and intracellular lipid stores (e.g. Meng *et al.*, 1993). Type II fibres, on the other hand are predisposed to work anaerobically with high concentrations of glycolytic enzymes, including

lactate dehydrogenase, PFK and pyruvate kinase (e.g. Pette & Spamer, 1986), with a continuum of enzyme concentration increasing alongside the MHC IIX content.

The rate at which metabolism occurs in different fibre types has been monitored during various forms of exercise in human subjects. The first of these investigations was carried out by Tesch *et al* (1989) during knee extensor exercise with muscle biopsies before and after exercise. This investigation found that after 30 maximal knee extensions there was a greater PCr degradation in type II compared to type I fibres, in these early studies the type II fibres were not subdivided into IIA and IIX (Tesch *et al.*, 1989). Several other studies have demonstrated similar patterns during short-term intense exercise, with type II fibres having a greater PCr and glycogen degradation during 30 s of sprint running (Greenhaff *et al.*, 1994) and during 10 and 20 s of electrical stimulation (Söderlund *et al.*, 1992). A greater PCr and ATP degradation during 10 and 25 s of sprint cycling has also recently been demonstrated (Karatzaferi *et al.*, 2001a;Karatzaferi *et al.*, 2001b).

During more prolonged submaximal exercise the opposite pattern of metabolism between fibre types has been found. Three hours of cycling exercise at approximately 30 % $\dot{V}O_{2peak}$ resulted in a greater glycogen degradation in type I fibres compared to type II fibres, with the relative glycogen degradation of type II fibres increasing as exercise progressed (Gollnick *et al.*, 1973;Gollnick *et al.*, 1974). Similar findings, of a greater type I compared to type II glycogen degradation, have been found during 2 hours cycling at the higher intensity of 75 % $\dot{V}O_{2peak}$ (Vollestad *et al.*, 1984).

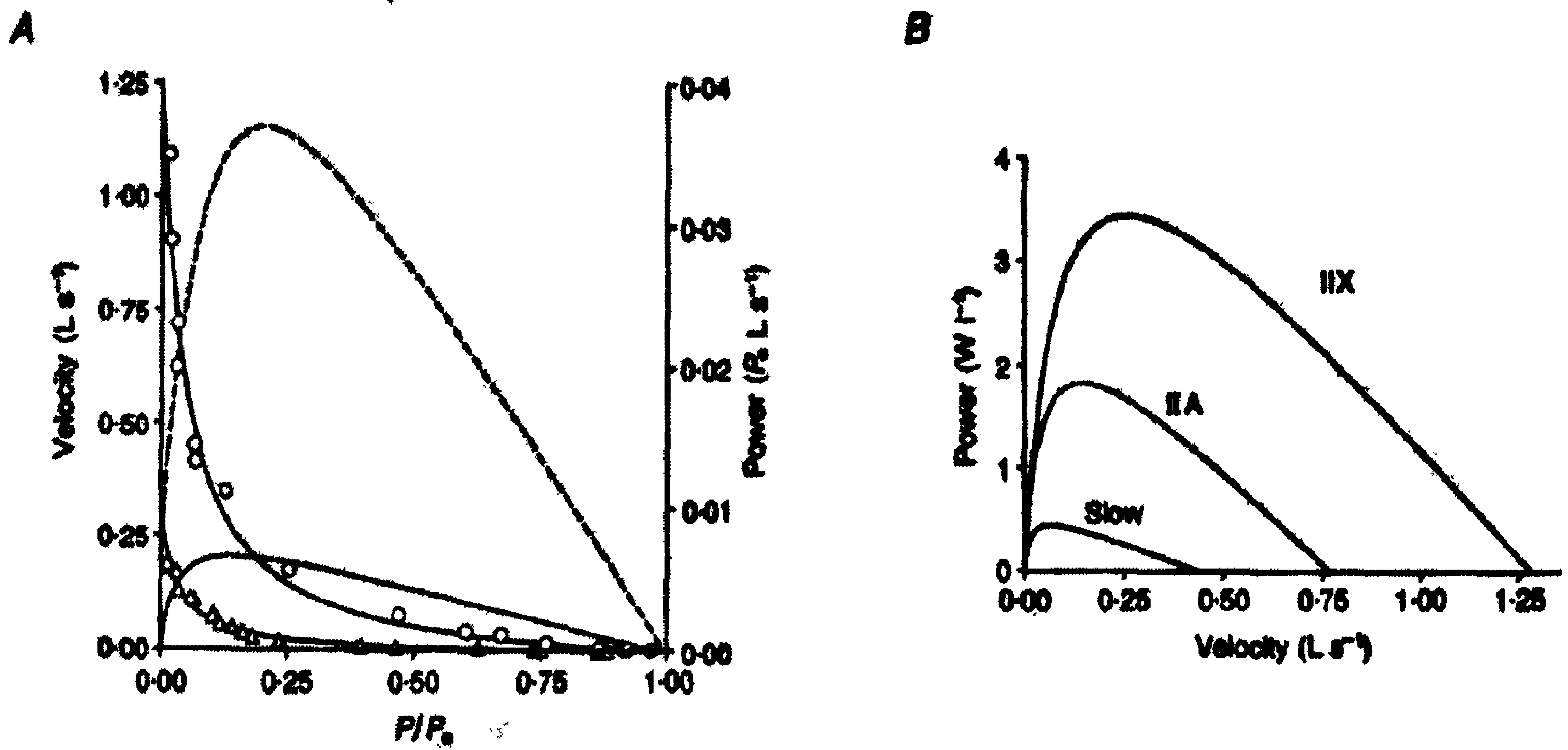


Fig 2.7. *Force-velocity, force-power (A) and power-velocity curves of different fibre types. In A in the force-velocity curve triangles are type I fibres and circles type IIX fibres. In the force-power curve type I fibres are the solid line and type IIX are the dashed line. In B power-velocity curves are represented as shown. (Bottinelli et al., 1996).*

More recent work, using single fibre PCr measurements, has yielded similar results with Krustrup *et al* (2004b) finding that during 10 min of cycling at 85 % $\dot{V}O_{2peak}$ there is a greater PCr and glycogen degradation in type I compared to type II fibres. These differences in metabolism between type I and type II fibres during short-term high-intensity and more prolonged submaximal exercise can be explained by the way in which these fibres are recruited during exercise, which is discussed in the following section.

2.3.2 *Muscle fibre type recruitment*

Human muscle fibres are organised into motor units, consisting of a single motoneurone and the group of fibres it innervates which will be all the same type (i.e. all type I, all IIA or all IIX fibres). It is generally agreed that motor units are recruited hierarchically dependent on motor unit size. This recruitment order is known as the size principle, and was originally proposed by Henneman *et al* (1965;1974), and states that at low forces small motor units (Type I fibres) are recruited and as force increases more large motor units (Type IIA followed by type IIX fibres) are recruited. Support of this theory comes from single motor unit electromyography (EMG) studies where it can, however, be difficult to interpret recruitment patterns from data encompassing several firing motor units in the vicinity of the electrode.

Moreover, there is some convincing evidence suggesting that this principle does not hold under all situations with some authors finding evidence of rate coding. This is the process whereby force is increased not by the recruitment of more muscle fibres but through an increase in the frequency or rate of action potentials. During cycling at an intensity equivalent to around 50 % of maximal dynamic force it has been found

that all fibres have been metabolically active (Vollestad & Blom, 1985;Greig *et al.*, 1985), demonstrated by glycogen depletion patterns, and the greater force that can be produced by the muscle suggested to be achieved through rate coding. Further work has also shown that in the biceps brachii new motor units are recruited up to around 90 % of maximal force, but in the adductor pollicis all motor units are recruited at 50 % of maximal force with rate coding providing the rest of the force increase (Kukulka & Clamann, 1981).

Asides from the use of EMG measuring glycogen depletion patterns from muscle biopsy samples is the other commonly used tool in investigations of fibre recruitment. Estimating fibre recruitment from glycogen depletion should, however, be interpreted with some care (Kernell *et al.*, 1995) as a decline in Periodic Acid Schiff (PAS) staining intensity may be a combined effect of both intensity and duration of exercise with a decrease in glycogen content having been observed in non-exercising muscles over time (McDermott *et al.*, 1987). For that reason new methods have been developed to assess muscle fibre activation using the PCr/Cr ratio (measured using high-performance liquid chromatography (HPLC) (Beltman *et al.*, 2004b)) and PCr measurements (luminometrically determined (Wibom *et al.*, 1991)) in single muscle fibres.

These novel techniques are useful since PCr splits immediately on activation of the muscle and in amounts energetically sufficient to account for the external work performed by the muscle fibre (Infante *et al.*, 1965). Further work has shown that changes in PCr content and thus the PCr/Cr ratio are good indicators of fibre activation and recruitment patterns (Conjard & Pette, 1999;Beltman *et al.*, 2004b). In fact studies using these methods have already provided details of fibre recruitment

during brief isometric and lengthening exercise (Beltman *et al.*, 2004a;Beltman *et al.*, 2004b;Beltman *et al.*, 2004c) and during more prolonged cycling and knee extensor exercise (Krustrup *et al.*, 2004a;Krustrup *et al.*, 2004b;Krustrup *et al.*, 2004c). Indeed Krustrup *et al* (2004b) has found that both type I and II fibres are recruited at the onset of moderate intensity cycling, providing further support for some rate coding during exercise. With the work of Beltman and colleagues also showing recruitment on the size principle with some evidence of rate coding during isometric exercise (Beltman *et al.*, 2004a) and also demonstrating no deviation from this hierarchical recruitment scheme during lengthening exercise (Beltman *et al.*, 2004c).

2.4 ELEVATION OF MUSCLE TEMPERATURE

2.4.1 Contractile properties

This thesis has so far described the basic metabolic and contractile processes occurring within skeletal muscle during exercise. The majority of these processes are, however, not fixed but demonstrate plasticity in both the short and long term. One factor which is known to alter muscular performance in the short term is a change in T_m, an area which has received relatively little attention in spite of the knowledge that *in vivo* T_m, may vary over a broad range depending on the environmental conditions and the heat liberated in the muscle itself (e.g. Asmussen & Boje, 1945;Saltin *et al.*, 1968) and increasing to approximately 40 °C during exercise (Robinson *et al.*, 1965;Saltin *et al.*, 1968).

The sensitivity of skeletal muscle contractile properties to changes in T_m, has been known since 1945, when Asmussen and Boje concluded that, "...a higher temperature

in the working organism facilitates the performance of work." In other words, raising T_m leads to an improvement in muscular performance. Further research, however, has revealed somewhat conflicting results, with the majority of workers finding that "warm-up" does indeed enhance athletic performance, via an increase in T_m (e.g. Asmussen *et al.*, 1976;Binkhorst *et al.*, 1977;Bergh & Ekblom, 1979;De Bruyn-Prevost & Lefebvre, 1980;Davies & Young, 1983) although some investigators reported little or no advantageous effect of 'warm-up' upon performance (e.g. Karpovich & Hale, 1956;Genovely & Stamford, 1982). These conflicting results are most likely due to the warming procedure and the contraction frequency at which the exercise was performed, with this discussed in more depth later on. Further work has led to the general agreement that T_m is a major determinant of skeletal muscle function (for reviews see Bennett, 1984;Rall & Woledge, 1990;Ranatunga, 1998).

Looking at specific aspects of contractile performance it has been found that as the temperature of mammalian muscles increases P_o of the muscle also increases (Ranatunga, 1982;Ranatunga & Wylie, 1983;Ranatunga, 1984;Stephenson & Williams, 1985;Ranatunga *et al.*, 1987;Bottinelli *et al.*, 1996;Steinen *et al.*, 1996) although not always (Davies *et al.*, 1982;Stewart *et al.*, 2003). The aforementioned studies which demonstrated a greater P_o as temperature increases, did so *in vitro* and at the low end of the physiological temperature range (up to 35 °C) and so these findings may not be transferable to the higher temperatures achieved during *in vivo* exercise. It would therefore appear that although P_o is only temperature dependent in the lower physiological range this is not be the case at the high physiological temperatures (37-40 °C) reached during exercise (Davies *et al.*, 1982;Stewart *et al.*, 2003).

As discussed previously skeletal muscle function conforms to a number of relationships, including the force-velocity relationship. The most temperature sensitive parameters of this relationship are the V_{max} and *a*/Po (describes the curvature of the force-velocity relationship), observed alongside an increased activity of mATPase (Steinen *et al.*, 1996). An increase in V_{max} and a decrease in *a*/Po (flattening of the relationship) with temperature have been demonstrated in rat (Ranatunga, 1982;Ranatunga, 1984) and also in human muscle (Binkhorst *et al.*, 1977;Davies *et al.*, 1982;He *et al.*, 2000;De Ruiter & de Haan, 2000). One might logically expect, therefore, that the mechanical power output of the muscle will increase, since as mentioned previously power is the product of both force and velocity, with a rise in T_m. Indeed, Ranatunga (1998) observed that the mechanical power output of rat intact fibre bundles increases significantly with warming within a physiological, although at the lower end, temperature range (25-35 °C). The temperature co-efficient (Q_{10}) within this temperature range was found to be 2-2.5. Similarly, whilst investigating the effects of temperature on handgrip muscles Binkhorst *et al* (1977) found that maximal power, Ft/Fo (value of force at which power is max), a/Fo and H (both parameters describing the shape of the power-velocity curve) increase, i.e. the curve becomes flatter, after raising the temperature in a water bath at 39 °C for half an hour. Similar findings have, also, been found studying the rabbit inferior oblique muscle (Asmussen *et al.*, 1994).

Further research with single human muscle fibres has demonstrated that increasing temperature resulted in a marked increase in maximum shortening velocity (2.7-2.9 times) and in peak power (3.8 times)(He *et al.*, 2000), Fig 2.8. Similar results have also been found by Bottinelli *et al* (1996) and Steinen *et al* (1996) using similar

muscle preparations. Furthermore, isometric tension and ATPase rates increased by around 20 %, which at velocities of up to ~60 % V_{max} increased in proportion to shortening velocities (He *et al.*, 2000). Interestingly, at each velocity the ATP consumption rate, relative tension and power was higher in fast than in slow muscles (He *et al.*, 2000).

During whole body exercise, which is of most relevance in the context of this thesis Sargeant (1987) observed a 4 % increase in power output per °C rise in quadriceps T_m during isokinetic maximal cycling at 95 revs.min^{-1} (Fig 2.9). Similarly Asmussen *et al* (1976) found a 4.2 % per °C and 5.1 % per °C increase in power output, during jumping and cycling respectively, after warming. Further work has also found similar increases in power output during a standing jump (Davies & Young, 1983). Interestingly, a greater rate of fatigue associated with the increase in maximal power has also been reported (Edwards *et al.*, 1972;Segal *et al.*, 1986;Sargeant, 1987;Prezant *et al.*, 1990), although some investigators have reported equal rates of fatigue at various T_m (Blomstrand *et al.*, 1985;Gerrits *et al.*, 2000).

The aforementioned study by Sargeant (1987) was the first study to clearly demonstrate that passively elevating the temperature of the working muscle leads to an increase in peak force and power output during whole-body dynamic cycle exercise, as demonstrated in Fig 2.9.

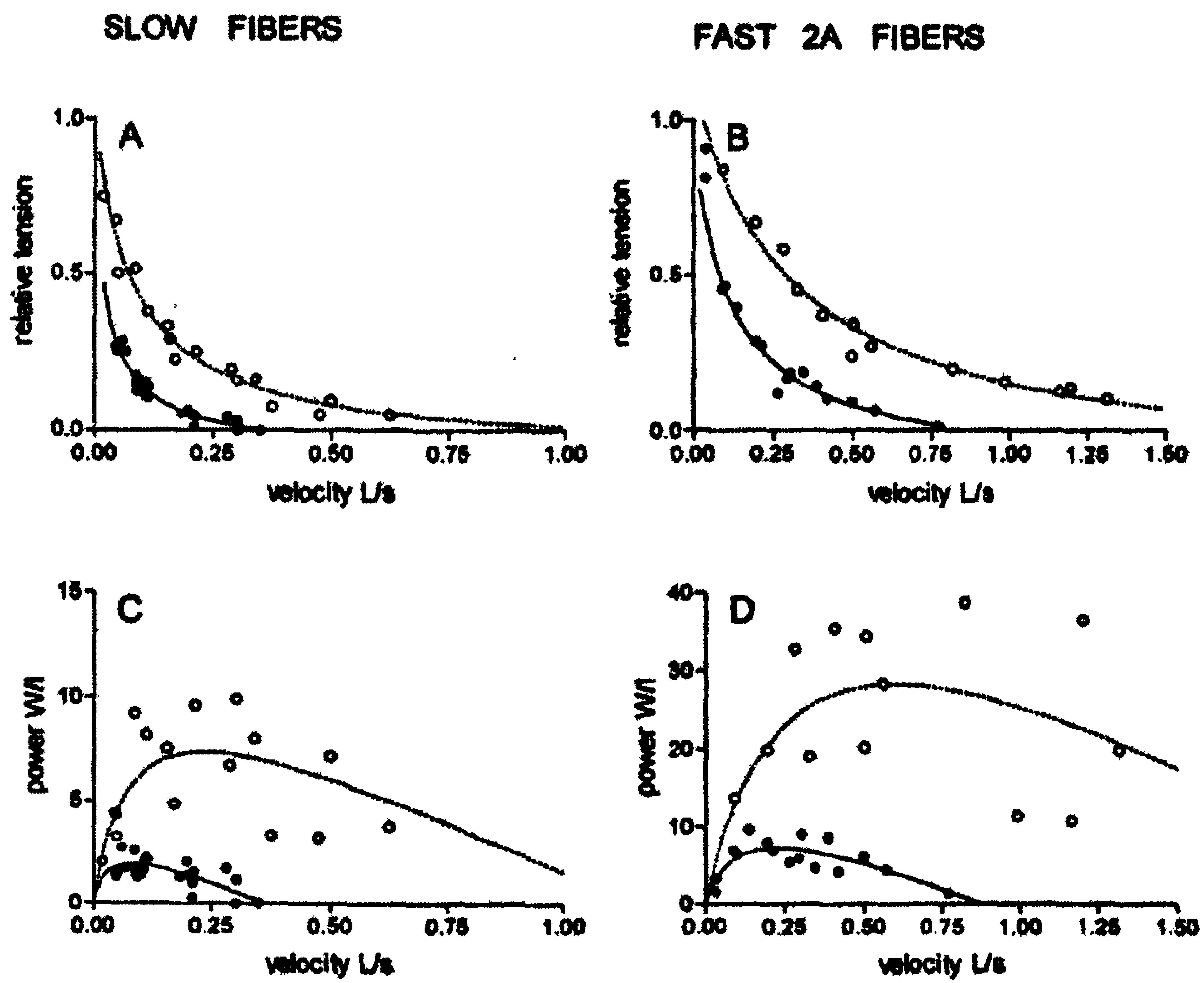


Fig 2.8. *Force-velocity and power-velocity relationships of slow and fast IIA fibres at 12 °C (closed circles) and 20 °C (open circles). (Taken from He et al., 2000).*

2.4.2 Effect of contraction frequency

The study of Sargeant (1987) also investigated the effect of T_m at different cadences during maximal sprint cycling. It was discovered that whilst power output was increased by approximately 4 % per °C at 95 revs.min^{-1} this fell to 2 % per °C at 54 revs.min^{-1}and increased to 10 % per °C at 140 revs.min^{-1}, demonstrating a velocity-dependent effect of temperature (Fig 2.10). Angular calculations have shown that during isokinetic cycling at 140 revs.min^{-1} (Sargeant, 1987) the knee was operating at an angular velocity of around 400 °s^{-1}, at the point of peak power production. The critical importance of the velocity of contraction may go some way to explaining why Davies and Young (1983) found a significant effect of temperature on power output generated during a standing jump, where the plantar flexion of the ankle will probably exceed 1000 °s^{-1} (Bobbert et al, 1986) and on the other hand were unable to demonstrate any temperature effect during cycling exercise, which was at an unspecified and possibly slow pedalling rate.

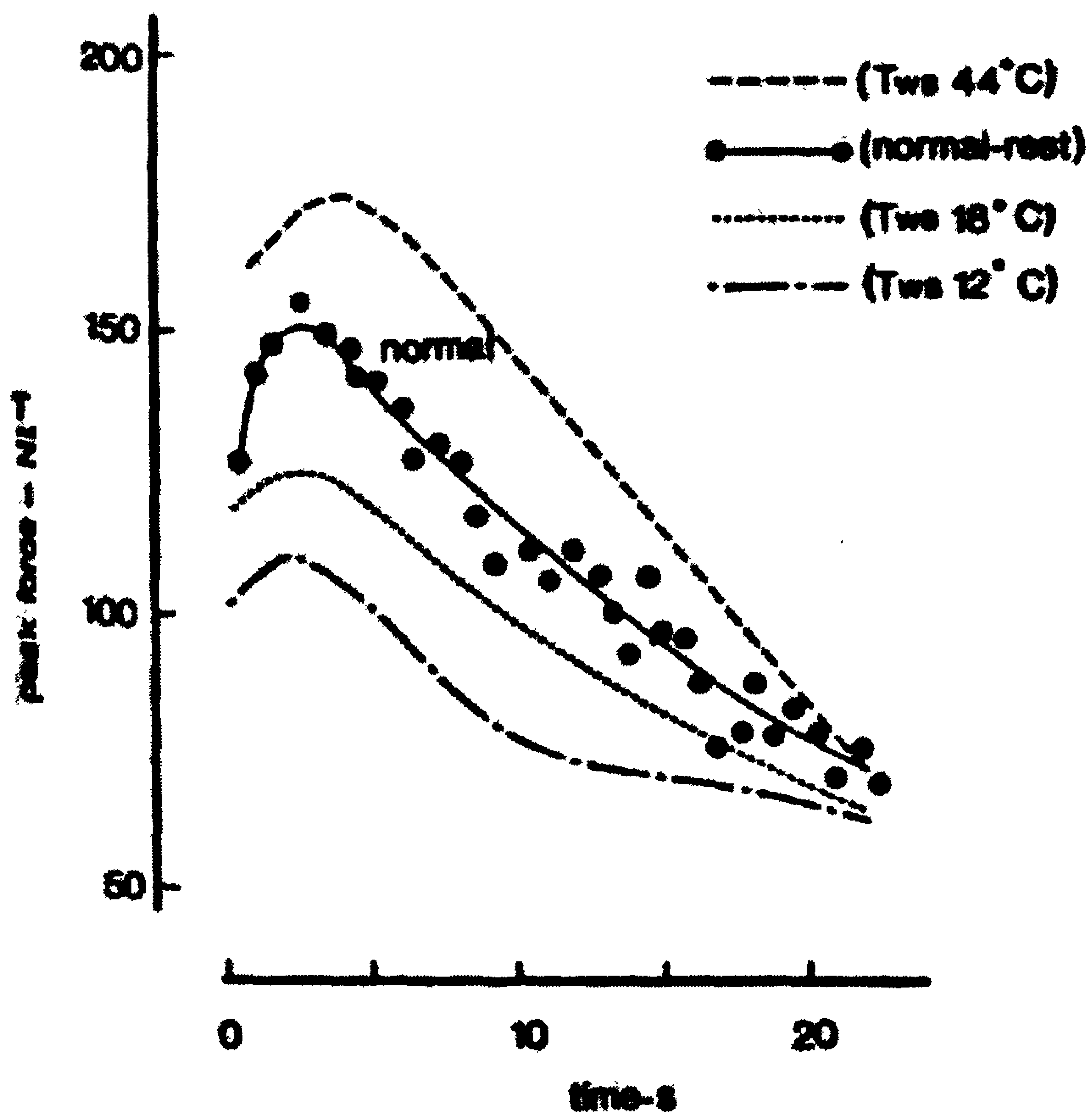


Fig 2.9. *Peak force during 20 s maximal isokinetic cycling at 95 revs.min^{-1} after immersion in water baths at different temperatures. (Taken from Sargeant, 1987).*

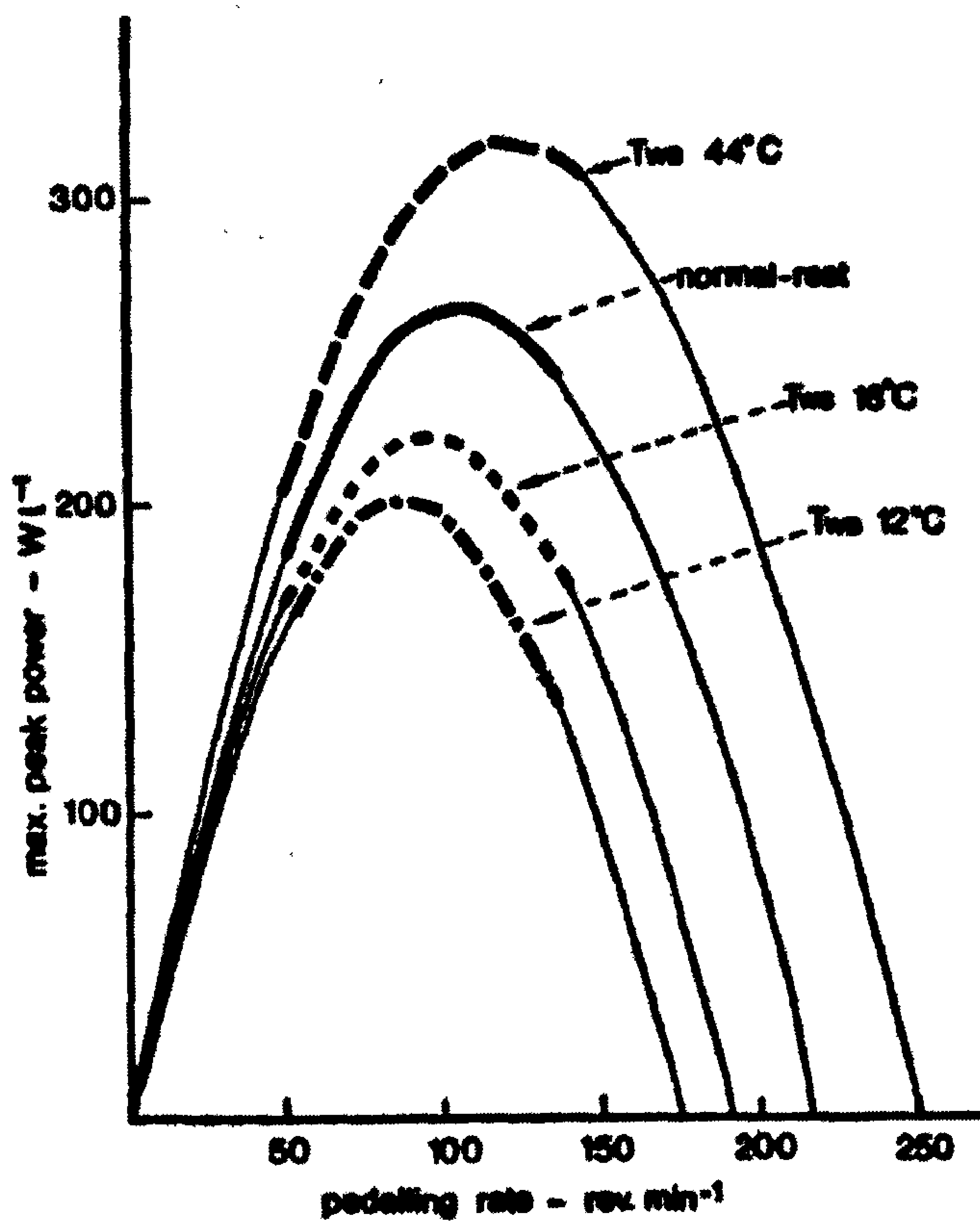


Fig 2.10. *Peak power produced during 20 s of maximal isokinetic cycling at 54, 95 and 140 revs.min^{-1} after immersion in water baths at various temperatures. Curves are extended through regression calculations. (Taken from Sargeant, 1987).*

2.5 PROPOSED MECHANISMS BEHIND ALTERATIONS IN CONTRACTILE PROCESSES

Essentially, previous work has shown that V_{max} and the a/Po of the force-velocity are most sensitive to changes in temperature and that the maximal power output of the muscle is also increased. Whilst this knowledge itself is useful in the world of sport and exercise the mechanism/or mechanisms behind the temperature dependent changes remain unknown, and may well have important implications in both the athletic and clinical world.

2.5.1 Increased oxygen delivery to the muscles

It has been suggested that changes in muscular performance following raised T_m may result from increased oxygen delivery, and therefore oxidative phosphorylation, to the muscles via a rightward shift in the oxyhaemoglobin dissociation curve and vasodilation of muscle blood vessels (McCutcheon *et al.*, 1999), thus providing more ATP to fuel the muscle towards a higher power output. Any increase in oxygen delivery to the muscles would only improve aerobic energy production if it was actually limited by the delivery of oxygen to the working muscle, as oxygen delivery may already be more than sufficient in supplying the working muscle.

The evidence that is cited to support a limitation in oxygen delivery is drawn from:

- The reported slowing of $\dot{V}O_2$ kinetics against a background of β-adrenergic

blockade, by propranlol and metoprolol (Twentyman *et al.*, 1981;Petersen *et al.*, 1983;Hughson, 1984) and during supine rather than upright exercise (Hughson *et al.*, 1993) – conditions considered to also slow muscle blood flow ($\dot{Q}_M$).

- Gausche et al (1984) reported that above the lactate threshold $\dot{V}O_2$ kinetics could be quickened by a prior warm-up bout of heavy exercise. They argued that this was likely to reflect an improved muscle perfusion due to the vasodilatory effects of the acidosis.

There is, however a great deal of evidence to suggest that $\dot{V}O_2$ kinetics are not limited by the delivery of oxygen to the working muscle at the transition from rest to exercise. The findings of Gausche et al (1984) have been contradicted by several authors (e.g. Burnley *et al.*, 2000) who found that phase II $\dot{V}O_2$ kinetics are not speeded by prior heavy exercise in humans. Furthermore, from the findings of De Cort and colleagues (1991) it can be reasoned that as a person's cardiac output has a more rapid response to exercise onset than $\dot{V}O_2$, then muscle blood flow must be sufficient to match $\dot{V}O_2$ at exercise onset.

Further research by Grassi *et al* (1998) has shown that artificially increasing blood flow to a pre-determined exercising value, in isolated canine gastrocnemius muscle, had no effect on $\dot{V}O_2$ kinetics (Fig 2.11), again suggesting that blood flow, and thus O_2 delivery, does not limit $\dot{V}O_2$ kinetics.

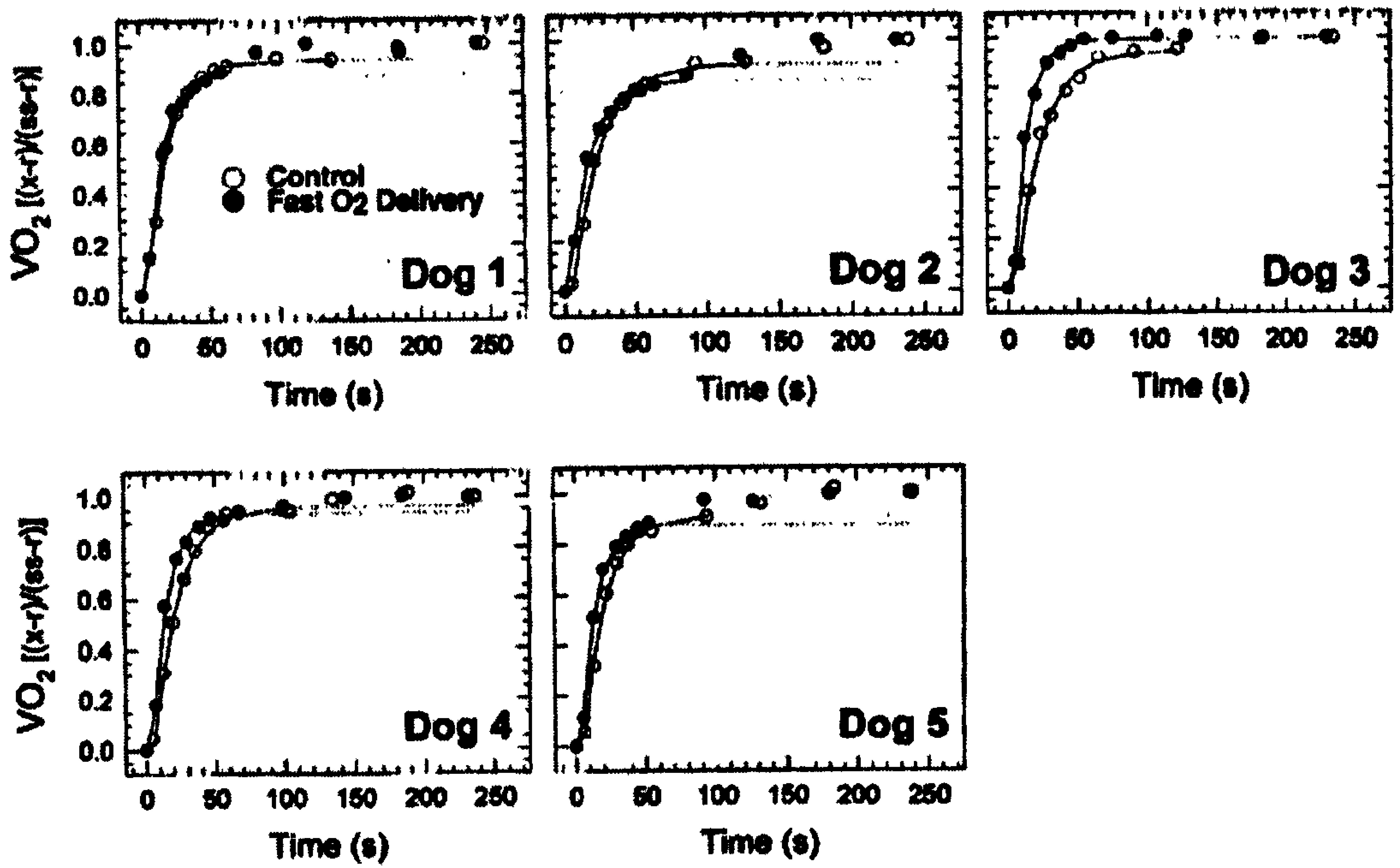


Fig 2.11. *Oxygen uptake during moderate intensity exercise under control and artificially fast oxygen delivery in dogs. (Taken from Grassi et al., 1998).*

It has also been shown that imposition of a lower-body positive pressure to levels that are known to reduce blood flow to the muscle does not slow $\dot{V}O_2$ kinetics (Williamson *et al.*, 1993). Furthermore, passive heating of the quadriceps, to around 40 °C, has also been reported not to speed $\dot{V}O_2$ kinetics (Koga *et al.*, 1997;Burnley *et al.*, 2002b). These findings all give evidence suggesting that $\dot{V}O_2$ kinetics are not limited by blood flow to the muscle. Similar findings have been shown at a microvascular level (Behnke *et al.*, 2001;Behnke *et al.*, 2002a;Behnke *et al.*, 2002b), demonstrating that the delivery of oxygen is sufficient to meet the metabolic demands of the exercising muscle across the rest to contraction transition.

On the other hand, all of the aforementioned studies investigated $\dot{V}O_2$ kinetics during moderate intensity exercise, with the response to heavy exercise being somewhat more complex (Whipp & Wasserman, 1972;Hughson & Morrissey, 1982;Barstow & Mole, 1991;Ozyener *et al.*, 2001). Another study by Grassi *et al* (2000) demonstrated that in the transition from rest to muscle $\dot{V}O_{2peak}$ eliminating all delays in convective O_2 delivery resulted in faster $\dot{V}O_2$ kinetics, a lower oxygen deficit and a decrease in muscle fatigue. On the other hand, Bangsbo *et al* (2000) found that muscle oxygen delivery to the muscle far exceeds $\dot{V}O_2$ at the onset of intense dynamic exercise and thus does not limit oxygen uptake. This is clearly still an area of much debate.

2.5.2 *Increased neuromuscular activity*

It has been proposed that an increase in T_m may contribute to improved performance by augmenting the function of the nervous system (Bishop, 2003). The contractile processes of skeletal muscle are initiated by Ca^{2+} release from the sarcoplasmic reticulum as a result of an action potential propagating along the muscle fibre

membrane (Melzer *et al.*, 1995). A measure of this action potential propagation is the muscle fibre conduction velocity (MFCV) which reflects the rate of muscle activation (Andreassen & Ardent-Nielsen, 1987). Indirect measures of EMG parameters have indicated that MFCV increases with T_m (Petrofsky & Lind, 1980;Winkel & Jorgensen, 1991;Van der Hoeven & Lange, 1994;Stewart *et al.*, 2003) leading to an increased power produced by the muscle. Recent advances in EMG analysis, allowing the direct calculation of MFCV during exercise, allowed Farina *et al* (2005) to observe a greater MFCV at higher T_m during low-force isometric contractions.

This increase in MFCV observed at higher T_m is likely due to a more rapid opening on voltage-gated Na^+ channels has been shown (Rutkove *et al.*, 1997;Rutkove, 2001) and reflects a more rapid action potential delivery to the muscle fibres and therefore a greater Ca^{2+} release from the sarcoplasmic reticulum. This increased Ca^{2+} release will allow individual muscle fibres within the muscle to be activated more rapidly, increasing the rate of cross-bridge cycling, requiring a greater supply of ATP, thus increasing the shortening velocity of the whole muscle. It may, therefore, be possible that an increased MFCV, a process likely to require a greater rate of ATP turnover, may drive the muscle to increase maximal velocity and power, although this has not been investigated during dynamic high-intensity exercise.

2.5.3 Increased anaerobic ATP turnover

As discussed in earlier sections, the rate at which ATP is provided is of great importance in supplying the muscle with the energy to support the development and maintenance of power output. It has been hypothesised, therefore, that the increase in power output and V_{max} reflects the increase in the activity of the mATPase since as

with other enzymatic processes mATPase activity is temperature dependent (Steinen *et al.*, 1996;He *et al.*, 2000) and is the most important determinant of shortening velocity (Barany, 1967). This increase in ATPase activity will probably reflect an increase in the rate of cross-bridge cycling at higher T_m, requiring an increased rate of ATP turnover within the muscle, although this has not yet been investigated.

As mentioned previously He *et al* (2000) observed that the ATP consumption rate during active shortening increased markedly after an increase in temperature from 12 to 20 °C. The results and model analysis of this investigation support the view that the increase in duty ratio with temperature at any given velocity of shortening is consistent with the view that the fraction of cross-bridges increases with temperature. Furthermore, these findings also suggest that force per myosin cross-bridge increases with increasing temperature, also suggested by Zhao and Kawai (1994). These studies, however, were carried out *in vitro* and at lower than 'exercising' T_m and are therefore not readily applicable to *in vivo* exercise.

The first experiment carried out in humans during *in vivo* exercise, at physiological temperature, was by Edwards and colleagues (1972) who looked at the effects of increasing T_m on metabolic processes during isometric contractions at $2/3^{rds}$ of maximum voluntary contraction. This study observed that when T_m was passively raised there is a greater rate of glycogen breakdown and lactate accumulation, representing an increase in glycolytic ATP turnover. The authors suggested at this time that the increase in T_m might increase the rate, and decrease the efficiency, of cross-bridge cycling. Although glycogenolysis was increased, however, this hypothesis was deemed unlikely since ATP and PCr stores were unaffected. Furthermore, this study also observed a rise in T_{rec} along with T_m which may have led

to the observed increase in glycogenolysis through a catecholamine response (Greenhaff *et al.*, 1991;Febbraio *et al.*, 1998) although this is by no means certain (Chasiotis, 1988;Wendling *et al.*, 1996).

Due to these possible drawbacks, Febbraio and colleagues (1996) conducted a study investigating the effects of T_m, without a rise in T_{rec}, on metabolism during 2 min cycling at approximately 115 % $\dot{V}O_{2max}$. This investigation revealed that when T_m is passively elevated there is an augmented decrease in the total adenine-nucleotide (TAN) pool. Furthermore, although the pre-exercise concentrations of lactate and glycogen were not different between the two trials (heated and control), post-exercise lactate was higher and glycogen was lower in the heated compared with the control trial. These changes along with ammonia accumulation, adenine nucleotide (AdN) degradation and IMP (ionosine 5'-momophosphate) accumulation insinuate that raising the temperature of the muscle increases both carbohydrate utilization and the energy contribution from anaerobic glycolysis and the AdN pool. From the data revealed in this experiment the researchers were not able to discover a precise mechanism for this phenomenon. However, it was hypothesized by the authors that either increased ATP turnover associated with the exercise and/or alterations to the anaerobic/aerobic contribution to energy turnover may be responsible for these metabolic alterations. Similar findings were also reported by Starkie *et al* (1999), although only glycogen utilisation was affected by temperature, with no differences in lactate or high-energy phosphate content, in this study where the exercise duration was 20 min. Although these studies provide detailed and useful information regarding the effects of T_m on the anaerobic energy turnover during constant load work no measure of aerobic energy contribution was made. Moreover, no studies have

investigated the effect of T_m on energy turnover when the external power output of the muscle is increased by the rise in T_m.

2.5.4 Increased efficiency

As previously mentioned alterations in energy turnover will clearly have an effect on mechanical efficiency if the work load on the muscle is constant, highlighting the close association between energy turnover and efficiency. The study mentioned previously by Edwards *et al* (1972) suggested that the efficiency of muscular contraction falls, demonstrated by the increase in energy utilization at a constant workload, when T_m was raised from 22.5 – 38.6 °C. This would mean that there is an increased cross-bridge cycling during isometric contraction at high T_m, requiring a greater energy turnover for the same level of sustained force. This is similar to the findings of Febbraio *et al* (1996), where workload was maintained constant at different T_m, although in neither of these studies aerobic energy production was accounted for. This is in spite of the fact that oxidative phosphorylation will be the main energy source (Medbo & Tabata, 1993;Parolin *et al.*, 1999) and therefore required for an accurate calculation of efficiency. It is important to note, at this point, that if exercise is to be sustained for longer than a few seconds; mechanical efficiency may well be an important determinant of performance and thus an important area of investigation.

In single human muscle fibres He and colleagues (2000) showed that slow and fast fibres have similar peak efficiencies despite the vast differences in power output and ATP consumption rate. These similar efficiencies, however, are reached at different contraction velocities, with the efficiency of slow twitch fibres being higher than fast

twitch fibres at low contraction velocities, and the opposite being observed at high velocities. Again, this is in general agreement with previous studies (Barclay, 1994;Barclay, 1996;Reggiani *et al.*, 1997). This leads to the hypothesis, on the basis of such observations both in the field and the laboratory, that type I muscle fibres and type II fibres have distinct efficiency/velocity relationships related to human movement frequencies, which are shown in Fig 2.12.

This schematic illustrates the hypothesized form of the mechanical efficiency/velocity relationship for both type I and type II muscle fibres. Again it is important to note that this efficiency/velocity relationship is not a fixed property but labile, and may be altered with changes in T_m, with different fibre types possibly showing variations in sensitivity at different T_m. At present there has been little work on the effect of T_m on the efficiency of human muscle, especially during whole body exercise and in single muscle fibres. The research of He et al (2000) also demonstrated that when T_m is raised both type I and IIA fibres show a similar increase in peak efficiency, with the magnitude of the change being dependent on the shortening velocity (He *et al.*, 2000).

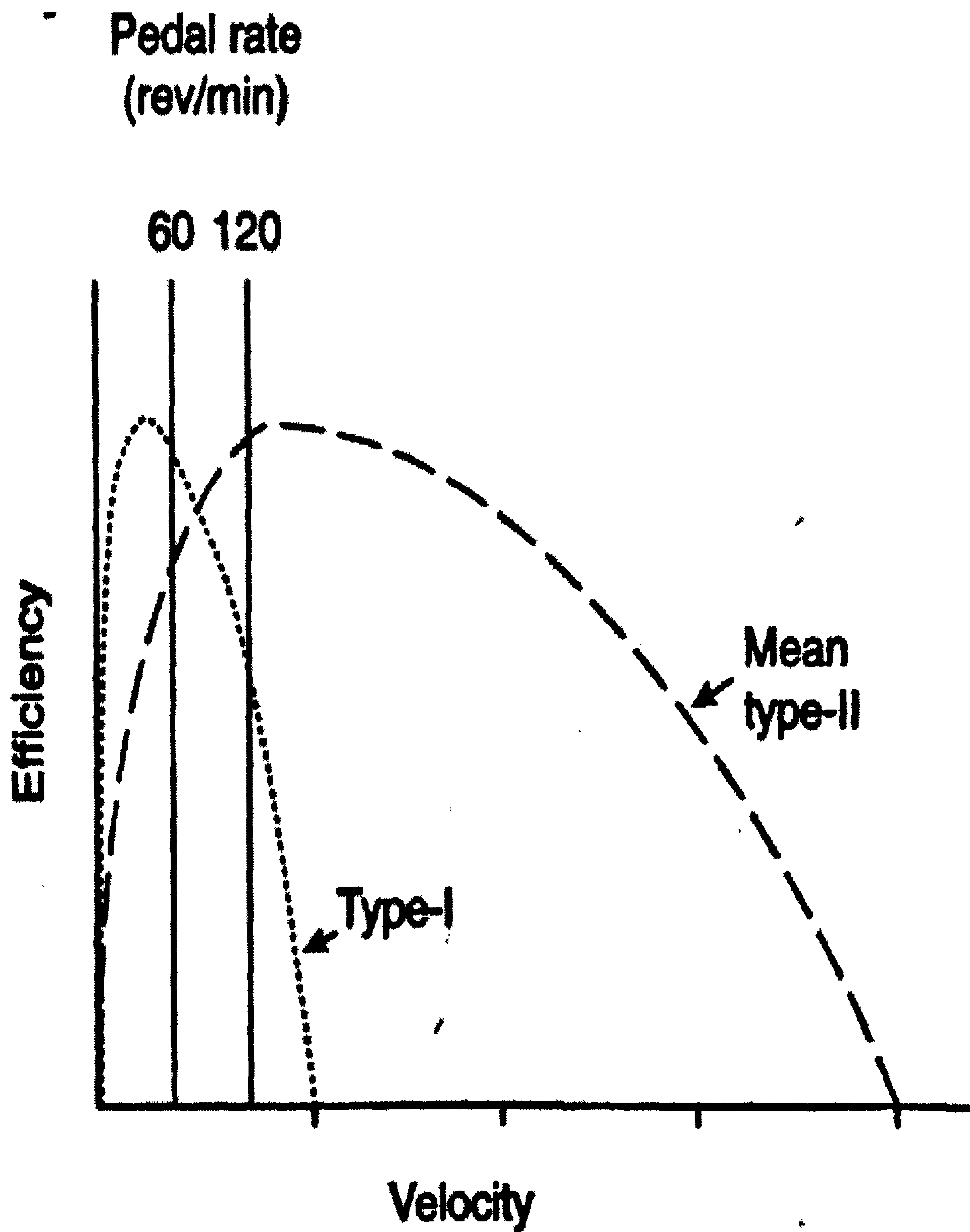


Fig 2.12. *Schematic representation of the relationship between mechanical efficiency and velocity in type I and type II fibres. Velocities equivalent to 60 and 120 revs.min^{-1} are included in the schematic. (Taken from Sargeant, 1999).*

During whole body exercise Ferguson and co-workers (2002) found that passive heating of the human skeletal muscle prior to cycling at 85 % $\dot{V}O_{2max}$ leads to an increase rate of energy turnover of 5.2 % (aerobic + anaerobic) at 60 revs.min^{-1} and a decrease of 5.9 % at 120 revs.min^{-1}. During these trials the total mechanical power (internal and external components) remained constant. This means that at 60 revs.min^{-1} there was a decrease in net mechanical efficiency of ~ 1 % and, on the contrary, a relative increase in net mechanical efficiency of just over ~ 1 % at 120 revs.min^{-1}. The authors of this study hypothesised that increasing the T_m leads to an increase in cross-bridge cycling which will be inappropriately fast at 60 revs.min^{-1} and thus leads to an increased energy turnover and decreased efficiency and that at 120 revs.min^{-1} type I fibres will be working closer to their optimum, due to a rightward shift in the efficiency velocity relationship. It must be noted, however, that estimates of efficiency in this work were made through blood lactate and $\dot{V}O_2$ measurements, with blood lactate not necessarily reflecting anaerobic muscle metabolism since lactate can be taken up and metabolised by various other tissues (Gladden, 2000;Gladden, 2004). During knee-extensor exercise at 60 kicks per minute, where efficiency was estimated through thigh oxygen uptake and muscle metabolites, a passive increase in T_m was found to have no significant effect on mechanical efficiency, although these differences may be due to the mode of exercise (Ferguson *et al*, 2006).

2.5.5 Single fibre sensitivity

There has been no research, as yet, investigating the effects of elevated T_m on the efficiency or ATP turnover of specific fibre types during whole body exercise, although previous work has suggested a greater sensitivity of type I fibres to temperature changes.

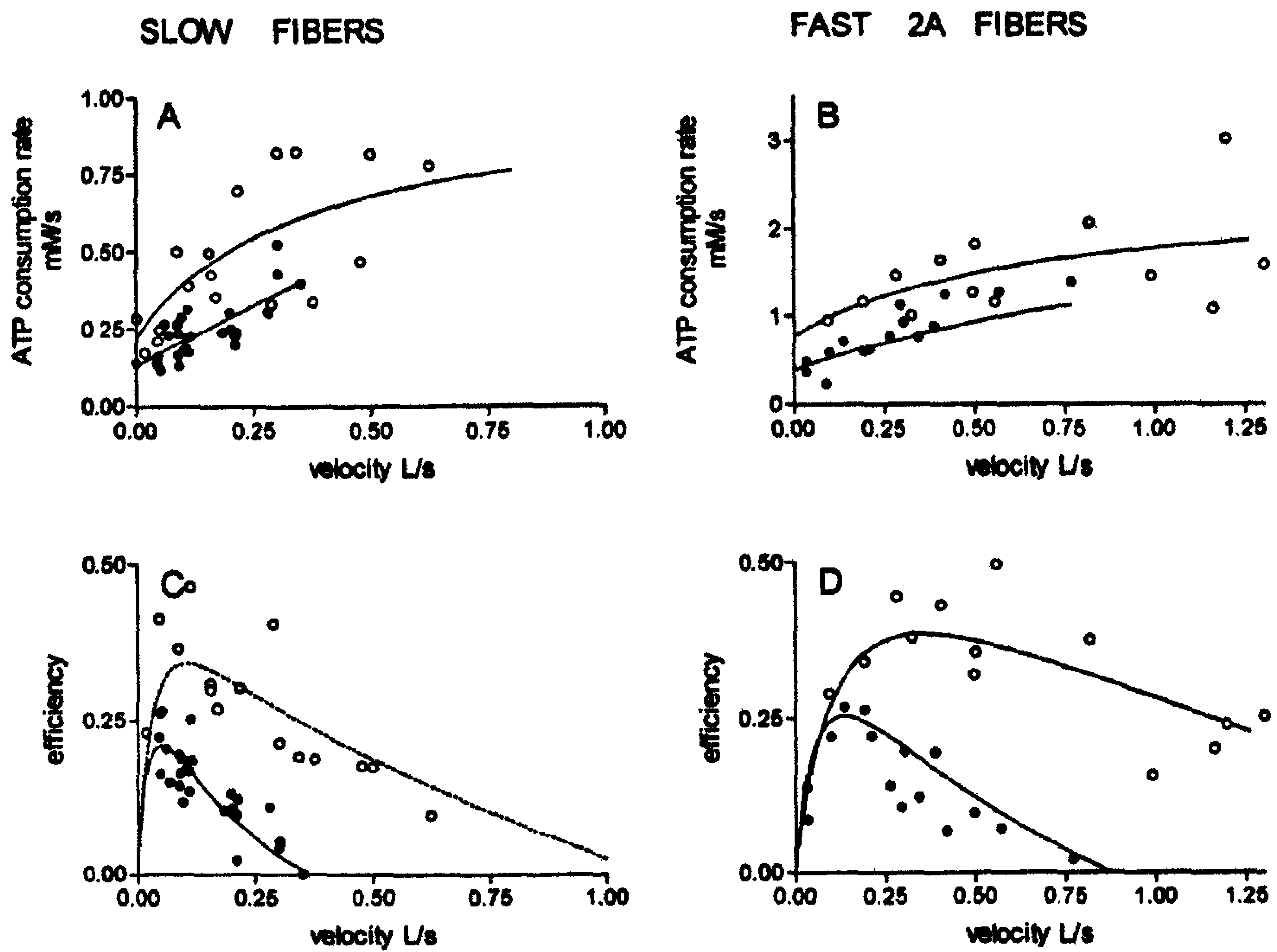


Fig 2.13 *ATP consumption rate and efficiency velocity relationships in slow and fast fibres at 12 (filled circles) and 20 °C (open circles), (Taken from He et al., 2000).*

Blomstrand *et al* (1985) indicated that when the temperature of the rat muscle was 36 °C compared to 28 °C, there was an increase in glycogenolysis and glycolysis in slow twitch but not fast twitch muscles, in response to electrical stimulation, indicating that the type I fibres may have a greater ATP turnover at higher temperatures and a greater contribution to power output. Similar studies have found a greater temperature sensitivity of soleus compared to the EDL muscle (Ranatunga, 1984).

During whole body exercise, in humans, it has been inferred that at contraction frequencies of 60–140 revs.min^{-1} increasing temperature will have the greatest impact on type I fibres, with respect to power production (Sargeant & Rademaker, 1996;Rademaker, 1997). During this study the percentage of type I fibres from biopsy samples was closely correlated with the Q_{10} values for power output in this cadence range, suggesting a greater power contribution of type I fibres may lead to a greater power output. This study however did not make any measure of the ATP turnover, efficiency or activation levels of the different fibre types leaving any conclusions speculative at best.

2.6 AIMS AND HYPOTHESES

In summary it has been known for many years that increasing the temperature of the muscle can have profound effects on contractile performance. The main effects are to increase maximal shortening velocity, the curvature of the force-velocity relationship and ultimately to increase the power output of the contracting muscle. The main aim of the present thesis is to determine the metabolic consequences of this rise in muscle temperature in an attempt to uncover the mechanisms behind said contractile changes and to determine whether this affects efficiency during exercise. Indeed, there have

been no previous attempts to investigate the effects of muscle temperature on efficiency, measured at the level of the muscle, during dynamic exercise.

In these investigations the main hypotheses are that the greater power output obtained at higher muscle temperatures will occur alongside an enhanced rate of anaerobic ATP turnover, MFCV and a greater PCr degradation in type I fibres. During more sustained exercise, at 60 revs.min^{-1}, the effect of a passive increase in T_m on mechanical efficiency and single fibre metabolism will be investigated, with the hypothesis being that increasing T_m will decrease mechanical efficiency due to a preferential effect on type I fibres. Finally, when T_m is elevated by prior exercise its effect on mechanical efficiency was determined at both 60 and 120 revs.min^{-1}. It was hypothesised in this experiment that efficiency would decrease at 60 revs.min^{-1} after prior exercise, with little or no effect at the faster pedal rate.

Chapter 3

GENERAL METHODS

3.1 SUBJECTS AND ETHICAL APPROVAL

In all the experimental studies in this thesis male volunteers were recruited to participate. Subjects were, at the time of participation, between 18 and 35 years of age, in good health and habitually physically active though not specifically trained. Before any participation the purposes, nature, any possible discomfort and risks of the study were fully explained both orally and in writing before subjects signed an informed consent form (Appendix A). Each experiment was approved by the University of Strathclyde Ethics committee prior to its onset.

Prior to attending the laboratory for an experimental trial subjects were asked to follow certain standard procedures. They were asked to refrain from any strenuous physical activity and ingestion of alcohol and caffeine for the 48 h period prior to each experimental trial. During this period subjects were also asked to record their dietary intake and to replicate this prior to any subsequent trials.

3.2 PRE-EXPERIMETAL TRIALS

3.2.1 Measurement of $\dot{V}O_{2peak}$ and LT

$\dot{V}O_{2peak}$ and LT were determined in chapters 6 and 7 using a continuous incremental cycling test, modified from the treadmill protocol described previously (Jones, 1998), to volitional exhaustion on an electronically braked cycle ergometer (Excalibur Sport, Lode, BV, Groningen). The starting workload for this test was dependant upon the activity levels of the subject and in chapter 7 on the pedal cadence, and ranged from between 50 and 100 W. The power output was increased every three minutes by

between 20 and 35 W with a fingertip blood sample taken at the end of each 3 min stage and analysed immediately for lactate concentration (Lactate Pro, Akray KDK, Koyota, Japan). HR and RPE were also recorded at the end of each stage with $\dot{V}O_2$ measured throughout the test. Approximately three stages were completed before and after LT, which was later calculated through the linear regression of two lines of blood lactate plotted against workload (Fig 3.1), according to the method described by Beaver *et al* (1985).

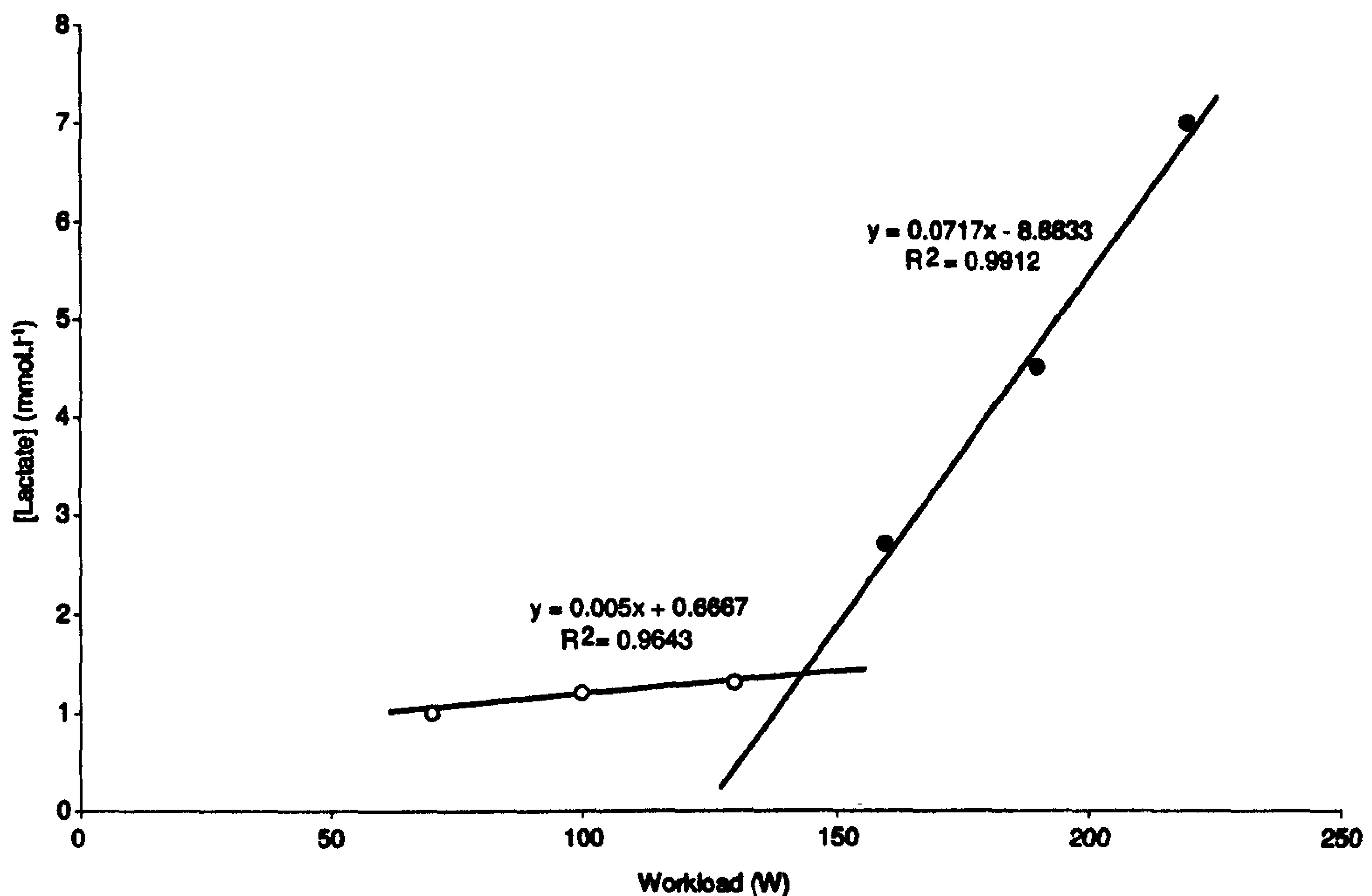


Figure 3.1 *Example of the determination of lactate threshold from one subject in chapter 7, with the regression equations for each line, used to calculate LT, shown.*

Three stages after LT the workload was increased every minute until subjects could no longer continue, or maintain the desired pedal rate. $\dot{V}O_{2peak}$ was taken as the highest $\dot{V}O_2$ measured over a 30 s period.

3.2.2 Familiarisations

Prior to participation in the experimental trials of chapter 4 and 5, which involved a 6 s maximal sprint on a friction-braked cycle ergometer (Monark, Sweden), subjects practised the sprint on at least 3 occasions. In chapters 6 and 7 subjects completed a familiarisation of the experimental protocol, with $\dot{V}O_2$ measured, to accustom the subjects to the exercise workload and to exercising with the mouthpiece in. This familiarisation is necessary in experiments where an accurate characterisation of oxygen uptake is required (e.g. Whipp *et al.*, 1982;Ozyener *et al.*, 2001).

3.3 ELEVATION OF MUSCLE TEMPERATURE

3.3.1 Passive heating

In chapters 4, 5 and 6 the temperature of the legs was passively elevated using the following protocol. In the first instance subjects stood in a hot water tank for 30 min, up to the gluteal fold, with the water at a temperature of approximately 42 °C. Subjects then exited the water tank, towelled dry, changed their shorts and then lay on an examination couch where their legs were wrapped in electrically heated blankets and a foil survival sheet. The use of electric blankets allowed T_m to be measured, biopsy sites to be prepared (chapters 4, 5 and 6) and an EMG electrode and goniometer to be attached (chapter 4), whilst maintaining T_m. Subjects would then lay

at rest with their legs wrapped until a T_m of around 37-37.5 °C was reached.

3.3.2. *Prior exercise*

In chapter 7 prior exercise was used to increase the temperature of the legs. This involved subjects cycling on the electronically braked cycle ergometer at a pedal rate of 90 revs.min^{-1} at the workload at which $\dot{V}O_{2peak}$ was obtained. Two 2 min bouts were performed with 2 min recovery period between each bout with a third bout to exhaustion completing the prior exercise (Fig 3.2).A further 2 min rest was given followed by 2 min unloaded cycling and then the 6 min experimental trial.

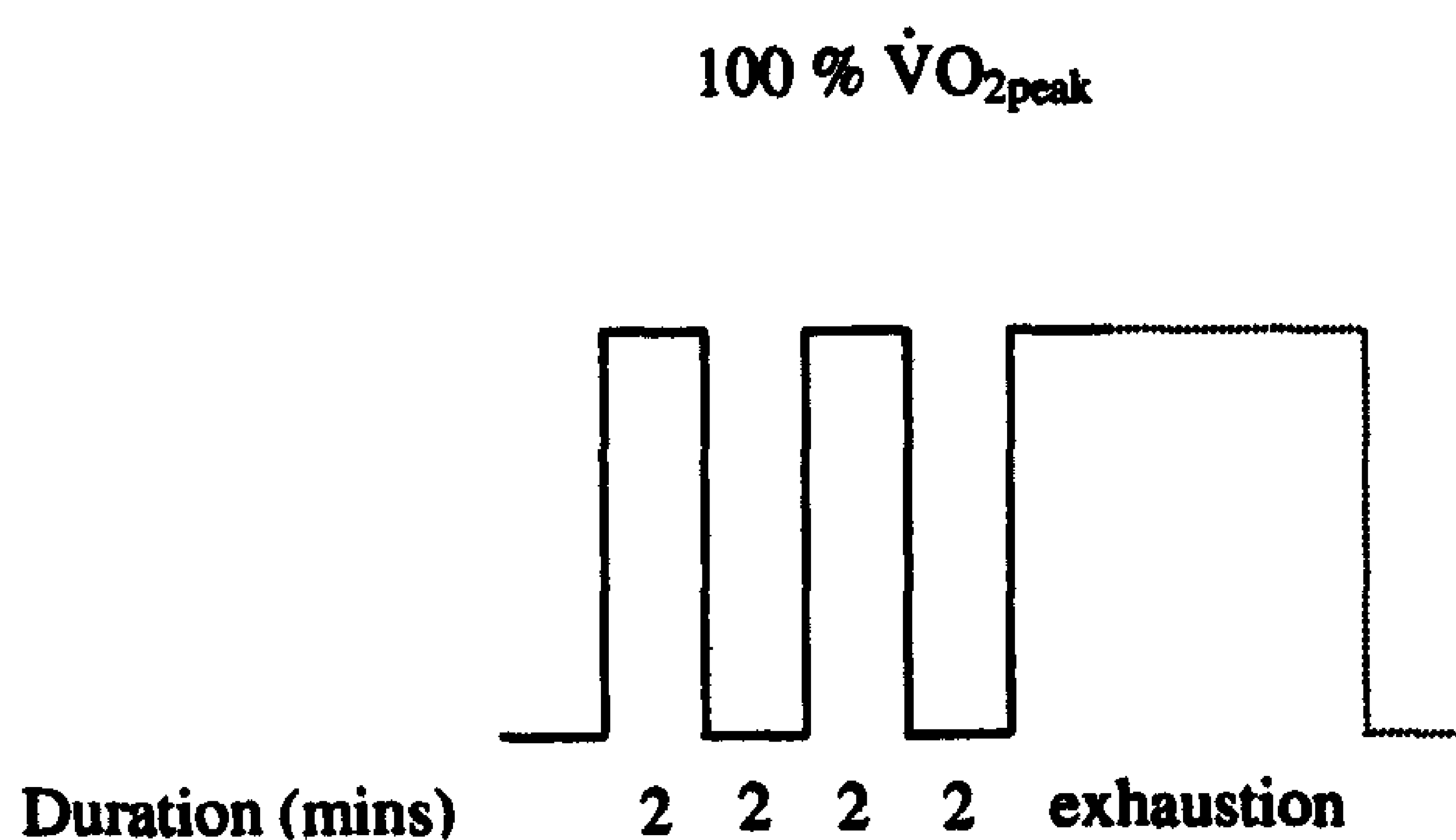


Figure 3.2. *Schematic diagram of the prior exercise protocol employed in chapter 8.*

3.4 PHYSIOLOGICAL MEASUREMENTS

3.4.1 Pulmonary gas exchange

Pulmonary gas exchange was measured breath-by-breath using an on-line gas analysis system (Oxycon Pro, Jaeger, Hoechberg, Germany) (chapter 6 and 7) through a low-dead space (30 ml), low resistance mouthpiece (0.75 $mmHg.l^{-1}.s^{-1}$ at 15 $l.s^{-1}$) and impeller turbine assembly (Jaeger Triple V) during the familiarisation and main trials. Each breath was analysed for inspired and expired gas volume and through a capillary line leading directly from the mouthpiece to the analyzer for expired O_2 (paramagnetic analysis - 0.05 % accuracy) and CO_2 (infrared analysis - 0.05 % accuracy). The gas analyzer was calibrated prior to each test with a gas mixture with known concentrations of O_2 (16.00 %) and CO_2 (5.00 %) and the volume calibrated automatically with different air flows (0.2 and 2.0 $l.s^{-1}$) passed through the mouthpiece. During this automatic calibration the gas transit delay in the capillary tube and the analyser rise time relative to the volume signal were accounted for allowing the calculation of $\dot{V}O_2$, $\dot{V}CO_2$ and $\dot{V}_E$ using standard methods (Beaver *et al.*, 1973). During all tests a nose-clip was worn to ensure all inspired and expired gases were passed through the mouthpiece.

The breath-by-breath data collected in chapters 6 and 7 were used to estimate the kinetics of the $\dot{V}O_2$ response. Firstly any outlying breaths, defined as any breath which deviated by more than three standard deviations from the five preceding breaths, were manually removed. Following this the values were interpolated to give second-by-second values for each exercise bout. The two data sets (as each experimental condition was repeated once) were time aligned and averaged to give

one set of values for each condition for each subject, which was used to characterise the $\dot{V}O_2$ response. Data were modelled with a mono-exponential function using a procedure described by Burnley *et al* (2006), modified from the earlier work of Rossiter *et al* (2002). The first 20s of data following the onset of exercise were removed, thus eliminating phase I of the response. The data between 20-120s (the first 2min) was then modelled with eq 9 using commercially available software (Graph Pad Prism, version 2.01):

$$\dot{V}O_2(t) = \dot{V}O_2(b) + A \times (1 - e^{-(t-TD)/\tau}) \qquad \text{(eq 9)}$$

where $\dot{V}O_2(t)$ is the $\dot{V}O_2$ at time t, $\dot{V}O_2(b)$ is the baseline $\dot{V}O_2$ measured in the 120 s (chapter 7) and 180 s (chapter 6) preceding the transition in work rate. *A*, *TD* and τ are the amplitude, time delay and the time constant of the phase II response, respectively.

The amplitude of the slow component in these chapters was determined by subtracting the absolute amplitude of phase II ($\dot{V}O_2(b)$+ A) from the end exercise $\dot{V}O_2$ (the average $\dot{V}O_2$ in the last 30 s of the exercise bout). Fitting this model to 120 s of data is used in this thesis in an attempt to isolate the phase II component of the response, rather than iteratively optimizing the fit (Rossiter *et al.*, 2001;Koga *et al.*, 2005a), which cannot be confidently employed to responses which contain a small slow component. This analytical method provides functionally identical parameter estimates (Burnley *et al.*, 2006) and on top of that increases the confidence in the phase II parameters (Whipp & Rossiter, 2005). On top of this rational using a 20-120 s time period to fit the data means that the parameter estimates will be minimally

affected if a slow component were to emerge prior to 120 s since its contribution to the response amplitude will be very small in this period of time.

3.4.2. Rectal and muscle temperatures

Rectal temperature was monitored throughout all experimental trials by the insertion of a rectal thermistor probe (Grants Instruments ltd, Cambridge, UK) 10 cm beyond the anal sphincter, connected to a 1000 series 8-bit squirrel data logger (Grants Instruments ltd, Cambridge, UK). After use rectal probes were cleaned with 70 % isopropyl alcohol and then sterilised and a 1 % Virkon™ solution (Antec International, Suffolk, UK) overnight.

In chapters 4, 5 and 6 pre-exercise T_m was monitored with a flexible temperature probe (Ellab UK ltd, Norfolk, UK). This probe was fed through, and advanced beyond the end of, a flexible venflon cannula (18 G) inserted into the vastus lateralis muscle to a depth of approximately 3 cm, in the direction of the muscle fibres. In chapter 7 a solid temperature probe (Ellab UK ltd, Norfolk, UK) was used to measure the temperature of the muscle. Again the probe was inserted to a depth of 3 cm into the vastus lateralis muscle and on removal the temperature also recorded at depths of approximately 2 and 1 cm. Both T_m probes were connected to a precision thermometer (Ellab UK ltd, Norfolk, UK) to give an accurate (± 0.1 °C) measure of T_m. Both temperature probes were calibrated at various water temperatures against a precision mercury thermometer before the onset of each experiment. Furthermore, T_m probes were also sterilised at 126 °C for a minimum of 11 min in an autoclave (Prestige Medical, Series 2100, Lancashire, UK) prior to each use.

3.4.3 Electromyography

In chapter 4 the motor unit innervation zone of the vastus lateralis was detected and marked in 4-5 test contractions with a dry array of 8 electrodes (silver bars, 5 mm long, 1 mm diameter, 10 mm inter-electrode distance), placed along the estimated fibre orientation. This motor unit innervation zone was identified as the point of inversion of the propagation of the detected potentials (Masuda *et al.*, 1985;Merletti *et al.*, 2003). Multichannel surface EMG signals were detected from the vastus lateralis of the non-biopsy leg, in chapter 4, with a linear adhesive array (model ELSCH008, SPES Medica, Salerno, Italy) positioned between the motor unit innervation zone and the knee. Each array consisted of eight electrodes with 5 mm interelectrode distance, in bipolar configuration. Before array placement the skin was lightly abraded with an abrasive paste (Spes Medica, Battipalgia, Italy) and 30 μl of conductive gel was pipetted (Eppendorf AG-multipipette plus, Hamburg, Germany) into each groove of the array to ensure 'clean' electrode-to-skin contact. The EMG signals were amplified (16 channel sEMG amplifier, EMG-16, LISiN, Prima Biomedical and Sport, Treviso, Italy), band pass-filtered (–3 dB bandwidth, 10–500 Hz, 40 dB decade), and sampled at 2 kHz. An electrogoniometer (Biometrics, Gwent, UK) was also positioned around the knee joint, secured with tape and connected to the EMG recording box with a cable specifically designed for this purpose by Enrico Merlo of Politecnico di Torino.

Average MFCV was then calculated from the multichannel sEMG signals during the cycling exercise. This off-line analysis of sEMG signals was performed kindly by Dr Dario Farina from Aalborg University, as described in detail previously (Farina *et al.*, 2004). The algorithm used is based on the computation of the mean square error between the signals of the array to which a time shift is applied. The time shift leading

to the minimum mean square error is that for which the signals are best aligned and is assumed as the estimated delay of propagation. MFCV is computed as e/t_d, where e is the interelectrode distance and t_d is the estimated delay (time shift in the alignment procedure). The method applies a Gaussian window to the mean square error, centered at the time instant at which MFCV is estimated, so that the action potentials with increasing distance from the center of the Gaussian window (i.e., the time instant of MFCV estimation) contribute to the delay estimation with a progressively decreasing weight. The standard deviation of the Gaussian window (set to 30 ms) defines the time interval from which MFCV is estimated. For each subject, the same channels were used for estimation of MFCV in the heated and control trials, avoiding any bias in the comparison of MFCV in the two conditions.

3.4.4 Maximal power output.

In chapters 4 and 5 maximal power output was determined during a 6 s sprint performed on a mechanically braked cycle ergometer (Ergomedic 824E, Monark, Verburg, Sweden), with a load of 7.5 % of the subjects body mass applied to the basket. From the sprint power output was calculated (BBC Acorn Computer, London, UK) every second from the known frictional load and the measurement of flywheel velocity and corrected for the acceleration of the flywheel (Lakomy, 1986). Mean power output, maximal pedal rate, and mean pedal rate were also calculated during the 6 s sprint.

3.5 VENOUS BLOOD SAMPLING

Venous blood samples (Chapter 7) were collected from an antecubital vein via an

indwelling cannula (20 G, Becton Dickinson, Cowley, Oxford, England). This was kept patent by flushing with small volumes of sterile saline solution (0.9 % sodium chloride BP). Samples (5 ml) were withdrawn from the cannula into sterile plastic syringes and mixed in tubes containing K^+EDTA (1.75 $mg.ml^{-1}$). Prior to all blood sampling the residual saline in the dead space of the cannula and tubing was withdrawn and discarded.

3.6 BLOOD ASSAYS

3.6.1 Blood Lactate

Principle:

This method for the determination of blood lactate is modified from the fluorometric method described by Maughan (1982) and was carried out on a Hitachi F-2500 fluorescence spectrophotometer (Hitachi Scientific Instruments, Berkshire, England).

$$\text{Lactate} + NAD^+ \xrightarrow{LDH} \text{Pyruvate} + NADH_2 + H^+$$

In this assay the above reaction is pushed to its end-point through the addition of an excess NAD^+, addition of the enzyme LDH, high pH for the removal of H^+, and the use of hydrazine to trap the pyruvate produced in the reaction. The $NADH_2$ produced in this reaction is highly fluorescent and is measured at an excitation wavelength of 340 nm and an emission wavelength of 450 nm to give the concentration of lactate present in the samples.

Reagents and enzymes:

Buffer: Hydrazine 1.1 $mol.l^{-1}$, pH 9.0 consisting of,

5.0 ml hydrazine hydrate

1.3 g hydrazine sulphate

0.2 g Na_2EDTA

made up to 100 ml with de-ionised H_2O (stored at 4 °C)

Cofactor: NAD^+, made up to a final concentration of 5 $mmol.l^{-1}$.

Enzyme: LDH from rabbit muscle (5 mg/ml, 550 U/mg, Roche Cat. No. 127 876)

Reaction Mix: 15 ml of reaction mix, containing 0.0497g NAD^+, 150 µl LDH (412.5 Units), made up to 15 ml with the hydrazine buffer, was sufficient to run 5 standards in triplicate and 17 samples in duplicate. The reaction mix was prepared fresh prior to each run of the assay.

Standards:

Standards were prepared every four weeks from a stock solution (4.44 $mmol.l^{-1}$, Sigma, Cat. No. 826-10 (from Trinity Biotech)) and stored refrigerated.

Stock solution (4.44 $mmol.l^{-1}$) (ml)	0.0	0.1	0.3	0.5	1.0
H_2O (ml)	5.0	4.9	4.7	4.5	4.0
Lactate concentration ($mmol.l^{-1}$)	0.0	0.09	0.26	0.44	0.88

Procedure:

1. After collection in the K^+EDTA tubes duplicate 100 µl aliquots of whole blood were immediately pipetted into 1 ml of ice-cold 2.5 % (v/v) PCA and mixed thoroughly. Samples were then centrifuged (Eppendorf 5403, Cambridge, UK) for 10 min at 4000 revs.min^{-1} and 4 °C and the supernatant of the de-proteinised blood removed and stored at -20 °C until analysis.

2. 20 µl of either standard or sample were added to 200 µl of the reaction mix, mixed thoroughly and incubated, in the dark, for 30 min at room temperature.

3. After incubation the fluorescence of each standard/sample was read. A calibration curve was constructed for the standards and the concentration of the samples calculated, taking into account the original dilution of sample in PCA.

3.6.2 Haemoglobin

Principle:

The concentration of Hb was determined from whole blood samples, collected in K^+EDTA, using a commercially availably kit (Randox Laboratories Ltd, Crumlin, UK). When in the presence of alkaline potassium, ferricyanide Hb is oxidised to methaemoglobin which will react with potassium cyanide to form cyanmethaemoglobin which absorbs at 540 nm. The intensity of this absorbance is directly related to the total Hb concentration.

Reagents:

Drabkin's reagent	Potassium Phosphate	52 mmol.l^{-1}
	Potassium Ferricyanide	30.4 mmol.l^{-1}
	Potassium Cyanide	38.4 mmol.l^{-1}
Brij-35 Solution		25 %

made up to 1 l with de-ionised H_2O (stored at room temperature in a dark bottle).

Standards:

Standards were prepared by dilution of the provided 18 g/dl stock solution of methaemoglobin with drabkin's solution.

Standard solution (ml)	0	2	3	4	5	6
Drabkin's solution (ml)	6	4	3	2	1	0
Haemoglobin concentration (g/dl)	0	6	9	12	15	18

Procedure:

1. The sample preparation for this assay was carried out on a semi-automatic pippettor (Microlab ® 500 diluter, Hamilton, Birmingham, UK), with 10 µl of sample being mixed with 2.5 ml of Drabkin's solution in a cuvette. Cuvettes were then left to incubate in the dark for 15 min at room temperature.

2. The blank was used firstly to zero the spectrophotometer (Aquarius CE-7400, Cecil

Instruments, Cambridge, UK) at 540 nm, after which the absorbance of the standards and the samples were measured in duplicate.

3. A calibration curve was then constructed from the standards, from which the Hb concentration of the samples was calculated.

3.6.3 Haematocrit

Principle:

Haematocrit describes the proportion of the total blood volume which is erythrocytes. After centrifugation the haematocrit is measured as the height of the red cells as a percentage of the total height of the blood. Samples are then corrected for plasma trapped within the packed red cells (0.98) (Chaplin & Mollison, 1952) and for venous sampling (0.91) (Chaplin *et al.*, 1953).

Procedure:

Whole blood, from the K^{+}EDTA tubes, was drawn up into 75 mm micro-capillary tubes (Bilbate Ltd, Daventry, UK), which were then sealed with a capillary sealing compound. These samples were then micro-centrifuged (Hawksley and Son Ltd, Sussex, UK) for 4 min and the HCt determined in triplicate for each sample. The mean CV for triplicate determination of HCt in chapter 7 was 0.5 %.

3.7 PLASMA VOLUME CHANGES

The change in plasma volume between samples before and after exercise was determined by the method of Dill and Costill (1974), from Hb and corrected HCt. The calculation of plasma volume change was carried out using the calculations detailed below, with a working example given in the aforementioned paper of Dill and Costill.

$BV_A = BV_B\ (Hb_B/Hb_A)$ $\qquad$ $\Delta BV = 100\ (BV_A - BV_B)/BV_B$

$RCV_A = BV_A\ (Hct_A)$ $\qquad$ $\Delta RCV = 100\ (RCV_A - RCV_B)/RCV_B$

$PV_A = BV_A - RCV_A$ $\qquad$ $\Delta PV = 100\ (PV_A - PV_B)/PV_B$ $\qquad$ (eq 10)

Where BV represents blood volume, RCV red cell volume and PV plasma volume; with subscripts A and B demoting after and before exercise. BV_B was taken to be 100.

3.8 MUSCLE SAMPLES

3.8.1 Biopsy and storage

All muscle biopsies were taken from the vastus lateralis muscle using the needle biopsy technique described by Bergstrom (1962). The vastus lateralis muscle is the most frequently used muscle in exercise physiology studies due to its major contribution during cycling, its accessible location and the absence of any major blood vessels or nerves. A large portion of the quadriceps was initially cleaned with a betadine solution (Seton Healthcare Group plc, Oldham, England) before local

anaesthetic (1 % lidocaine, Astra Pharmaceuticals, England) was injected under the skin. After confirming anaesthesia, a small incision (~1 cm) was made in the skin and the muscle fascia. When two biopsies were taken in the same trial two separate incisions were made approximately 2-3 cm apart. Bleeding from the incisions was stopped by the application of pressure and the incisions were then covered with sterile gauze until the biopsy was taken. Resting samples were taken with subjects lying in the supine position on an examination couch, with post exercise samples taken with subjects remaining seated on the bike. Muscle samples were immediately frozen (<10 s) in liquid nitrogen, placed in plastic screw top vials and stored at -80 °C. Prior to analysis samples were freeze-dried (Thermo Savant, Micro Modulo, Basingstoke, UK) for 36-48 h, any visible blood and connective tissue removed, powdered and prepared for extraction and analysis.

3.8.2 Metabolite extraction

For the determination of ATP, PCr and lactate content between 4 and 10 mg of powdered muscle was extracted in 0.5 M PCA/1mM EDTA in the ratio of 1 ml to every 12.5 mg muscle. After centrifugation (5000 $revs.min^{-1}$ for 6 min at 4 °C) the supernatant of each sample was neutralised with 2.2 M $KHCO_3$. Samples were again centrifuged and the supernatant pipetted to a clean eppendorf and stored at – 80 °C for subsequent analysis.

In order to determine the glycogen content of muscle samples, 1-4 mg of powdered muscle was digested in 0.1 M NaOH in a water bath at 80 °C for 10 min, neutralised with HCl-citrate buffer and hydrolysed with amyloglucosidase (from *Aspergillus niger*, Sigma Chemical Co., Cat No. A-3042). Samples were then incubated for an

hour, and following centrifugation (14000 revs.min^{-1} for 1 min at 4 °C) the supernatant was pipetted into a clean eppendorf, kept on ice and used immediately for the glycogen assay.

3.9 HOMOGENATE MUSCLE ASSAYS

3.9.1. ATP and PCr

Principle:

The method used for the determination of both ATP and PCr concentration is described by Harris *et al* (1974) and is carried out spectrophotometrically (Aquarius CE-7400, Cecil Instruments, Cambridge, UK) at a wavelength of 340 nm. The three reactions are involved in this assay:

G6PDH

HK

CPK

Reagents and enzymes: volume per sample

Reaction Mix:			
	TEA	100 mmol.l^{-1}	75 µl (TEA, $Mg(Ac)_2.4H_2O$, $EDTA.Na.2H_2O$)
	$Mg(Ac)_2.4H_2O$	10 mmol.l^{-1}	
	$EDTA.Na.2H_2O$	1.0 mmol.l^{-1}	
	DTT	1.0 mmol.l^{-1}	15 µl
	$NADP.Na_2.4H_2O$	1.0 mmol.l^{-1}	30 µl
	$ADP.Na.2H_2O$	0.4 mmol.l^{-1}	3 µl
	Glucose	5.0 mmol.l^{-1}	30 µl
	G-6-PDH		2.25 µl
	H_2O		522 µl

Enzymes: HK (From bakers yeast, Sigma Chemical Co. H-4502)

CPK (From rabbit muscle, Sigma Chemical Co. C-3755)

Standards: ATP 2.5 mmol.l^{-1} (Sigma Chemical Co. A-2383)

PCr 4.0 mmol.l^{-1} (Sigma Chemical Co. P-7936)

Procedure:

1. 75 µl of standard, sample or blank was added to the 675 µl of the reaction mixture and mixed thoroughly. After about 5-10 min at room temperature the initial absorbance was read.

2. 6 µl of HK (approximately 3 units per sample) was then added to the mixture and mixed gently for 20 min and the absorbance read again.

3. 6 μl of CPK (approximately 6 units per sample) was then added to the mixture which was then left to incubate for a further 30 min before reading the final absorbance allowing ATP and PCr concentration to be calculated.

3.9.2 Lactate

Principle:

This method of determination of lactate was described by Lowry and Passonneau (1972) and was carried out on a fluorescence spectrophotometer at an excitation wavelength of 340 nm and an emission wavelength of 450 nm. As with the blood lactate assay the addition of an excess NAD^+, the enzyme LDH, high pH and the use of hydrazine to trap the pyruvate forces this reaction to an end-point. The highly fluorescent $NADH_2$ yielded is then measured, which is proportional to the lactate concentration within the sample.

Reagents and enzymes:

Reaction mixture:	2-amino-2-methyl-1-propanol (pH 10)	1000 $mmol.l^{-1}$
	Hydrazine	1000 $mmol.l^{-1}$
	NAD^+	100 $mmol.l^{-1}$

10 ml of 2-amino-2-methyl-1-propanol, 5 ml hydrazine and 50 μl of NAD^+ were made up to 100 ml with H_2O for each assay.

Enzyme: LDH (from beef heart, Sigma Chemical Co. L-2625)

Standards: Stock solution of L-lactic acid, sodium salt (Sigma Chemical Co, L-7022) 500 μM, aliqouted and frozen at -20 °C.

Lactate stock (μl)	1	3	5	10	15	20
Reaction mixture (μl)	500	500	500	500	500	500
Lactate concentration (μM)	0.998	2.98	4.95	9.80	14.56	19.23

Procedure:

1. To 500 μl of reaction mix 10 μl of blank/sample were added and the standards were added in the volumes described previously. Samples were mixed thoroughly and left to sit in the dark at room temperature for 5 min before the initial fluorescence was measured.

2. 10 μl of enzyme (5.5 units per sample) was then added and the cuvettes were mixed and left to incubate, for 30 min, in the dark and at room temperature after which the final fluorescence was read.

3. A calibration curve was constructed from the standards to allow the calculation of lactate concentration in the samples.

3.9.3 Glycogen

Principle:

The method employed to measure glycogen content is the spectrophotometric method

G6PDH

described by Lowry and Passonneau (1972). Glycogen content was measured at a wavelength of 366 nm. The two reactions involved in this assay are:

glucose-6-phosphate + NAD^+ → P-gluconolactone + NADH

glucose + ATP →(HK) glucose-6-phosphate + ADP

Reagents and enzymes: volume per sample

Reaction mixture:	TEA	100 $mmol.l^{-1}$	180 µl (combined)
	KOH	40 $mmol.l^{-1}$	
	$Mg(Ac)_2.4H_2O$	30 $mmol.l^{-1}$	
	$EDTA.Na_2.2H_2O$	1 $mmol.l^{-1}$	
	Adjusted to pH 8.2 with KOH (Hanna Instruments, Bedfordshire, UK)		
	ATP	0.75 $mmol.l^{-1}$	11.5 µl
	DTT	1 $mmol.l^{-1}$	11.5 µl
	NAD	1 $mmol.l^{-1}$	22.5 µl
	H_2O		390 µl

Enzymes: G-6-PDH (Sigma Chemical Co. G-5885)
HK (Sigma Chemical Co. H-4502)

Standards: 1.5 $mmol.l^{-1}$ glucose (Sigma Chemical Co. 49159)

Procedure:

1. To 615 µl of reaction mix 60 µl of either sample/standard/blank was added and then mixed before sitting for 5 min after which the initial absorbance was read.

2. 5 µl of G-6-PDH:HK mixture (1:1 ratio; approximately 2.5 units of each enzyme) was then added, mixed and then left to incubate for 10 min at room temperature.

3. The final absorbance was then read and glycogen content calculated.

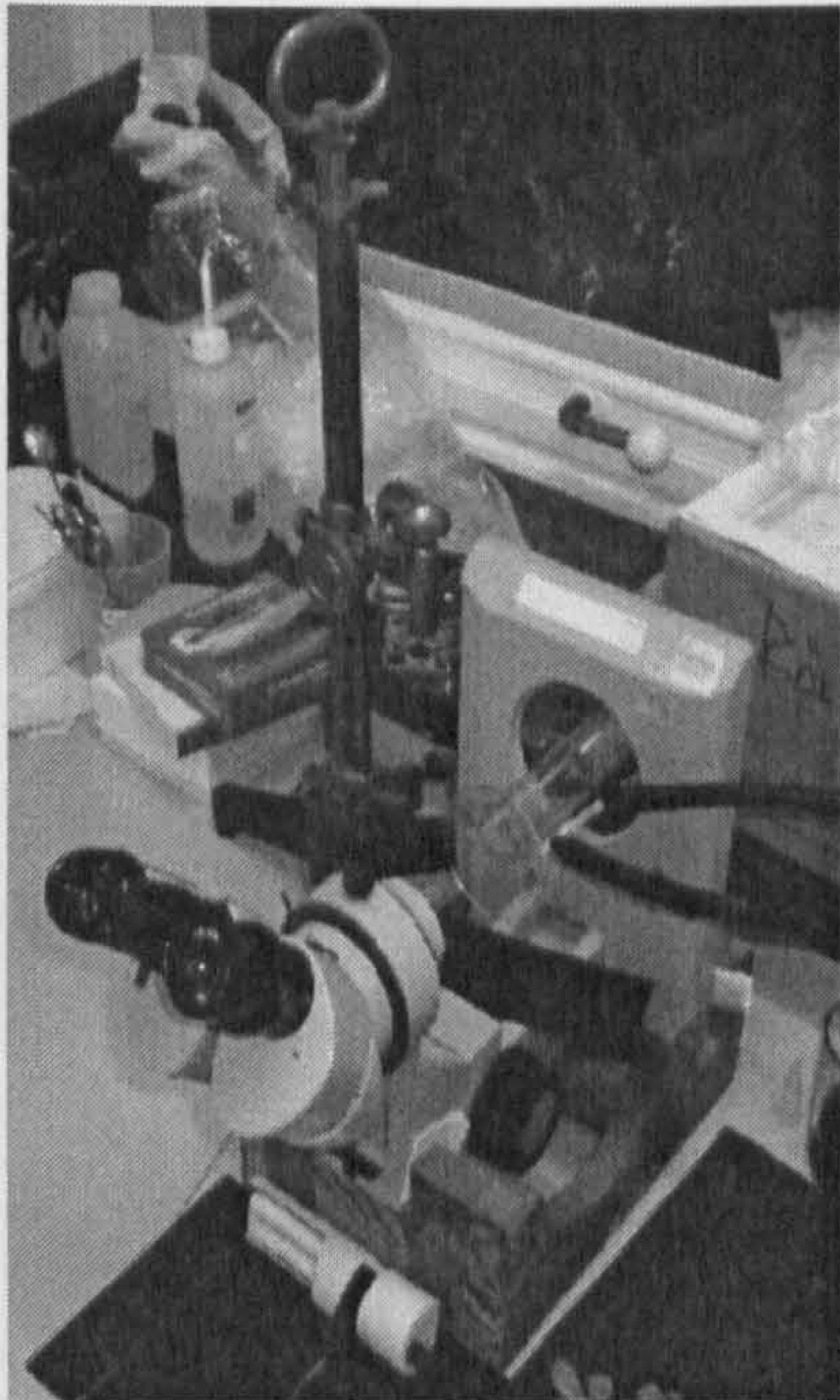
3.10 SINGLE FIBRE ANALYSIS

3.10.1 Dissection and weighing of single fibre fragments

Fragments of single muscle fibres were manually dissected under a low power light microscope (Leica Microsystems, Wetzlar, Germany). Approximately 50 fibres were dissected from each biopsy, depending on the quality and size of the tissue.

Single fibre fragments were weighed using a quartz-fibre fishpole balance similar to that described by Lowry and Passonneau (1972). A quartz fibre is attached to a small piece of copper wire which is secured with epoxy cement to the plunger of a 50 cc glass syringe which has the end of the syringe barrel cut off. Quartz is used instead of glass as it will retain its position indefinitely. The plunger was then inserted into the syringe, with the fibre then concealed within the syringe, which is held in place with a metal clamp stand. A glass cover is positioned over the open end of the syringe to remove any air currents whist making measurements (Fig 3.3). A light microscope,

with a 10 mm (0.1 mm divisions) ocular, is used to measure the deflection of the quartz fibre when the fibre fragment was placed upon the quartz fibre by simply touching the muscle fibre against the end of the quartz fibre. The balance was zeroed prior to each measurement simply by adjusting the clamp stand until the quartz fibre was at the zero point in the ocular.



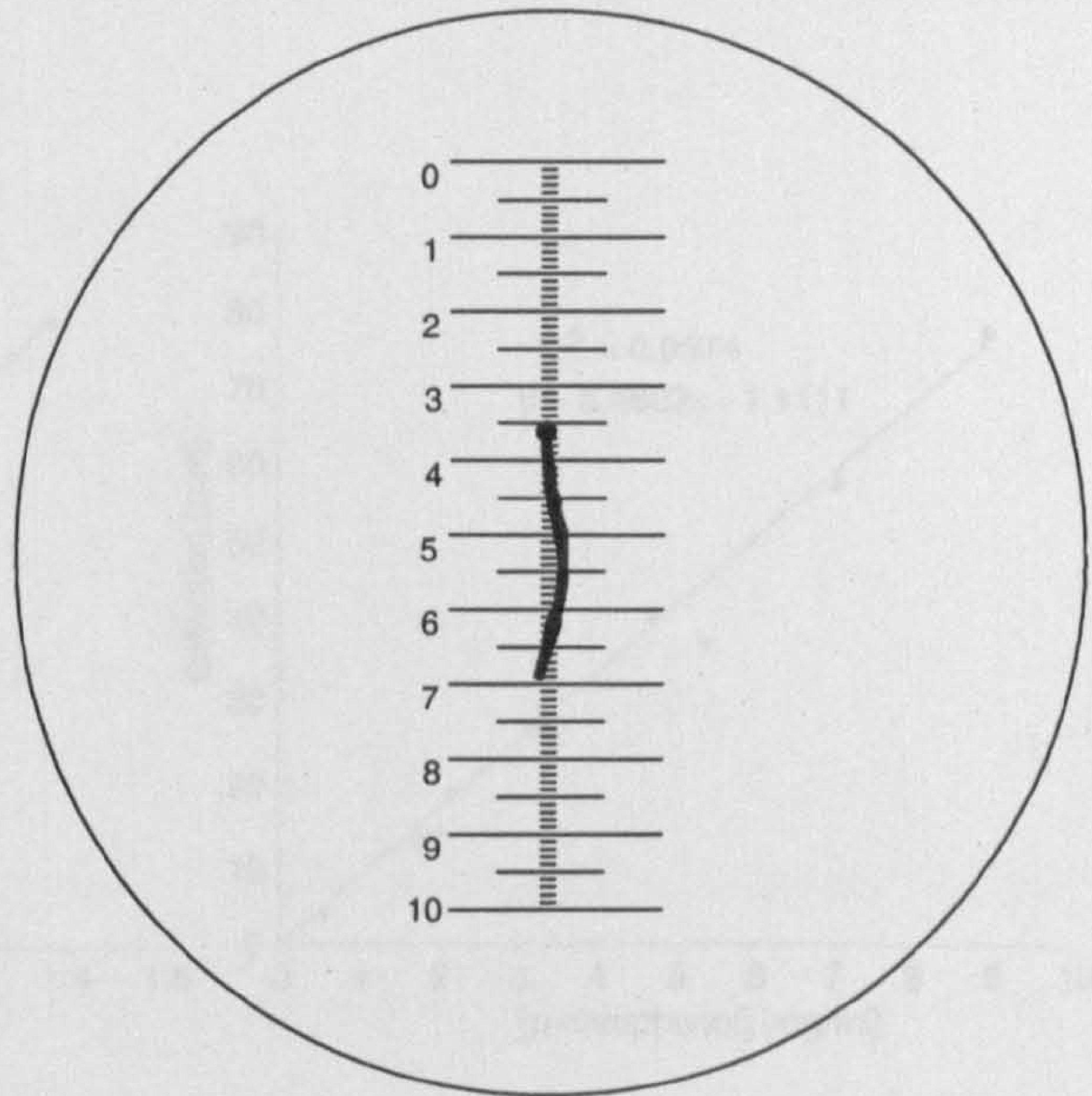


Figure 3.3 *Photograph of balance used to weigh single fibre fragments (left) and schematic of view threw ocular with the muscle fibre attached to the quartz fibre and deflecting the quartz by approximately 3.5 mm (right).*

The balance was calibrated colormetrically with *p*-nitrophenol crystals. A 1 g/l solution of *p*-nitrophenol was made and a standard curve created with various dilutions of this stock in a carbonate buffer (50 mM Na_2CO_3, 50 mM $NaHCO_3$) spectrophotometrically at a wavelength of 400 nm. *p*-nitrophenol crystals, of various

sizes, were then weighed using the balance and dissolved in 0.5-1.5 ml of the carbonate buffer after which the absorbance was measured. The formula from the standard curve was used to calculate the μg/ml of *p*-nitrophenol in each sample (Fig 3.4). This was then compared to the deflection measured on the balance and a conversion factor calculated to convert deflection into weight for the balance. The deflection factor for the balanced used in chapter 5 was 8.08, this will obviously vary with individual balances.

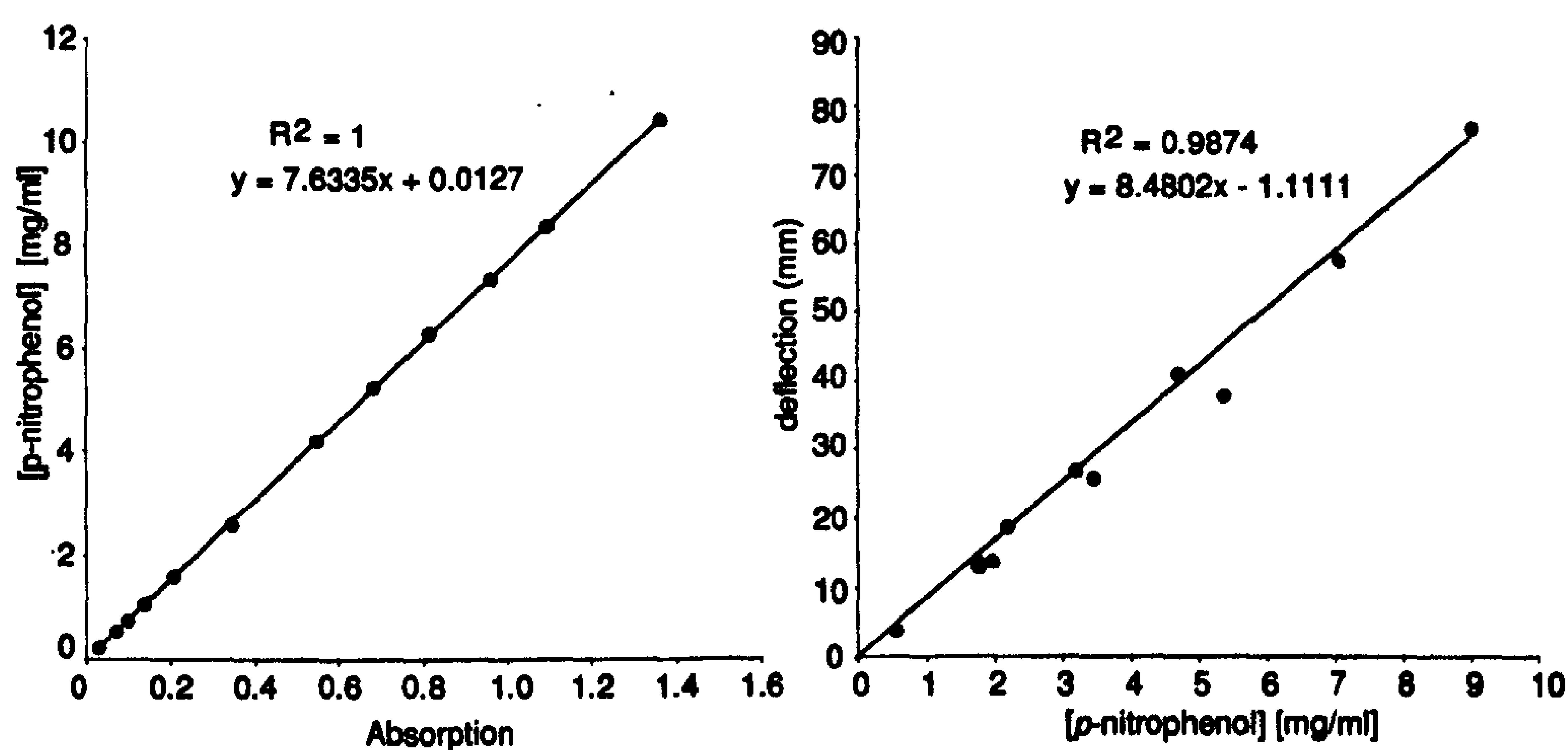


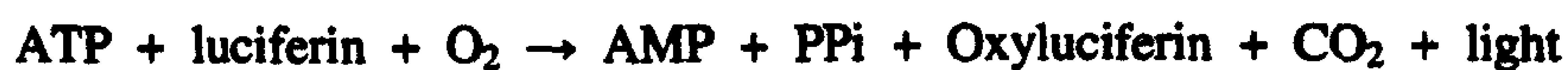
Figure 3.4 *Calibration curve for p-nitrophenol of various concentrations from stock solution (1g/dl – left hand graph) and for p-nitrophenol crystals with deflection on the balance against the concentration (right hand graph), calculated from measured absorption converted to concentration from initial standard curve formula. This was then used to calculate the conversion factor for weighing of single fibres.*

3.10.2 ATP and PCr content

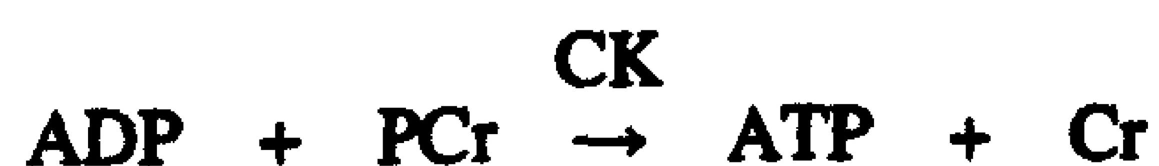
Principle:

Prior to running the assay each fibre was extracted in 200 µl of 2.5 % (w/v) TCA followed by neutralisation with 20 µl of $KHCO_3$. This assay is based on the luminometric method detailed by Wibom *et al* (1991) and is based upon the light produced from the following reactions.

Firefly
luciferase



$$ATP + luciferin + O_2 \rightarrow AMP + PPi + Oxyluciferin + CO_2 + light$$



$$ADP + PCr \xrightarrow{CK} ATP + Cr$$

In the first reaction light is produced directly in proportion to the ATP content of the fibre. In the second reaction PCr content is measured by the addition of CK and a high concentration of ADP.

Reagents and Enzymes

Reaction mixture	Sucrose	180 $mmol.l^{-1}$
	KH_2PO_4	15 $mmol.l^{-1}$
	EDTA	1 $mmol.l^{-1}$
	pH adjusted to 6.67 with KOH	
	ATP monitoring agent (Bio Orbit Oy, Turku, Finland)	
	ADP substrate (Bio Orbit Oy, Turku, Finland)	

Enzyme CK (Sigma Chemical Co. C-9858)

Standard ATP (Bio Orbit Oy, Turku, Finland)

<u>Procedure:</u>

1. The lyophilized ATP monitoring reagent was reconstituted with 47.5 ml of the sucrose solution and 925 µl pipetted into each cuvette and left to incubate at 25 °C for 10 min.

2. To the reaction mix 50 µl of sample/blank were added and the light emitted measured by the luminometer (Model 1251, Bio Orbit Oy, Turku, Finland) to give ATP concentration.

3. ADP substrate (10 µl) and then CK (10 µl or 35 units) were added with the emitted light measured before and after CK addition.

4. 10 µl of ATP was then added for internal standardisation.

Each run was carried out using an automated system with 25 cuvettes in the luminometer carousel each time. Further details regarding the calculation of final concentrations can be found in the paper of Wibom *et al* (1991).

3.11 COEFFICIENT OF VARIATION FOR ASSAYS

The CV for assays was calculated using the method of Bland (1991):

CV = (SD of the difference between duplicate samples/sample mean) * 100

Table 3.1. *CV for metabolite assays based on the values from duplicate samples*

Chapter	Metabolite	CV (%)
4	Muscle ATP	1.1
4	Muscle PCr	1.2
4	Muscle lactate	1.4
4	Muscle glycogen	1.4
5+6 (38 fibres)	Single fibre ATP	6.1
5+6 (38 fibres)	Single fibre PCr	4.1
6	Muscle ATP	1.3
6	Muscle PCr	1.4
6	Muscle lactate	1.5
6	Muscle glycogen	1.2
7	Blood lactate	1.3
7	Hb	0.9

The CV value for single fibre ATP and PCr determination is based on the duplicate measurements from 38 duplicate fibre extracts made in different runs.

3.12 MYOSIN HEAVY CHAIN DETERMINATION

3.12.1 SDS-PAGE

Principle:

Electrophoresis is the migration of molecules in solution in response to an electric field. In SDS-PAGE proteins are denatured in SDS which confers a negative charge to each protein. Since SDS binds to protein fairly specifically in a mass ratio of 1.4:1, and through further denaturing with 2-mercaptoethanol, the proteins migrate according to their molecular weight, with the smallest proteins migrating fastest. These proteins are then silver stained (Oakley *et al.*, 1980) and the relative percentage of different MHC isoforms is determined, densitometrically, in relation to the total MHC present.

Sample Preparation

Muscle samples were denatured in the SDS reducing buffer, described by Staron and Pette (1986), and heating for 10 min at 75 °C and centrifugation (10 min at 4 °C and 4000 revs.min^{-1}) . For each mg of homogenate muscle sample 1 ml of SDS buffer was added (Chapters 4 and 6) and in single fibres 10 µl of buffer added for each mm of fibre (Chapter 5).

SDS reducing buffer: 10 % glycerol

5 % 2-mercaptoethanol

2.3 % SDS

62.5 mM Tris

pH set to 6.8 with HCl

0.05 % bromophenol blue

Electrophoresis

All electrophoresis was carried out with sample loaded onto a stacking gel, which is a non-restrictive large pore gel, poured on top of a separating gel known as the resolving gel. This method is derived from the work of Fauteck and Kandarian (1995).

Resolving gel:
30 % glycerol
T 6 % acrylamide (cross-linking 2.7 %)
0.375 M Tris
ultra pure H_2O
0.1 % SDS
0.1 % TEMED
0.03 % APS

Stacking gel:
30 % glycerol
T 4 % acrylamide (cross-linking 2.7 %)
0.125 M Tris
H_2O
0.1 % SDS
0.15 % TEMED
0.045 % APS

Gels were then submerged in a running buffer made up from:

25mM Tris (base)

0.192 M Glycine

0.1 % SDS

Gels were electrophoresed for ~ 3 h at a constant 100 V and then for ~ 14 h at a constant 6 mAMP on a Mini-Protean III system (Bio-Rad, Hercules, California, U.S.A.), with 4 gels being run simultaneously in a water bath at ~ 8 °C.

Staining

After electrophoresis stacking gels were removed and the resolving gels silver stained using a method modified from that of Oakley *et al* (1980).

Fixing:	1hr	50 % ethanol
		10 % acetic acid
		40 % H_2O
Wash:	>3 hr	5 % ethanol
		5 % acetic acid
		90 % H_2O
	5min	H_2O

	30 min	10 % Gluteraldehyde
	3 * 30 min	H_2O
Stain:	20 min	0.8 g silver nitrate 1 ml 2N NaOH 1.4 ml ammonia made up to 100 ml with H_2O
Wash:	3 * 5 min	H_2O
Develop:		0.02 g citric acid 540 µl formaldehyde
Stop:		Acetic acid

Gels were then left for 30 min with glycerol added, to stop gel cracking, to the solution before being transferred to cellophane sheets and left to dry at room temperature for 48 h. Each band was then expressed as a percentage of the total MHC content of the corresponding lane using a calibrate densitometer (Bio-Rad GS8000 calibrate densitometer). The densitometer software allows each individual band to be isolated (either automatically or by hand) and the relative optical density calculated relative to other bands in that lane, with a subtraction for background staining from the lane also included. MHC isoforms were identified according to their migration rates compared with molecular weight standards and characterised as type I, IIA and IIX.

3.13 STATISTICAL ANALYSES

All statistical analyses were carried out on SPSS version 12 or Microsoft excel. Each experimental chapter in this thesis has a repeated measures design, with subjects performing both control and heated (or prior exercise in chapter 7) experimental trials. For data with repeated measures in each trial a two-way (trial * time) repeated measures ANOVA was used for analyses. In chapter 7 a three-way repeated measures ANOVA (time * pedal rate * condition) was used for blood lactate analysis. Where a significant effect was observed *post-hoc* t-tests with bonferroni correction were used to locate these differences. When a variable was measured once in each trial student's paired t-tests were used for analyses. Data are presented as mean ± SD with the critical alpha level (P) set at 0.05.

Chapter 4

ANAEROBIC ATP TURNOVER AND MFCV DURING THE DEVELOPMENT OF MAXIMAL POWER OUTPUT AT DIFFERENT MUSCLE TEMPERATURES

This chapter has been published as an abstract and a paper.

Abstract. Gray, S. R., De Vito, G., Nimmo, M. A., & Ferguson, R. A. (2005). Skeletal muscle ATP turnover is elevated at higher muscle temperatures during the development of maximal power output in humans. *J Physiol* **567P**, C68.

Paper. 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. *Am J Physiol* **290**, R376-R382.

4.1 INTRODUCTION

Temperature is an important determinant of skeletal muscle contractile and metabolic properties (Bennett, 1984;Rall & Woledge, 1990;Ranatunga, 1998). One major effect of elevated T_m is to alter both the force/velocity and power/velocity relationships which has been demonstrated both in mammals (Ranatunga, 1998) and humans (He *et al.*, 2000;De Ruiter & de Haan, 2000). Thus, a passive elevation of T_m results in an improved performance in terms of both work (Asmussen & Boje, 1945) and power output in humans during sprint cycling (Sargeant, 1987). Since the temperature dependent contractile properties are a function of mATPase activity (He *et al.*, 2000) which is itself temperature dependent (Steinen *et al.*, 1996), it is possible that an increased ATP turnover would contribute to the greater maximal power output under elevated T_m conditions. Indeed, a greater rate of ATP turnover at high T_m has been reported during sustained submaximal isometric contractions (Edwards *et al.*, 1972) and intense sustained dynamic exercise (Febbraio *et al.*, 1996).

Neuromuscular factors may also have an effect on performance and ATP turnover. MFCV, for instance, provides important information in relation to the muscle fibre membrane and contractile properties (Ardent-Nielsen & Mills, 1985). MFCV is the average value of conduction velocities of the motor units active during a contraction and thus reflects motor control strategies (Andreassen & Ardent-Nielsen, 1987). A greater MFCV under elevated temperature conditions may lead to individual sarcomeres being more rapidly activated meaning that the contractile speed of the whole fibre would be enhanced (Wickiewicz *et al.*, 1983). Associated with this would be a greater requirement for ATP turnover. It has been previously demonstrated that MFCV is elevated with increased T_m during low-force isometric contractions of the

tibialis anterior muscle (Farina *et al.*, 2005). Moreover, the increase in conduction velocity in single motor units is correlated to the increase in motor unit twitch force and rate of force development (Farina *et al.*, 2005). It may therefore be possible that the temperature-dependent increase in power output is caused by more rapid muscle activation and thus MFCV.

The main purpose of the present study therefore was to determine whether a passive increase in T_m altered ATP turnover and MFCV during the development of maximal power output during cycling. It was hypothesized that increasing T_m would lead to an increase in maximal power output due to increases in anaerobic ATP turnover, primarily from PCr utilization and glycogenolysis, and MFCV. Furthermore, it has previously been demonstrated that the temperature dependant increase in power output is fibre type specific, with type I fibres being most sensitive to an increase in temperature (Sargeant & Rademaker, 1996). Therefore, a further hypothesis was that the magnitude of increase in power with temperature would be dependent upon the percentage of MHC type I in the exercising muscle.

4.2 METHODS

4.2.1 *Subjects*

Eight healthy male subjects (age 25 ± 6 yr, height 1.82 ± 7 m, mass 77 ± 11 kg; means ± S.D.), with no history of muscle or metabolic disorders, volunteered for the study. Two of these subjects performed the experimental protocol but gave only one biopsy for MHC determination and are thus not included in any of the results or discussion of ATP turnover and MFCV.

4.2.2 *Experimental Protocol*

On arrival to the laboratory subjects inserted a rectal thermistor probe to allow continuous monitoring of T_{rec}. Following this, in the control condition of normal T_m (N), subjects rested for 30 min at normal room temperature (20–22 °C), while the muscle biopsy site was prepared, a flexible T_m probe inserted and an EMG electrode and goniometer attached. In the ET condition, T_m was passively increased as described in chapter 3 with the same preparation of the legs as in the control condition. After temperature manipulation or the 30 min rest at room temperature, a resting muscle sample was taken and the T_m probe removed. The subjects then mounted the cycle ergometer and performed a 6 s maximal sprint from a stationary position for the determination of maximal power output, immediately after which another muscle sample was obtained with the subject remaining seated on the ergometer. The resistance applied to the bike was 7.5 % of each subject's body mass as suggested by Bar-Or *et al* (1987), e.g. a subject weighing 100 kg had a weight of 7.5 kg used for resistance on the cycle ergometer. From the sprint power output was

calculated (BBC Acorn Computer, London, UK) every second from the known frictional load and the measurement of flywheel velocity, corrected for the acceleration of the flywheel (Lakomy, 1986).

4.2.3 Muscle analysis

After freeze-drying muscle samples were analysed for ATP, PCr, lactate, glycogen and MHC composition as described in chapter 3.

4.2.4 Calculations

Anaerobic ATP turnover was calculated as ΔPCr + 1.5 Δmuscle lactate + 2 ΔATP (Spriet, 1995), where Δ is the change in concentration. The small quantity of ATP produced, or utilized, relating to the accumulation of glycolytic metabolites (e.g., pyruvate and glyceraldehyde-3-phosphate) is neglected, as they represent <2 % of the rate of ATP turnover in all cases (Spriet *et al.*, 1987a). Furthermore, this calculation relies on the assumption that, during a 6 s maximal sprint, the amount of lactate efflux is not great (Bangsbo *et al.*, 2001), and there is minimal uptake of glucose from outside of the cell (Katz *et al.*, 1986). It is also assumed that thigh oxygen uptake is minimal during the 6 s exercise (Bangsbo *et al.*, 2000). Internal power output ($W.kg^{-1}$) was estimated as 0.153 $(\text{frequency})^3$, where frequency is converted to Hz from that measured on the cycle ergometer (Minetti *et al.*, 2001). This equation was originally calculated by Minetti *et al* in 2001 who used motion analysis to estimate the power generated by the muscles to overcome the inertial and gravitational forces opposing the movement of the limb. In brief using three-dimensional motion capture, with markers positioned around relevant joints on both sides of the body, subjects cycled at

several different pedal rates. From this the three-dimensional coordinates and standard anthropometric tables were obtained and the position of the body's centre of mass and the linear and angular speed of each body segment calculated. A software package, designed by Minetti *et al* (2001) was then used to estimate the internal mechanical power output required to accelerate the limbs with respect to the body centre of mass. This same method was used in subsequent experimental chapters requiring a measure of internal power output.

The temperature dependence of measured variables is presented as temperature coefficients (Q_{10}), calculated as follows (Bennett, 1984):

$$Q_{10} = (R^2/R^1)^{[10/T_2 - T_1]}$$

where R_2 and R_1 are rate processes, respectively, at temperatures T_2 and T_1, with T_2 being greater than T_1. $Q_{10} > 1$ indicates a positive thermal dependence; $Q_{10} = 1$ indicates a thermal independence; and $Q_{10} < 1$ indicates a negative thermal dependence.

4.2.5 Statistical Analysis

Data were analysed by either two-way (temperature and time) ANOVA with repeated measures or paired *t*-tests. Where a significant effect was detected, differences were located with post hoc paired *t*-tests with Bonferroni correction. Pearson correlation coefficient (*R*) was computed to assess linear relations between variables. Significance was accepted at $P<0.05$. Data are presented as means ± SD.

4.3 RESULTS

4.3.1 Temperature

The heating protocol resulted in a higher ($P<0.05$) T_m before the onset of exercise (37.5 ± 0.6 °C in ET vs. 34.2 ± 0.6 °C in N). Rectal temperature was also higher following the heating protocol (37.2 ± 0.2 °C in ET vs. 37.1 ± 0.2 °C in N; $P<0.05$).

4.3.2 ATP turnover and MFCV

Passive elevation of T_m did not affect the resting metabolite content but resulted in a greater ($P<0.05$) fall in PCr content and increase in lactate content (Table 4.1) during the 6 s sprint. Elevating T_m lead to an increase in both MFCV (3.79 ± 0.47 vs. 5.55 m/s ± 0.72 in N and ET conditions, respectively; Q_{10} of 3.8; $P<0.05$) and ATP turnover (Table 4.2) compared with N, the latter by 35 %. This represents a Q_{10} value of 2.7 for ATP turnover. The Q_{10} values for ATP resynthesis from PCr hydrolysis and glycolysis were 3.8 and 1.7, respectively. There was a close association ($P<0.05$) between MFCV and ATP turnover under both temperature conditions (Fig. 4.1).

Table 4.1. *Muscle metabolites before and after the 6 s maximal sprint under conditions of normal (N) and elevated (ET) muscle temperature.*

		Pre Exercise	Post Exercise
PCr	N	74.1 ± 5.7	41.7 ± 6.1*
	ET	76.9 ± 4.3	29.2 ± 9.3*†
Lactate	N	5.3 ± 0.8	22.0 ± 4.1*
	ET	5.6 ± 1.0	24.9 ± 5.1*†
ATP	N	23.3 ± 1.7	19.6 ± 1.4*
	ET	23.9 ± 1.9	18.1 ± 1.9*
Glycogen	N	483 ± 154	447 ± 161*
	ET	490 ± 167	440 ± 182*

Values are means ± S.D. (N=6). Significant difference ($P<0.05$) between pre- and post-exercise denoted by * and between conditions denoted by †. Values are $mmol.kg^{-1}$ (dm) except for glycogen which is in mmol glycosyl $units.kg^{-1}$ (dm).

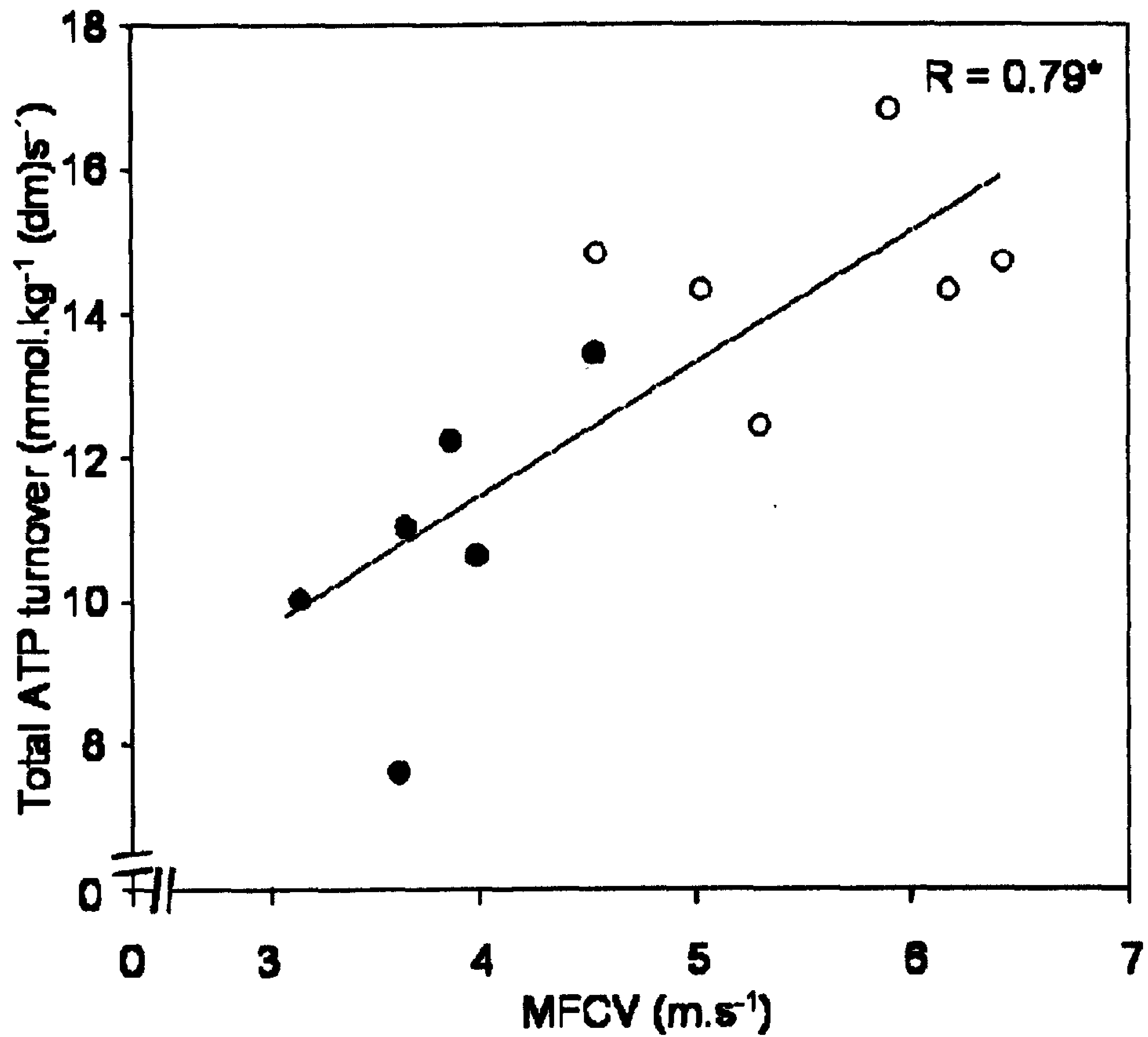


Fig 4.1. *Relationship between MFCV and total ATP turnover under conditions of normal (closed circles) and elevated (open circles) muscle temperature. * denotes a significant correlation (P<0.05).*

4.3.3 Power Output

Despite the greater rate of ATP turnover and the overall increase in both maximal and mean power output (Q_{10} of 2.2 and 1.7, respectively) being significant (Table 4.3), not all subjects demonstrated an increase in power output with heating (Fig. 4.2). For example, subjects 1, 2, 3, 6, 7, and 8 demonstrated an increase in maximal power output by an average of 30 % (10 % per 1 °C increase in T_m), whereas the power output of subjects 4 and 5 did not increase substantially. This is illustrated by examining the relationship between Q_{10} values for ATP turnover and mean power output (Fig. 4.3), although there are no data from subjects 7 and 8. All data points are above the line of identity, demonstrating that the magnitude of increase in ATP turnover was greater than the increase in power output in all cases.

Table 4.2. *Rates of anaerobic ATP turnover during 6 s maximal sprint exercise under conditions of normal (N) and elevated (ET) muscle temperature.*

	N	ET
PCr ATP turnover	5.4 ± 0.5	7.9 ± 1.3*
Glycolytic ATP turnover	4.1 ± 1.0	4.8 ± 1.2*
Anaerobic ATP turnover	10.8 ± 1.9	14.6 ± 2.3*

Values are means ± S.D. (N=6). Significant difference between conditions denoted by * ($P<0.05$). Values are expressed as $mmol.kg^{-1}$ $(dm)s^{-1}$).

Table 4.3. *Power output and pedal rate during 6 s maximal sprint exercise under conditions of normal (N) and elevated (ET) muscle temperature.*

	N	ET
Maximal external power (W)	1202 ± 347	1462 ± 435*
Mean external power (W)	878 ± 214	1006 ± 242*
Maximal pedal rate ($revs.min^{-1}$)	155 ± 21	176 ± 26**
Mean pedal rate ($revs.min^{-1}$)	124 ± 20	142 ± 25*

Values are means ± S.D. (N=8). Significant difference between conditions denoted by * ($P<0.05$) and ** ($P<0.01$

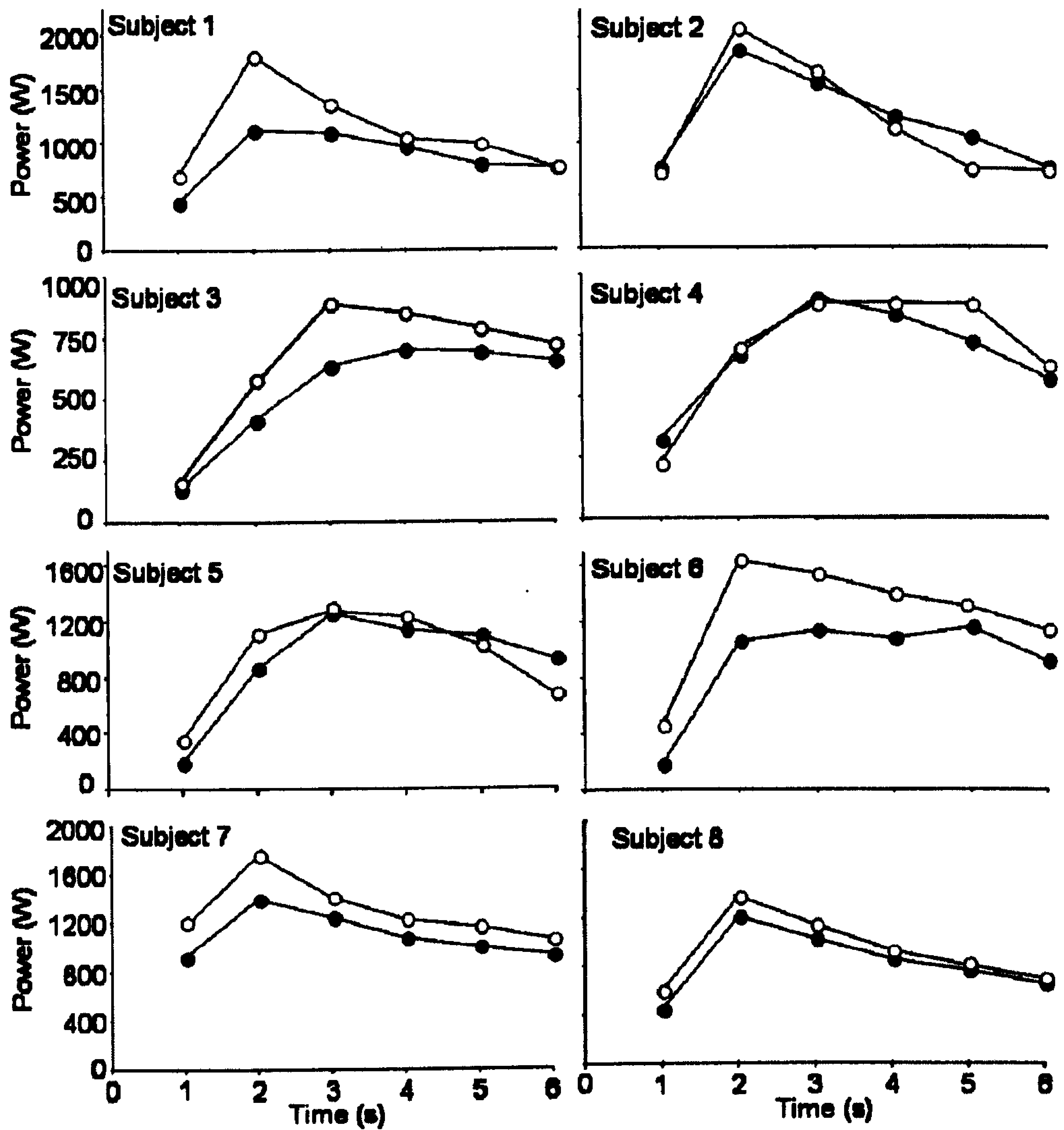


Fig 4.2. *Individual power output during 6 s maximal sprint exercise under conditions of normal (closed circles) and elevated (open circles) muscle temperature.*

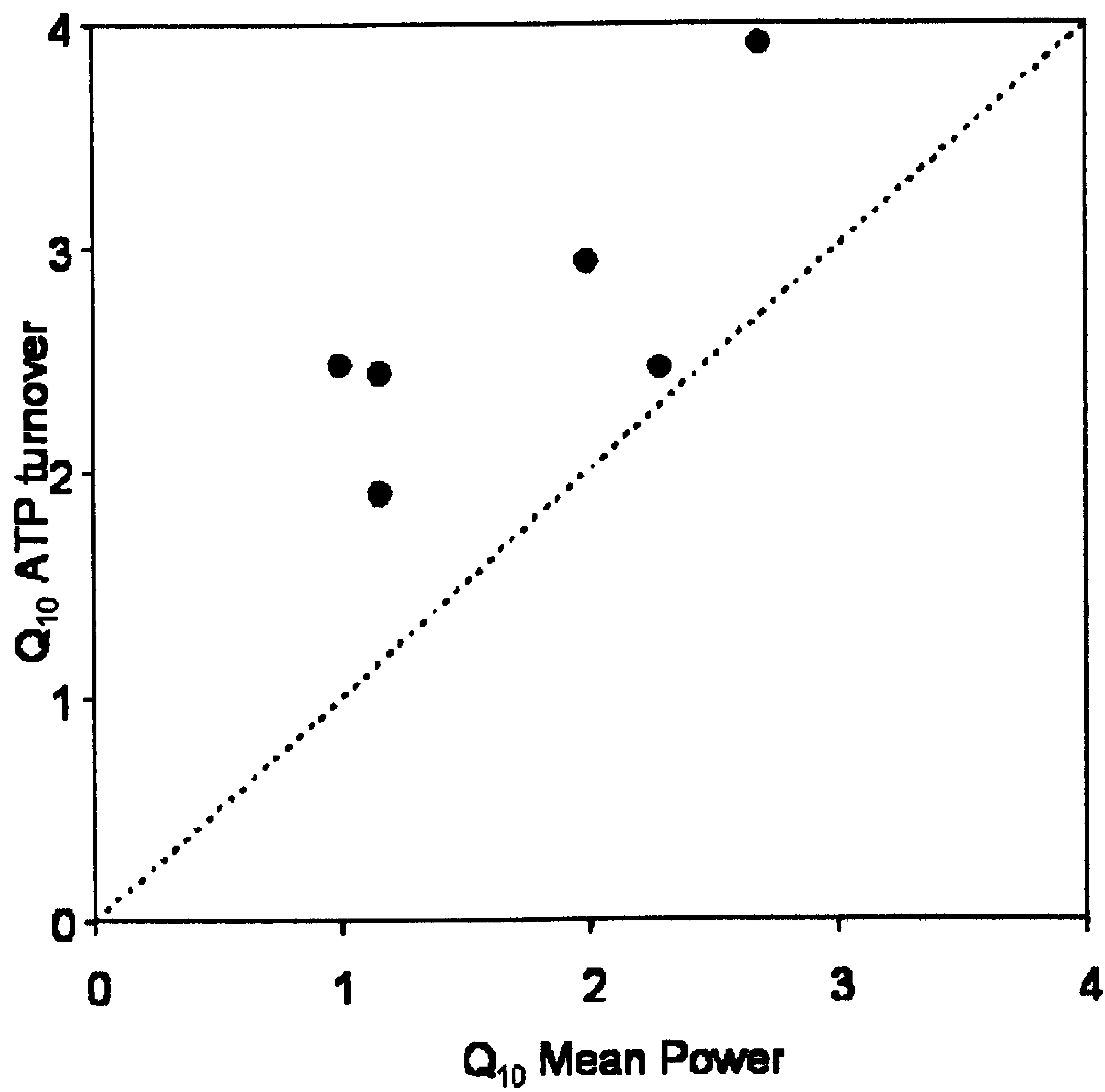


Fig 4.3. *Relationship between Q_{10} values for ATP turnover and mean power output (N=6)*

The MHC composition (type I 32 % ± 9, type IIA 53 % ± 6, and type IIX 15 % ± 9) dependent effect of T_m on power output is demonstrated in Fig. 4.4, where the magnitude of increase in both mean and peak external power, with the rise in T_m, is positively correlated with the percentage of MHC IIA ($P<0.05$), whereas there is no such relationship with percentage of MHC I.

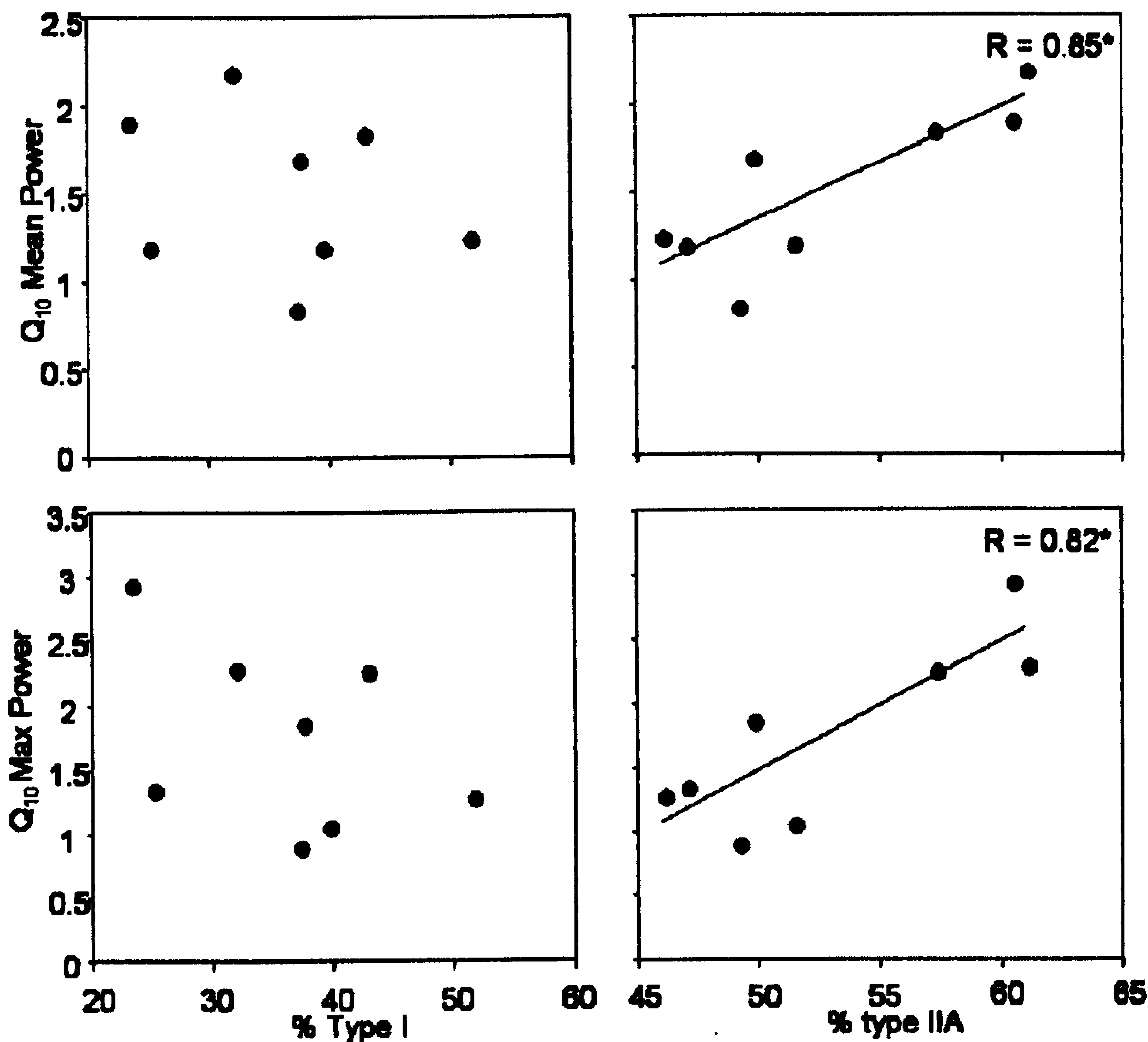


Fig 4.4. *Relationship between MHC I and IIA and Q_{10} for mean and maximal power output (N=8). * denotes a significant correlation ($P<0.05$).*

4.4 DISCUSSION

The present investigation has demonstrated that passively elevating the temperature of the exercising muscle prior to the performance of a maximal sprint increases the rate of ATP turnover and MFCV during the exercise. Furthermore, the magnitude of increase in power output that occurred as a result of the passive heating was correlated with the relative content of MHC IIA isoform.

The increase in ATP turnover with heating reflects an elevated rate of cross-bridge cycling and would occur since, as with other enzymatic processes, mATPase activity is temperature dependent (Steinen *et al.*, 1996;He *et al.*, 2000). This effect was previously shown during isometric contractions (Edwards *et al.*, 1972) and intense dynamic exercise (Febbraio *et al.*, 1996). It might have been expected that during a maximal sprint in which maximal power output is generated ATP turnover would be at its highest level. Whilst the subjects in the current study developed their maximal power output with a load of 7.5 % body mass the optimal load for power output has been found to be nearer 9.8 % body mass (Bar-Or, 1987). It may have, therefore, been prudent to carry out an optimisation for power output (Winter *et al.*, 1996) although such a procedure has recently been found to be ineffectual during cycling (Pearson *et al.*, 2004). Further options are to calculate the resistance as a percentage of lean body mass or leg volume although as the main focus of the current study was to determine the effect of muscle temperature and not to optimise power output such a procedure was not implemented.

Rates of ATP turnover during short term friction loaded cycle ergometery under control conditions have been shown previously to be 6.0 $mmol.kg^{-1}.s^{-1}$ (dm) for PCr

utilization and 4.8 mmol.$kg^{-1}.s^{-1}$ (dm) for glycolysis leading to lactate formation (Boobis *et al.*, 1982). These values are similar to those obtained during N in the present study, although other studies have reported higher values (Gaitanos *et al.*, 1993;Parolin *et al.*, 1999). On the contrary the highest rate of human skeletal muscle ATP turnover from the individual metabolic pathways have been reported to be ~9 mmol.$kg^{-1}.s^{-1}$ (dm) for both PCr utilization during electrical stimulation (Hultman & Sjöholm, 1983) and glycolysis during 30 s isokinetic cycling at 140 revs.min^{-1} (Jones *et al.*, 1985). On this basis there is, therefore, a clear capacity for ATP turnover to increase.

The close association between ATP turnover and MFCV (Fig 4.1) highlights the relationship between muscle activation and energy turnover. The effect of elevating T_m was to increase MFCV, probably due to a temperature-mediated effect on voltage gated Na^+ channels (for review see Rutkove, 2001), alongside the elevated ATP turnover. At higher temperatures the opening and closing of these channels accelerates allowing less Na^+ to enter the cell. A corresponding decrease in action potential amplitude, duration and area follows leading to a more rapid onset depolarisation producing a faster MFCV (Rutkove *et al.*, 1997). The more rapid action potential delivery to the muscle fibres will lead to a greater Ca^{2+} release from the sarcoplasmic reticulum (SR), this increase in the rate at which a sarcomere is activated will result in an enhancement of the shortening velocity of the whole muscle (Wickiewicz *et al.*, 1983) requiring the greater rate of ATP turnover observed. It might also be considered that this would lead to a greater ATP turnover by SR Ca^{2+}ATPase. This is unlikely however since the activity of the Ca^{2+}ATPase has been found to be slightly depressed in rat muscle fibres after 30 min incubation at 37 °C

(Schertzer *et al.*, 2002) making it likely that the majority, if not all, of the elevated ATP turnover comes from an increased ATP hydrolysis by mATPase at the myosin head. Further work is, however, required to further investigate and confirm or refute the existence of such an association between MFCV and the rate of ATP turnover.

We hypothesized, that the greater power output achieved as a result of heating would occur in conjunction with a greater ATP turnover. However, although greater ATP turnover was observed in all subjects during ET, not all subjects increased power output. This is in contrast with the fact that for a 1 °C increase in T_m there is a 10 % increase in power output (Sargeant, 1987). Furthermore, the change in ATP turnover was not reflected by similar magnitude of change in power output (Fig 4.3). There are several possible reasons for this. One explanation might be that the efficiency of contraction is lower at the higher T_m.

Mechanical efficiency can be estimated using values of molar enthalpy during muscle contraction when ATP re-synthesis is powered by a net hydrolysis of ATP (35 kJ per mole of ATP used), PCr hydrolysis (55 kJ per mole of ATP used) and the anaerobic utilization of glycogen (67 kJ per mole of ATP used) (see Curtin & Woledge, 1978;Woledge & Reilly, 1988;Gonzalez-Alonso *et al.*, 2000 for further discussion). With an accurate measure of mechanical power output, that includes the so-called internal work performed to overcome inertial and gravitation forces of the lower limbs (Minetti *et al.*, 2001), we observed that mechanical efficiency was the same between the two temperature conditions (Table 4.4), although this may not appear to be the case looking at fig 4.3 a contradiction with no clear explanation . Of course, the absolute values are relatively low compared to measurements of mechanical efficiency during sustained moderate (~40 W) and intense (~65 W) knee extensor

exercise (e.g. ~25 % (Ferguson *et al.*, 2001), 35-50 % (Krustrup *et al.*, 2003), respectively), reflecting the fact that high power is achieved at the expense of efficiency (Curtin & Woledge, 1996;di Prampero & Piiper, 2003). The fact that the estimate of mechanical efficiency in the present study is not lower in the heated condition is partially supported by previous work where it was suggested that efficiency would increase at a relatively high speed of contraction under elevated T_m (Ferguson *et al.*, 2002) due to the rightward shift in the efficiency-velocity relationship at higher temperatures (He *et al.*, 2000). It would seem likely, therefore, that efficiency would increase at the high pedal rates attained in the present study. This would only be the case, however, if the pedal rate was maintained constant, which is not the case whilst using a friction-braked cycle ergometer. In the present study as the pedal rate increased by around 20 revs.min^{-1} in the heated trial, i.e. moving to the right on the efficiency-velocity relationship, this counteracts the rightward shift with temperature, the result being that the velocity is at about the same relative point on the curve and hence efficiency is not altered.

An alternative explanation may be related to the fact that with the friction-loaded cycle ergometer the load is fixed and any change in power output is achieved through a change in pedal rate. The maximal pedal rate under control conditions was ~160 revs.min^{-1} which is much higher than the optimal rate suggested for maximal power development (Sargeant *et al.*, 1981) and also approaching the highest possible pedal rate. To obtain a higher power output when heated, the pedal rate would have to increase, as was observed (Table 4.2). It is possible that the two subjects that did not increase power output under elevated temperature conditions had reached, at ~160 revs.min^{-1} in the normal temperature condition, their own biomechanical limit of knee

angular velocity. Thus, no further increase in velocity was possible when the muscles were heated.

Table 4.4. *Muscle energetics during 6 s maximal sprint exercise under conditions of normal (N) and elevated (ET) muscle temperature.*

Energy Source	N	ET
Net ATP hydrolysis (J s^{-1})	262 ± 113	402 ± 136*
Net PCr hydrolysis (J s^{-1})	3518 ± 625	5144 ± 1171*
Lactate accumulation (J s^{-1})	3204 ± 900	3704 ± 990*
Metabolic input[1] (J s^{-1})	6984 ± 1520	9251 ± 2115*
Total mechanical work[2] (J s^{-1})	961 ± 298	1144 ± 347*
Mechanical Efficiency[3] (%)	13.7 ± 2.4	12.3 ± 2.2

Values are means ± S.D. (N=6). Significant difference between conditions denoted by * ($P<0.05$).

[1] calculated as the sum of energy turnover from the net ATP hydrolysis, PCr hydrolysis and lactate accumulation. [2] calculated as the sum of external and 'internal' power output.

[3] calculated as the ratio between total mechanical work and metabolic input

So far in this discussion has been concerned with the effect of passive, local heating on power output and ATP turnover of the active muscles as a whole. It is clear, however, that the whole of the available musculature would be active in the present study (Karatzaferi *et al.*, 2001b) so consideration must be given to the contribution of individual muscle fibres, which have diverse contractile and metabolic properties (e.g. Larsson & Moss, 1993;Bottinelli *et al.*, 1996). It was observed that the magnitude of the temperature dependent increase in power output is correlated with the percentage of MHC IIA (Fig 4.4) which is in contrast to the previous suggestion that type I fibres are more sensitive to changes in temperature in the pedal cadence range of 60-140 revs.min^{-1} (Sargeant & Rademaker, 1996). Since all fibres are equally affected by temperature (He *et al.*, 2000) and these effects are velocity specific, this discrepancy can be explained by the higher contraction velocities achieved with the use of the friction-loaded cycle ergometer. The V_{max} of fibres with predominantly MHC I is equivalent to approximately 165 revs.min^{-1} (Sargeant, 1994) with a V_{opt} of approximately 60 revs.min^{-1}. In the present study these fibres are likely to be working close to or beyond their V_{max}, thus having a minimal contribution to power output, even in the control condition. The V_{max} ratio between fibres with a predominance of MHC I and MHC IIA is approximately 2.3 (Bottinelli *et al.*, 1996). With a V_{opt} at approximately one third of V_{max} (Bottinelli *et al.*, 1996;He *et al.*, 2000), the hypothetical V_{opt} of MHC IIA fibres would be around 130-140 revs.min^{-1}, well within the range of pedal velocities achieved. It is therefore likely that these fibres had the dominant contribution to power output in the present study and will be working beyond their optimum on the descending right arm of the power-velocity relationship. A rightward shift in this relationship, with an increase in T_m, will therefore result in a substantial increase in the power output of these fibres.

In a practical sense, Jones and colleagues (2004) suggested that warm up would be of the greatest benefit to the power output of athletes with a high proportion of type I fibres. This was considered an unfortunate paradox since those athletes with the most to gain from an increased power output (e.g. sprinters) will have large type II content and thus gain little from increasing the temperature of the muscle. The findings of the present study, however, contradict this and suggest that type IIA fibres are affected more by temperature at relatively high velocities. In the present study knee angular velocity was approximately 1000 $°s^{-1}$ which is actually close to the values achieved at 95% of maximal sprinting velocity (Kivi *et al.*, 2002).

In conclusion, we have demonstrated that passive elevation of T_m increases the rate of ATP turnover as well as MFCV, without any changes in mechanical efficiency, which helps explain the greater power output achieved as a result of an increased T_m. The present data also suggests that fibres with a high proportion of MHC IIA are most sensitive to temperature at the relatively fast cadences reached in the present study. Furthermore, the possibility that type I fibres have little contribution to power output at these contraction velocities requires further investigation.

Chapter 5

SINGLE FIBRE METABOLISM DURING THE DEVELOPMENT OF MAXIMAL POWER OUTPUT AT DIFFERENT MUSCLE TEMPERATURES

This chapter has been published as an abstract.

Gray, S. R., Söderlund, K., & Ferguson, R. A. (2006). Greater PCr utilisation in single human type IIA muscle fibres during the development of maximal power output at higher muscle temperatures. *Proc Physiol Soc* 3, C40.

5.1 INTRODUCTION

It is well established that passive elevation of T_m leads to a greater power output in human skeletal muscle (Asmussen & Boje, 1945;Asmussen *et al.*, 1976;Sargeant, 1987;De Ruiter & de Haan, 2000). This increase in power output occurs alongside an elevated mATPase activity (Steinen *et al.*, 1996). It was demonstrated in chapter 4 that the greater power output also occurs alongside a greater rate of skeletal muscle anaerobic ATP turnover. Since all muscle fibre types are recruited during such short-term exercise (Karatzaferi *et al.*, 2001b), it is important to investigate the effect of elevating T_m on the activity of different fibres during such exercise.

Skeletal muscle fibres can be characterised on the basis of their MHC isoform expression. It is now known that human muscle fibres may express only one, but frequently co-express two, isoforms of MHC resulting in the existence of a continuum of fibre types and contractile properties (Larsson & Moss, 1993;Schiaffino & Reggiani, 1996). Each individual fibre will also have an individual force-velocity and power-velocity relationship dependant on its MHC expression, both of which will be affected to a similar extent by temperature (Bottinelli *et al.*, 1996;He *et al.*, 2000). During *in vivo* exercise, in humans, it has been hypothesized that the greater power output, at higher T_m, is due to the greater sensitivity of type I fibres in the cadence range of 60-140 revs.min^{-1} (Sargeant & Rademaker, 1996). However, in chapter 4 it was shown that as pedal rate is increased to around 160-180 revs.min^{-1} subjects with a greater proportion of MHC IIA appear to be more sensitive to temperature perturbations. Both this assertion and that of Sargeant and Rademaker (1996), however, were made through correlations between the Q_{10} values for power output and the percentage of the respective fibre type, and not through a direct measurement

of single fibre metabolism.

In order to gain further insight into this area it is necessary to investigate the metabolic response of these differing fibre types through single fibre analysis. For this purpose methods allowing measurement of ATP and PCr content in single fibre fragments have been developed (Wibom *et al.*, 1991) and have been used to provide insight into the metabolic responses of single fibres to various modes of exercise (Söderlund & Hultman, 1991;Sahlin *et al.*, 1997;Conjard & Pette, 1999;Karatzaferi *et al.*, 2001a;Karatzaferi *et al.*, 2001b). The aim of the present study, therefore, was to determine the ATP and PCr content at rest and post-exercise in characterised single human muscle fibres during the development of maximal power output at different muscle temperatures. We hypothesised that there would be a greater ATP and PCr degradation in fibres with a predominance of MHC IIA after the elevation of T_m, contributing to the greater power output under these conditions.

5.2 METHODS

5.2.1 Subjects

Six healthy male subjects (age 25 ± 6 years, height 1.82 ± 0.07 m, body mass 77 ± 11 kg; means ± S.D.), with no history of muscle or metabolic disorders, volunteered for the study.

5.2.2 Experimental protocol

The experimental protocol is the same as that described in chapter 4, with the remainder of the biopsy samples from chapter 4 being used for the current chapter.

5.2.3 Muscle analyses

After freeze drying, single fibres were manually dissected from muscle samples, weighed and analysed for ATP, PCr and MHC content. Fibres were then characterised into one of seven groups dependent upon their MHC content; type I, type IIA, IIAX25 (0-25 % IIX isoform), IIAX50 (26-50 % IIX), IIAX75 (51-75 % IIX), IIAX100 (76-100 %) (Fig 5.1). This characterisation was carried out on a with each SDS-PAGE run of single fibres carried out alongside a molecular weight marker and a homogenate sample, the three bands of which have previously been shown to correspond to MHC I, IIA and IIX (Bottinelli *et al.*, 1994), known to have a substantial concentration of MHC IIX. Each band was then expressed as a percentage of the total MHC content of the corresponding lane using a calibrate densitometer (Bio-Rad GS8000 calibrate densitometer). The densitometer software allows each individual band to be isolated

(either automatically or by hand) and the relative optical density calculated relative to other bands in that lane, with a subtraction for background staining from the lane also included. This correction involves the selection of a small area on the gel from which the average density is calculated and subtracted from the density of the band prior to calculations.

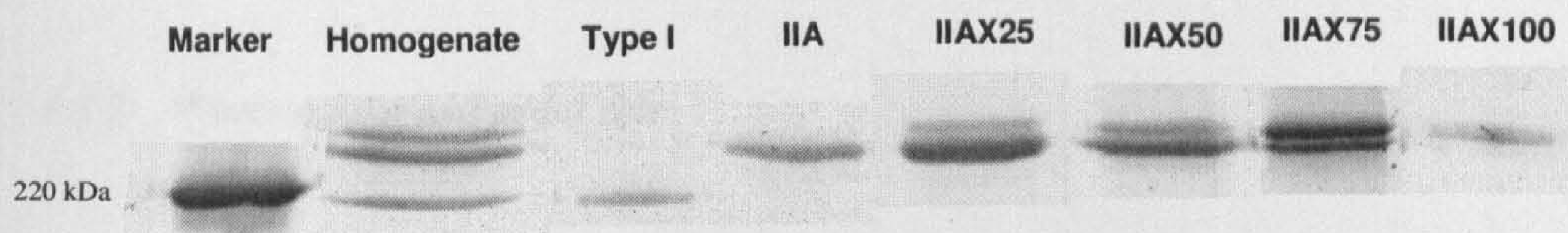


Figure 5.1 *Example of SDS-PAGE gels showing the different fibre groups, which were quantified through calibrated densitometry, and the molecular weight marker for myosin heavy chain.*

5.2.4 Statistical analyses

Data were analysed by either two-way (temperature and time) analysis of variance (ANOVA) with repeated measures or paired *t*-tests. Where a significant effect was detected, differences were located with *post-hoc t*-tests with Bonferroni correction. Significance was accepted at $P<0.05$. Data are presented as means ± S.D.

5.3 RESULTS

5.3.1 Temperature

T_m was 3.0 ± 0.4 °C higher ($P<0.05$) prior to ET (37.3 ± 0.2 °C) compared to N (34.3 ± 0.6 °C). T_{rec} was also higher ($P<0.05$) in ET (37.1 ± 0.2 °C) compared to N (37.2 ± 0.2 °C).

5.3.2 Power output and pedal rate

Maximal power output during the sprint was 23 % greater ($P<0.05$) in ET compared to N (1427 ± 493 W vs. 1169 ± 398 W, respectively) (Fig 5.2). Maximal pedal rate was also greater ($P<0.05$) in ET compared to N (179 ± 30 revs.min^{-1} vs. 157 ± 24 revs.min^{-1}, respectively), increasing by 14 %. Mean power output and mean pedal rate were also 14 and 15 % greater ($P<0.05$), respectively.

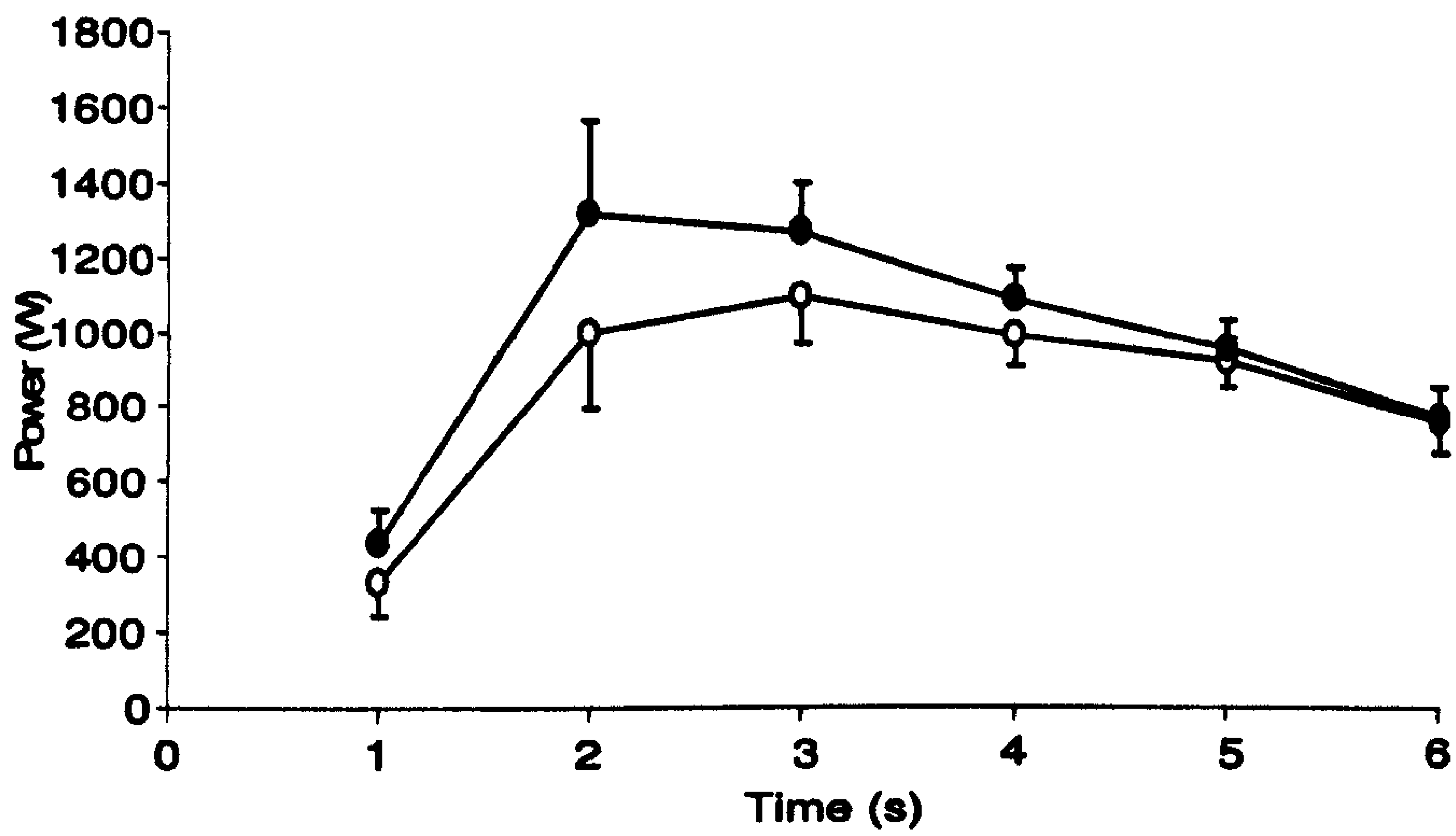


Figure 5.2. *Power output during 6 s maximal sprint exercise under normal (N; open circles) and elevated (ET; filled circles) muscle temperature conditions. Values are mean ± SD, (N=6).*

5.3.3 *Resting ATP and PCr content*

In N, both ATP and PCr content was lower (P<0.05) in type I fibres compared to the five type II fibre groups (Tables 5.1 and 5.2). Within these type II fibre groups ATP and PCr content were the same (P>0.05). In ET, PCr content was also lower in type I compared to type II fibres (Table 5.2). ATP content, however, was the same in type I and II fibres (Table 5.1). Resting ATP and PCr content remained the same (P>0.05) following elevation of T_m.

Table 5.1. *ATP content in MHC characterized single muscle fibres before and after a 6 s maximal sprint under normal (N) and elevated (ET) muscle temperature conditions.*

	N				ET			
Table	Rest	(n)	Post-Ex	(n)	Rest	(n)	Post-Ex	(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*	(78)	23.2 ± 4.3†	(84)	23.9 ± 3.9	(59)	20.8 ± 4.1†‡	(86)
IIAX25	25.1 ± 3.7*	(26)	22.6 ± 4.5	(24)	23.1 ± 2.6	(9)	22.8 ± 4.8	(26)
IIAX50	25.8 ± 3.0*	(18)	20.4 ± 4.4†	(42)	23.5 ± 4.4	(32)	22.3 ± 3.7†	(15)
IIAX75	25.8 ± 4.7*	(18)	19.8 ± 2.7†	(8)	22.7 ± 3.1	(8)	19.8 ± 1.4	(8)
IIAX100	26.0 ± 2.6*	(3)	17.4 ± 4.7†	(12)	26.3 ± 4.6	(9)	19.4 ± 4.8	(5)

Values are expressed as mean ± S.D with the number of fibres analyzed represented by (n). * denotes a significant difference compared to type I fibres (P<0.05), † denotes a significant difference compared to rest (P<0.01) and ‡ denotes a significant difference compared to N (P<0.01). Values are mmol.kg.$^{-1}$ (dm).

5.3.4 Post-Exercise ATP content

After exercise, in N, ATP content decreased ($P<0.01$) in type IIA, IIAX50, IIAX75 and IIAX100 fibre groups, whereas in ET ATP content decreased ($P<0.01$) in type IIA and IIAX50 fibres only. There was no difference in post-exercise ATP content between trials except in the type IIA fibres (Table 5.1). In these fibres ATP content was lower ($P<0.01$) in ET compared to N, decreasing by 3.1 $mmol.kg^{-1}$ (dm) (13 %) in ET and 1.6 $mmol.kg^{-1}$ (dm) (6 %) in N.

5.3.5 Post-Exercise PCr content

PCr content was lower ($P<0.01$) after exercise in all fibre groups under both conditions. Post-exercise PCr content was lower ($P<0.01$) in type IIA fibres in ET compared to N (Table 5.2), decreasing by 57.1 $mmol.kg^{-1}$ (dm) (71.2 %) in ET and 48.7 $mmol.kg^{-1}$ (dm) (59.3 %) in N. In the other fibre groups there were no differences in post-exercise PCr content, between conditions, although there was a tendency for PCr content to be lower in IIAX25 fibres in ET compared to N ($P=0.02$, not significant due to Bonferroni correction).

Table 5.2. *PCr content in MHC characterised single muscle fibres before and after a 6 s maximal sprint under normal (N) and elevated (ET) muscle temperature conditions.*

	N				ET			
Table	**Rest**	**(n)**	**Post-Ex**	**(n)**	**Rest**	**(n)**	**Post-Ex**	**(n)**
I	67.6 ± 12.1	(42)	34.1 ± 10.0†	(48)	72.5 ± 16.8	(30)	30.7 ± 6.5†	(68)
IIA	82.2 ± 14.5*	(78)	33.5 ± 14.1†	(84)	80.3 ± 14.4*	(59)	23.1 ± 9.3†‡	(86)
IIAX25	87.6 ± 18.0*	(26)	35.2 ± 14.2†	(24)	88.5 ± 15.8*	(9)	23.5 ± 12.4†	(26)
IIAX50	86.0 ± 18.1*	(24)	31.4 ± 12.8†	(42)	84.1 ± 18.4*	(32)	28.4 ± 14.5†	(15)
IIAX75	84.3 ± 20.5*	(18)	32.6 ± 8.5†	(8)	83.2 ± 15.4*	(8)	24.3 ± 4.0†	(8)
IIAX100	93.1 ± 9.3*	(3)	30.6 ± 15.0†	(12)	82.7 ± 9.1*	(9)	19.5 ± 13.4†	(5)

Values are expressed as mean ± S.D with the number of fibres analysed represented by (n). * denotes a significant difference compared to type I fibres ($P<0.05$), † denotes a significant difference compared to rest ($P<0.01$) and ‡ denotes a significant difference compared to N ($P<0.01$). Values are $mmol.kg^{-1}$ (dm).

5.4 DISCUSSION

The present study has demonstrated that passive elevation of T_m prior to maximal sprint exercise results in a greater maximal power output, as would be expected, and also a greater ATP and PCr degradation in type IIA fibres, compared to the control condition.

Resting PCr content was ~20 % greater in type II fibre groups compared to type I fibres, which is in agreement with several previous studies (Tesch *et al.*, 1989;Söderlund & Hultman, 1991;Söderlund *et al.*, 1992;Sahlin *et al.*, 1997). However, within the type II hybrid groups PCr content was the same, in the present study, which is in contrast to previous observations of a progressive increase in PCr content from type IIA through to IIX fibres (Sant'Ana Pereira *et al.*, 1996) although this has not always been found to be the case (Karatzaferi *et al.*, 2001b). There was a 10 % greater resting ATP content in type II fibre groups compared to type I fibres in the control condition. This is in contrast to previous work which found similar resting ATP content in type I and II fibres (Greenhaff *et al.*, 1993;Greenhaff *et al.*, 1994;Karatzaferi *et al.*, 2001b) although some work has found similar results to the present study (Söderlund *et al.*, 1992). Furthermore, as previously observed, there was no difference in the resting ATP content of the type II hybrid fibres in both conditions (Sant'ana Pereira *et al.*, 1996;Karatzaferi *et al.*, 2001b). Whilst the present data agrees with several previous studies, the differences in single fibre metabolite content between these studies highlights the natural variation in ATP and PCr content observed through single fibre analyses.

Six seconds of maximal sprint exercise (in the control condition) resulted in large

reductions in PCr content (from 49 % in type I fibres up to 67 % in IIAX100, respectively), supporting the previous finding that type II fibres, and in particular those with a high proportion of the MHC IIX isoform, are more active than type I fibres during short-term high-intensity exercise (Karatzaferi *et al.*, 2001b). The decline in ATP content was also greater as the proportion of MHC IIX increased (although was statistically insignificant in the IIAX25 group). This pattern and magnitude of the decline in ATP is consistent with those observed during a 10 s maximal sprint (Karatzaferi *et al.*, 2001b).

The effect of passively elevating T_m was to increase ATP and PCr degradation in fibres expressing predominantly MHC IIA during the maximal sprint exercise, when power output had increased. PCr is a sensitive marker of the degree of activity of single muscle fibres (Conjard & Pette, 1999) and would represent a greater metabolic stress acting upon these fibres. Furthermore since PCr hydrolysis is directly related to the amount of external work performed (Infante *et al.*, 1965) it can be concluded that the greater power output observed in the elevated temperature condition, in the present study, is in part the result of a greater degree of activity, or activation, of MHC IIA fibres. This can also account for the greater rate of ATP turnover observed previously at higher T_m (chapter 4) and may also be the product of an increased activity of creatine kinase as this enzyme approaches its optimum temperature of approximately 42 °C (Wyss *et al.*, 1990). In fact in the heated condition, where the change in homogenate PCr was 47.7 $mmol.kg^{-1}$ (d.m.), the change in PCr in type I fibres was 41.8 $mmol.kg^{-1}$ (d.m.) and 57.2 $mmol.kg^{-1}$ (d.m.) in type IIA fibres (average type I + IIA = 49.5 $mmol.kg^{-1}$ (d.m.), calculated from chapter 5: Table 5.2) demonstrating that the increased PCr hydrolysis in IIA fibres can indeed account for

the increase in homogenate PCr hydrolysis after the elevation of T_m.

The greater activity of specifically type IIA fibres, at higher T_m, may be explained by considering the individual power-velocity relationships of different fibre types. In the present study subjects reached pedal rates of 160 – 180 revs.min^{-1}, a velocity close to the estimated V_{max} of ~165 revs.min^{-1} for type I fibres (Sargeant, 1994) and just beyond the estimated V_{opt} for type IIA fibres of ~130 revs.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 these cadences will, therefore, have little influence on cross-bridge cycling and consequently ATP utilisation and power producing capability of type I fibres, 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. Consequently the temperature shift will have its largest effect on ATP utilisation and power production of these fibres. Muscle fibres containing the type IIX isoform are likely to be working on the ascending left-side of their power-velocity relationship where temperature will have little effect on these fibres. From these estimations, therefore, it becomes clear why at the relatively high velocities reached in the present study, an increased activity of type IIA fibres would be responsible for the increase in power output at higher temperatures, rather than type I fibres as suggested by Sargeant and Rademaker (1996), although this may be the case at the slightly lower cadences of between 60 and 140 revs.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 PCr/Cr ratio as a measure of fibre activity (e.g. Beltman *et al.*, 2004b), 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 co-efficient of variations of 6.1 and 4.1 %, and thus accurate measures of ATP and PCr content, respectively. Like any study utilising the muscle biopsy technique there will still be natural variation between samples taken from the same person, which is also the case for single fibres since different fibres, although of the same type, are analyzed and compared in the different conditions. It has also proved useful to investigate hybrid fibres in greater depth in the present study; however, because one is limited by the number of hybrid fibres found in each sample to differentiate more hybrid groups would have been problematic. The present study, nevertheless, supports 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 a greater ATP and PCr degradation in type IIA fibres after passive elevation of muscle temperature. This greater activation of these fibres resulted in the greater power output at high muscle temperatures during short-term maximal exercise where pedal rates reached 160 – 180 $revs.min^{-1}$.

Chapter 6

THE EFFECT OF PASSIVELY INCREASING MUSCLE TEMPERATURE ON MECHANICAL EFFICIENCY AND SINGLE FIBRE METABOLISM DURING HEAVY EXERCISE

6.1 INTRODUCTION

As discussed in the preceding chapters T_m is a factor known to moderate both the metabolic and mechanical performance of skeletal muscle (e.g. Bennett, 1984;Rall & Woledge, 1990). Indeed in chapters 4 and 5 it was demonstrated that passively increasing T_m prior to short-term maximal exercise leads to an increased rate of anaerobic ATP turnover and type IIA fibre activity, alongside a greater power output. When the force of the muscle is maintained constant elevating T_m has also been shown to lead to a greater anaerobic ATP turnover during isometric contractions (Edwards *et al.*, 1972), which would suggest that efficiency may be reduced at higher T_m. During isometric contractions, however, energy turnover is known to be different than during dynamic exercise (Fenn, 1923), which will be more related to daily activities and athletic events.

This led Febbraio and colleagues (1996) to study the effect of an elevated T_m on skeletal muscle metabolism during 2 min cycling at 115 % VO_{2peak} and found a greater glycogenolysis, glycolysis and ATP hydrolysis after elevation of T_m. Furthermore, as exercise progresses (20 min cycling at 70 % $\dot{V}O_{2peak}$) increasing T_m was shown to result in a greater glycogenolysis, with no change in high energy phosphate degradation (Starkie *et al.*, 1999). During exercise of this duration, however, the main energy source is oxidative phosphorylation (e.g. Medbo & Tabata, 1993;Parolin *et al.*, 1999) which was not measured in any of the previous studies meaning mechanical efficiency could not be accurately reported.

One study has reported the effect of elevating T_m on mechanical efficiency during 6 min cycling at 85 % $\dot{V}O_{2max}$, and found that after elevation of T_m mechanical

efficiency was reduced at 60 $revs.min^{-1}$ and increased at 120 $revs.min^{-1}$ (Ferguson *et al.*, 2002). This study, however, used measures of $\dot{V}O_2$ and blood lactate concentration to calculate efficiency, with no measure of muscle metabolism. The authors of this study suggested that the decrease in efficiency, at 60 $revs.min^{-1}$, was the result of a decrease in the efficiency of type I fibres although no research to date has investigated the effects of T_m on single fibre metabolism during heavy cycling exercise of this duration. Furthermore, whilst blood lactate gives an indication of anaerobic energy turnover (di Prampero & Ferretti, 1999) it does not necessarily reflect the response within the muscle since lactate can be taken up and metabolised by various tissues, including the heart and inactive muscles (Gladden, 2000;Gladden, 2004).

The aim of this study, therefore, was to investigate the effects of passive elevation of T_m on skeletal muscle metabolism and $\dot{V}O_2$, and thus mechanical efficiency, during heavy cycling exercise performed at 60 $revs.min^{-1}$. It was hypothesised that elevating T_m would lead to a greater rate of anaerobic ATP turnover alongside an elevation in the amplitude of $\dot{V}O_2$, resulting in a decrease in mechanical efficiency at higher T_m. A further purpose of the present study was to investigate the metabolic response within single muscle fibres with the hypothesis being that there would be a greater PCr, and possibly ATP, degradation in type I fibres after the elevation of T_m.

6.2 METHODS

6.2.1 Subjects

Six healthy male subjects (age 25 ± 3 yrs, height 1.85 ± 0.06 m, mass 87 ± 16 kg; means ± S.D.) volunteered for the study.

6.2.2 Experimental protocol

Prior to the experimental trials subjects' $\dot{V}O_{2peak}$ and LT were determined on an electromagnetically braked cycle ergometer at 60 revs.min^{-1}, as described in chapter 3. An external power output half way between LT and $\dot{V}O_{2peak}$ (Δ50 %) was then calculated and used in the subsequent experimental trials. Approximately 7 d later a familiarisation trial was carried out to allow subjects to become accustomed to the intensity of the exercise bout and to breathing through the mouthpiece whilst exercising.

Around 7-14 d after habituation subjects attended the laboratory on 4 separate occasions to perform a 6 min cycling bout under normal (N) and elevated temperature (ET) conditions and a 2 min cycling bout also under both T_m conditions. The order of these trials was randomised and each trial was separated by a minimum of 7 d, shown schematically in Fig 6.1.

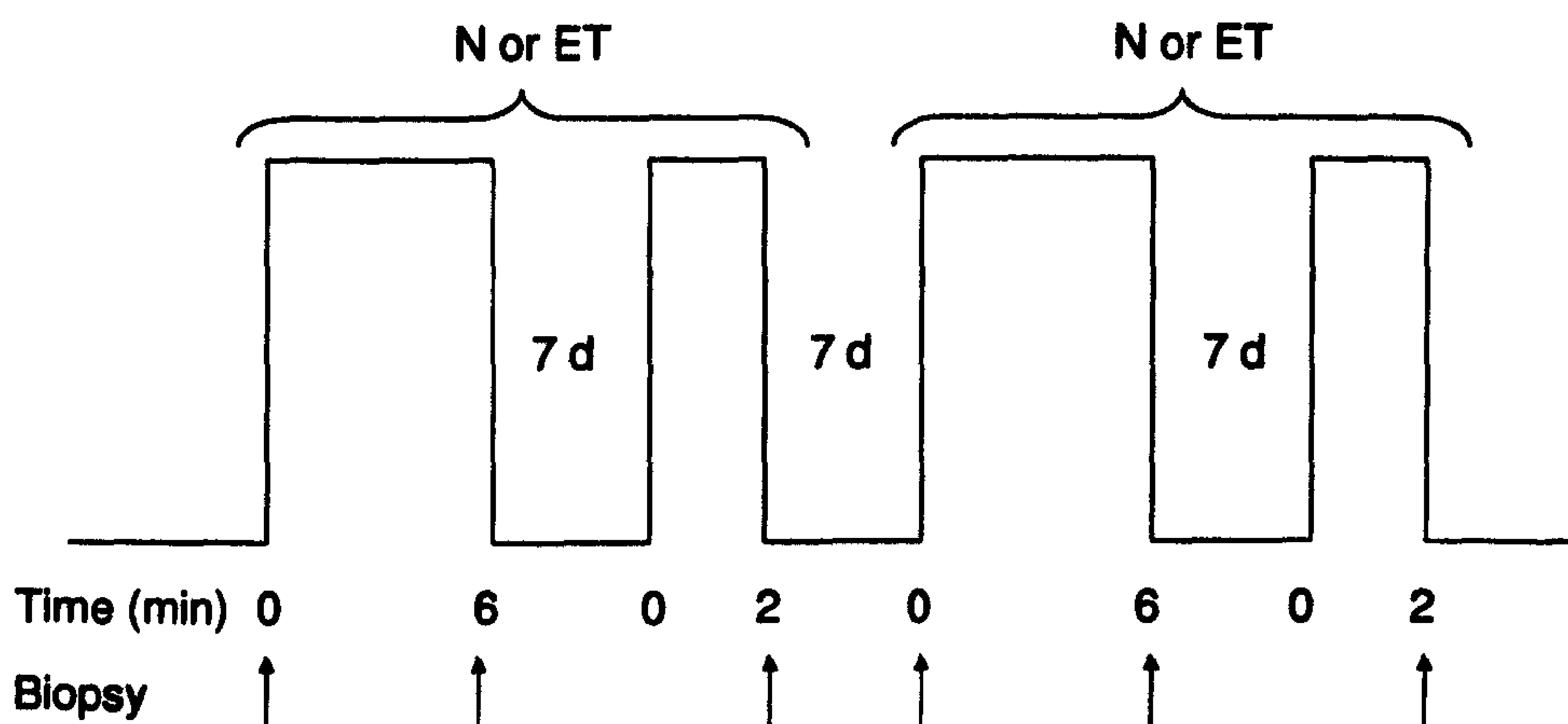


Fig 6.1. *Schematic diagram of experimental protocol.*

On arrival to the laboratory subjects inserted a rectal thermistor probe to allow continuous monitoring of T_{rec}. In the control condition, subjects then rested supine for 30 min at normal room temperature (20-22 °C) whilst the muscle biopsy site was prepared and a flexible T_m probe inserted. In ET, T_m was passively elevated as described in chapter 3 with the legs then prepared as in N.

In both 6 min trials a resting biopsy was taken at this point (during the 2 min trials a resting biopsy was not taken). Subjects then moved to the cycle ergometer where they sat at rest for 3 min, with the blankets still wrapped around the legs in ET, before cycling at 60 revs.min^{-1} (Δ 50%) for either 2 or 6 min. In all trials a post exercise biopsy was taken immediately after exercise with the subject remaining seated on the bike. Pulmonary gas exchange was recorded continuously at rest and throughout the 6 min trials (the mouthpiece was removed ~15 s before the end of exercise to allow the muscle biopsy to be taken). The $\dot{V}O_2$ data for each condition was averaged between two repeated trials, with muscle samples obtained on only the first occasion.

6.2.3 *Muscle analyses*

After freeze-drying, muscle samples were analysed for homogenate ATP, PCr, lactate, glycogen and MHC composition. Furthermore, from 2 subjects' singles fibres were dissected from the remainder of the muscle sample and analysed for ATP, PCr and MHC composition. Single fibres were characterised into one of three groups dependent upon their MHC content; type I, type IIA and IIAX as described in chapter 5.

6.2.4 *Calculations*

The kinetics of the $\dot{V}O_2$ response, the slow component and the O_{2def} were estimated as described in chapter 3. Aerobic ATP turnover was calculated using the RER and $\dot{V}O_2$ (Weir, 1949). It is worth noting that while pulmonary $\dot{V}O_2$ is a close reflection of muscle oxygen uptake they may differ by around 10 % (Barstow *et al.*, 1994). Furthermore, the small amount of oxygen released from myoglobin was not accounted for in the current study and so the "true" $\dot{V}O_2$ will be higher than that measured at the lung in this study.

Anaerobic ATP turnover was calculated as: ΔPCr + 1.5Δ muscle lactate + 2 ΔATP (Spriet, 1995). The small quantity of ATP produced, or utilized, relating to the accumulation of glycolytic metabolites (e.g. pyruvate and glyc-3-P) are neglected as they represent <2 % of the rate of ATP turnover in all cases (Spriet *et al.*, 1987a). The estimation of anaerobic ATP turnover in this chapter will be an underestimation since lactate efflux, nor the amount metabolised by other tissues, is not measured (Bangsbo *et al.*, 1990). Lactate release has, however, been shown to be the same when T_m is

passively elevated by about 3 °C during knee extensor exercise (Ferguson *et al.*, 2006) so this will not significantly affect comparisons between conditions.

For the calculation of anaerobic energy turnover ΔH (molar enthalpy change) values of 35, 55 and 67 kJ mol^{-1} of ATP produced were used for net ATP hydrolysis, phosphocreatine breakdown and glycolysis leading to lactate formation, respectively (Curtin & Woledge, 1978;Woledge & Reilly, 1988;Gonzalez-Alonso *et al.*, 2000). The total rate of energy turnover was determined as the sum of aerobic and anaerobic energy turnover, respectively. Internal power output ($W.kg^{-1}$) was estimated as 0.153 $(frequency)^3$, where frequency was converted to Hz from the $revs.min^{-1}$, as described by Minetti *et al* (2001). A brief description of the derivation of this equation is detailed in chapter 4 (page 102). The external power output was automatically monitored, in a velocity dependent manner, on the cycle ergometer and this gave a value of external power output used in further calculations. Mechanical efficiency was defined as the ratio between total mechanical power output, including the internal power output, and total energy turnover.

6.2.5 Statistical analysis

Data were analysed by either two-way (temperature and time) analysis of variance (ANOVA) with repeated measures (efficiency was analysed in this way, should I specify this?) or paired *t*-tests. Where a significant effect was detected, differences were located with *post-hoc* *t*-tests with Bonferroni correction. Significance was accepted at $P<0.05$. Data are presented as means ± S.D.

6.3 RESULTS

6.3.1 $\dot{V}O_{2peak}$ and LT

The mean $\dot{V}O_{2peak}$ was 3.4 ± 0.5 $l.min^{-1}$ and was achieved at an external power output of 281 ± 54 W. LT occurred at 164 ± 49 W (58 ± 11 % of $\dot{V}O_{2peak}$), with the external power output required to elicit Δ50 % was calculated to be 219 ± 50 W.

6.3.2 Rectal and muscle temperature

The warming protocol in the present study resulted in a 2.7 ± 1.1 °C elevation ($P<0.05$) in T_m (34.7 ± 1.1 °C in N and 37.4 ± 0.2 °C in ET) prior to the onset of exercise. T_{rec}, on the other hand, was not different ($P=0.07$) between trials (37.1 ± 0.2 and 37.3 ± 0.2 °C in N and ET, respectively).

6.3.3 Homogenate muscle metabolites and ATP turnover

Passively elevating the temperature of the legs had no effect ($P>0.05$) on the resting concentration of any metabolites (Table 6.1). After 2 mins of exercise PCr concentration was lower ($P<0.05$) in ET compared to N, decreasing by 32.0 ± 8.3 $mmol.kg^{-1}$ (dm) in N and by 37.8 ± 10.6 $mmol.kg^{-1}$ (dm) in ET. Lactate concentration was higher ($P<0.05$) at 2 min in ET compared to N, increasing by 8.6 ± 3.6 $mmol.kg^{-1}$ (dm) in N and by 13.4 ± 3.9 $mmol.kg^{-1}$ (dm) in ET. There was no effect of temperature or time on the ATP concentration after 2 min of exercise. Muscle glycogen content was not affected by T_m but ($P<0.05$) decreased as exercise progressed, except from between 2-6 min in the ET. After 6 min of exercise there

were no differences in the concentrations of metabolites between temperature conditions.

From these changes in metabolites it was calculated that in the first 2 min of exercise there was a 28 % greater ($P<0.05$) rate of total anaerobic ATP turnover. This increase was the result of a greater ($P<0.05$) rate of glycolytic ATP turnover and a trend ($P=0.05$) for a greater rate of ATP turnover from PCr hydrolysis. On the other hand, between 2-6 min and for the overall 6 min exercise period elevation of T_m had no effect on the rate of anaerobic ATP turnover within the muscle.

Table 6.1. *Muscle metabolites before and after 2 and 6 min of heavy exercise under conditions of normal (N) and elevated (ET) muscle temperature.*

		Rest	2 min	6 min
PCr	N	72.9 ± 7.0	40.9 ± 8.3†	29.4 ± 8.4†
	ET	72.8 ± 9.7	35.0 ± 8.8†*	26.9 ± 7.4†
Lactate	N	5.4 ± 0.7	14.0 ± 3.8†	26.2 ± 4.4†
	ET	6.0 ± 0.6	19.4 ± 3.7†*	32.6 ± 7.2†
ATP	N	22.7 ± 1.8	21.7 ± 1.9	19.8 ± 1.8
	ET	23.2 ± 0.8	22.8 ± 2.0	21.5 ± 1.7
Glycogen	N	399.7 ± 139.0	332.8 ± 79.1†	242.7 ± 78.8†
	ET	417.5 ± 125.7	302.3 ± 97.9†	262.7 ± 53.3

Values are means ± S.D. (N=6). Significant difference ($P<0.05$) from same time-point in N denoted by *, different from previous time point denoted by †. Values are expressed as $mmol.kg^{-1}$ (dm) except for glycogen which is in mmol glycosyl $units.kg^{-1}$ (dm).

Table 6.2. ***Rates of anaerobic ATP turnover between 0-2, 2-6 and 0-6 min during heavy exercise under conditions of normal (N) and elevated (ET) muscle temperature.***

		0-2 min	2-6 min	0-6 min
PCr ATP turnover	N	0.27 ± 0.07	0.05 ± 0.01	0.12 ± 0.03
	ET	0.31 ± 0.09	0.03 ± 0.02	0.13 ± 0.03
Glycolytic ATP turnover	N	0.08 ± 0.03	0.08 ± 0.03	0.08 ± 0.03
	ET	0.13 ± 0.03*	0.09 ± 0.04	0.10 ± 0.04
Anaerobic ATP turnover	N	0.36 ± 0.06	0.13 ± 0.03	0.21 ± 0.03
	ET	0.46 ± 0.08*	0.13 ± 0.07	0.24 ± 0.04

Values are means ± S.D. (N=6). Significant difference ($P<0.05$) between conditions denoted by *. Values are expressed as $mmol.kg^{-1}$ $(dm)s^{-1}$

6.3.4. Single fibre ATP content

As with the homogenate data elevating the temperature of the muscle had no effect on resting single fibre ATP content (Table 6.3). There was also no difference in the resting ATP content between fibre groups. After 2 min of exercise ATP content was reduced ($P<0.01$) compared to rest in all fibre types, in both N and ET. Thereafter ATP content was only reduced in type IIA fibres in both N and ET. The elevation of T_m resulted in a lower ($P<0.01$) ATP content, compared to the control trial, after 6 min of exercise in the type I fibres.

6.3.4. Single fibre PCr content

T_m had no effect on the content of PCr at rest. In N and ET resting PCr content was higher ($P<0.05$) in type IIAX fibres compared to type I fibres, and in ET was also higher ($P<0.05$) in IIA compared to type I fibres. From rest to 2 min of exercise PCr content was reduced ($P<0.01$) in all fibre types in both N and ET. In N PCr content was reduced ($P<0.01$) further from 2-6 min in type I and IIA fibres, with no further reduction in PCr content in ET. There was no effect of elevating T_m on PCr content although there was a trend ($P=0.06$) for a lower PCr content in type I fibres after 2 min of exercise in ET compared to N.

Table 6.3. *Single fibre ATP content in characterised muscle fibres under control (N) and elevated (ET) muscle temperatures*

	N			ET		
	0 min (n)	2 min (n)	6 min (n)	0 min (n)	2 min (n)	6 min (n)
I	23.9 ± 2.3 (15)	22.1 ± 5.3‡ (22)	24.6 ± 2.8 (21)	24.0 ± 5.2 (15)	20.1 ± 2.3‡ (17)	18.1 ± 2.6* (20)
IIA	25.5 ± 3.3 (18)	22.5 ± 2.9‡ (25)	19.6 ± 3.5†‡ (25)	24.1 ± 3.7 (24)	22.8 ± 2.1†‡ (17)	19.9 ± 5.1‡ (18)
IIAX	26.5 ± 3.3 (30)	21.6 ± 1.4‡ (8)	21.7 ± 4.0 (11)	27.0 ± 2.8 (9)	25.5 ± 5.3†‡ (5)	20.6 ± 6.7 (11)

Values are expressed as mean ± S.D with the number of fibres analyzed represented by (n). * denotes a significant difference compared N ($P<0.01$), † denotes a significant difference compared to type I fibres ($P<0.01$), ‡ denotes a significant difference from the preceding time point. Values are mmol.kg.$^{-1}$ (dm).

Table 6.4. *Single fibre PCr content in characterised muscle fibres under control (N) and elevated (ET) muscle temperatures*

	N			ET		
	0 min (n)	2 min (n)	6 min (n)	0 min (n)	2 min (n)	6 min (n)
I	75.0 ± 4.3 (15)	27.0 ± 11.4 ‡ (22)	16.6 ± 10.8 ‡ (21)	69.5 ± 13.2 (15)	21.3 ± 9.9‡ (17)	16.1 ± 6.5‡ (20)
IIA	83.7 ± 14.8 (18)	36.8 ± 17.4‡ (25)	23.3 ± 21.8‡ (25)	83.8 ± 9.9* (24)	34.7 ± 12.5*‡ (17)	27.5 ± 23.9 (18)
IIAX	90.5 ± 9.6* (30)	47.7 ± 29.1*‡ (8)	37.3 ± 18.1* (11)	86.9 ± 9.4* (9)	52.5 ± 31.7*‡ (5)	58.2 ± 27.2*† (11)

Values are expressed as mean ± S.D with the number of fibres analyzed represented by (n). * denotes a significant difference compared type I fibres ($P<0.01$), † denotes a significant difference compared to type IIA fibres ($P<0.01$), ‡ denotes a significant difference from the preceding time point. Values are $mmol.kg.^{-1}$ (dm).

6.3.6 Pulmonary $\dot{V}O_2$

Elevating T_m had no effect on any aspect of $\dot{V}O_2$ kinetics or the O_{2def} (Table 6.5; Fig 6.2). The 95 % confidence intervals for estimates of primary τ and primary $\dot{V}O_2$ amplitude, under both conditions, were 7.7 ± 3.0 s and 0.12 ± 0.5 $l.min^{-1}$, respectively.

Table 6.5. ***Pulmonary oxygen uptake responses to heavy exercise under conditions of normal (N) and elevated (ET) muscle temperature***

Parameter	N	ET
Baseline $\dot{V}O_2$ ($l.min^{-1}$)	0.44 ± 0.08	0.46 ± 0.09
Primary time constant (s)	30 ± 11	33 ± 11
Primary Amplitude ($l.min^{-1}$)	2.33 ± 0.56	2.34 ± 0.59
Absolute primary amplitude ($l.min^{-1}$)	2.76 ± 0.62	2.80 ± 0.66
Oxygen deficit (l)	2.2 ± 1.3	2.3 ± 1.2
Slow component amplitude ($l.min^{-1}$)	0.25 ± 0.20	0.10 ± 0.10
End-exercise $\dot{V}O_2$ ($l.min^{-1}$)	3.02 ± 0.67	2.96 ± 0.66

Values are means ± S.D. (N=6).

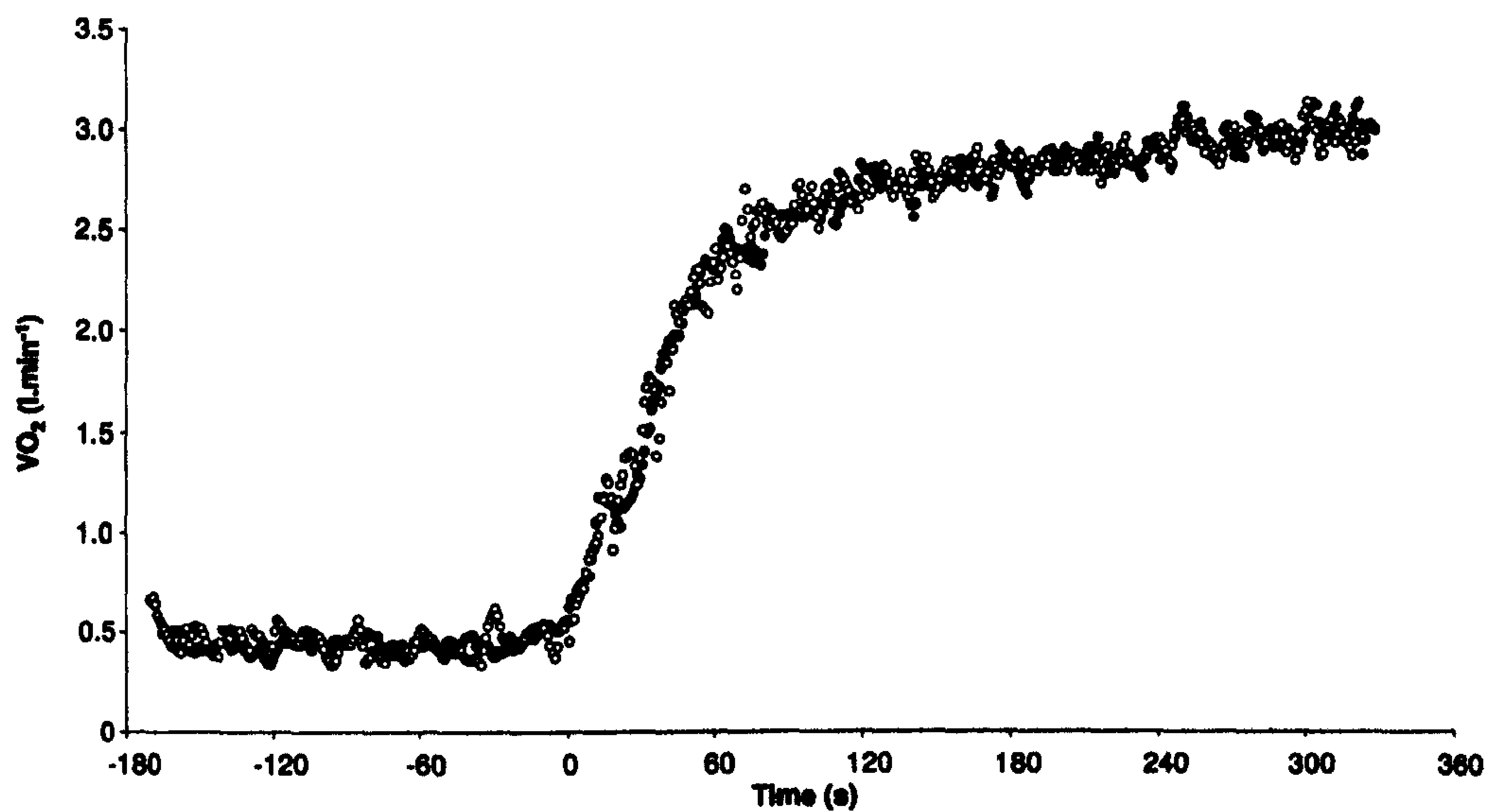


Fig 6.2. *Mean second-by-second $\dot{V}O_2$ response (after manual filtering, averaging and interpolating) to a 6 min of heavy exercise under control conditions (filled circles) and after elevation of T_m (closed circles) (N=6).*

6.3.7 *Energy turnover and mechanical efficiency*

As a consequence of the greater energy turnover ($P<0.05$) from PCr hydrolysis and lactate accumulation (Table 6.6) in the first two minutes of exercise, mechanical efficiency, calculated as the ratio between total mechanical power output (external + internal power output) and total energy turnover, was reduced ($P<0.05$) by 3.0 ± 1.0 % after increasing T_m. Between 2 and 6 min and over the full 6 min exercise period, however, there was no difference in mechanical efficiency between temperature conditions. In the control condition there was a lower ($P<0.05$) efficiency between 2-6 min compared to 0-2 min.

Table 6.6. *Energy turnover and mechanical efficiency between 0-2, 2-6 and 0-6 min under conditions of normal (N) and elevated (ET) muscle temperatures*

		0-2 min	2-6 min	0-6 min
Net ATP hydrolysis ($J.s^{-1}$)	N	7.3 ± 2.7	7.3 ± 4.1	7.3 ± 6.8
	ET	7.6 ± 3.9	1.4 ± 5.2	3.5 ± 5.7
Net PCr hydrolysis ($J.s^{-1}$)	N	196.7 ± 68.6	33.8 ± 11.1†	88.1 ± 23.6
	ET	232.5 ± 86.0*	23.1 ± 17.4†	92.9 ± 22.8
Lactate accumulation ($J.s^{-1}$)	N	94.6 ± 45.9	66.6 ± 35.5	76.1 ± 23.6
	ET	144.7 ± 42.2*	60.6 ± 46.4	88.6 ± 30.3
Aerobic energy turnover ($J.s^{-1}$)	N	530.5 ± 108.6	853.5 ± 177.4†	728.8 ± 153.0
	ET	539.4 ± 112.4	841.9 ± 174.8†	722.5 ± 148.7
Efficiency (%)	N	28.1 ± 1.4	24.1 ± 0.6†	25.6 ± 0.7
	ET	25.1 ± 1.1*	24.9 ± 1.1	25.5 ± 0.9

Values are means ± S.D. (N=6). Significant difference ($P<0.05$) between conditions denoted by * and from 0-2 min in the same condition by †.

6.4 DISCUSSION

The present study has demonstrated that passively elevating the temperature of the working muscles prior to heavy exercise results in an increase in the rate of anaerobic ATP turnover and a decrease in mechanical efficiency during the first two min of heavy exercise. This decrease in efficiency, shown for the first time in the present study, was associated with a tendency for a greater PCr degradation in type I fibres, although further work is required to confirm this.

The present study has demonstrated that at higher T_m there is a greater rate of anaerobic ATP turnover within the first two min of exercise, with no difference in the remainder of the exercise period. It must be pointed out that under both heated and control conditions the anaerobic ATP turnover will be underestimate as lactate release from the exercising muscle, nor the quantity taken up and metabolised by other tissues, was not measured. Lactate release has, however, been shown to be the same when T_m is passively elevated by about 3 °C during knee extensor exercise (Ferguson *et al.*, 2006) so this is unlikely to affect comparisons between conditions.

Moreover, taking the whole six min exercise into account there was no effect of increasing the temperature of the muscle on the rate of anaerobic ATP turnover within the muscle, which differs from the study of Ferguson *et al* (2002) who estimated a greater anaerobic energy turnover at higher T_m, from blood lactate measurements. This highlights that whilst blood lactate may give an indication of anaerobic ATP turnover within the muscle, it does not necessarily give an accurate measure of the muscular response. Previous work has shown that increasing T_m leads to a greater rate of anaerobic ATP turnover during isometric contractions (Edwards *et al.*, 1972) and a

greater accumulation of lactate, IMP, NH_3 and lower ATP after 2 min intense cycling at higher T_m (Febbraio *et al.*, 1996). These findings are similar to the response within the muscle, during the first two min of exercise in the present study, with a similar response absent in the remaining four min of exercise.

Previous work has shown that during the oxygen deficit the muscle are working more efficiently than when the steady state is achieved (Pahud *et al.*, 1980), a finding supported by the work of Krustrup *et al* (2003). These observations highlight the importance of making measurements of efficiency, both in the initial period (oxygen deficit) of exercise and during the steady state, as a true understanding of how efficiency is affected during exercise cannot be gained from examining the whole-exercise response. This is further supported by the present findings where efficiency was decreased at higher T_m in the initial period of exercise; an observation which would not have been picked up had measurements, e.g. muscle biopsies, only been made before and after the 6 min of exercise.

The reason for the greater rate of anaerobic ATP turnover observed at higher T_m may in part be the result of a Q_{10} effect on several other enzymes, along with mATPase (Steinen *et al.*, 1996;He *et al.*, 2000), including the non-contractile ATPases. There is, however, very little information regarding the effect of temperature on Na^+-K^+-ATPase with one study demonstrating a decrease in Ca^{2+}-ATPase activity after incubation at 41 °C (Schertzer *et al.*, 2002) suggesting, if anything, a decrease in non-contractile ATPase activity after elevation of T_m. Creatine kinase, which catalyses the hydrolysis of PCr, is also known to have an optimum temperature of around 42 °C (Wyss *et al.*, 1990) and its activity is ,therefore, likely to be enhanced after heating. There are clearly several other enzymes involved in the anaerobic energy production

pathways that may exhibit an increased activity at higher T_m, e.g. glycogen phosphorylase, PFK, lactate dehydrogenase, AMP deaminase and nucleoside phosphorylase which would increase adenine nucleotide metabolism (Tullson & Terjung, 1990) and glycogenolysis and glycolysis (Young *et al.*, 1985) although there is little data in human muscle concerning the effect of T_m on these key enzymes. Whilst it is clear, therefore, that increasing T_m leads to a greater anaerobic energy production in the initial period of exercise aerobic energy sources will be the major energy source (Medbo & Tabata, 1993;Parolin *et al.*, 1999) and thus necessary to make an accurate estimation of mechanical efficiency.

Previous work has shown that at 60 revs.min^{-1} passively elevating T_m leads to a greater $\dot{V}O_2$ (Ferguson *et al.*, 2002). The present study, however, found that increasing T_m does not lead to an elevation in $\dot{V}O_2$, an observation also found by Burnley *et al* (2002b) and Koga *et al* (1997) at slightly faster pedal rates. The reason for these contradictory results is unclear as the mode and intensity of exercise were similar between these investigations but may be due to the possibility of a raised T_{rec}, which was not measured, in the study of Ferguson and colleagues. It would appear therefore, that passively increasing T_m has no affect on any aspect of the $\dot{V}O_2$ response to heavy exercise. This has also recently been demonstrated across the exercising muscle during knee-extensor exercise (Ferguson *et al.*, 2006).

Although there was no difference in $\dot{V}O_2$ after the elevation of T_m in the present study mechanical efficiency was nevertheless reduced in the initial period of exercise. The fact that mechanical efficiency is decreased in the first two minutes of exercise and not for the whole exercise period may be due to a predominant effect of T_m on anaerobic energy sources, which are the dominant energy pathways at the onset of

exercise with a reduced anaerobic contribution as exercise continues. This may seem sensible since elevation of T_m has been associated with a greater anaerobic energy production during short-term exercise (Edwards *et al.*, 1972;Febbraio *et al.*, 1996) and a greater mATPase activity (Steinen *et al.*, 1996;He *et al.*, 2000) but has a lesser or no effect during more prolonged exercise (Starkie *et al.*, 1999), as in the present study, and has no appreciable affect on $\dot{V}O_2$.

Another possible explanation for the lack of T_m effect on efficiency as exercise progresses may be related to the exercise induced rise in T_m (e.g. Saltin *et al.*, 1968). It is possible that a temperature effect is observed at the onset of exercise but not at the end as T_m in N will approach the levels reached in ET. Whilst no measure of T_m was made post-exercise in the present study, previous work has shown that when T_m is raised by ~3 °C prior to exercise it is still approximately 1.5 °C higher at the end of heavy knee-extensor (Ferguson *et al.*, 2006) and cycling exercise (Koga *et al.*, 1997). It may therefore be the case that this smaller T_m difference may not be large enough to alter efficiency in the latter part of exercise, as was the case in the first two minutes.

It has previously been suggested that the decrease in mechanical efficiency at higher T_m is related to a decrease in the efficiency of type I fibres although this hypothesis has never been directly tested (Ferguson *et al.*, 2002). This suggestion arose from the hypothesis that at 60 $revs.min^{-1}$ the type I fibres will be working close to their optimum for efficiency (Sargeant & Jones, 1995) and that increasing T_m, leading to a rightward shift in the efficiency-velocity relationship, leads to a decrease in the efficiency of these fibres (Ferguson *et al.*, 2002). The study of Ferguson *et al* (2002) however made no measure of single fibre metabolism so whilst these assertions were arrived at using the available data further work was required in this area.

In that light the present study has shown that there is a greater ATP degradation in type I fibres from 0-6 min of the exercise period. This suggests that there is certainly a greater metabolic stress upon the type I fibres at higher T_m, although ATP content is not a good marker of the activity of muscle fibres (Conjard & Pette, 1999). On the other hand the PCr content of single muscle fibres does give a good indication of the activity of muscle fibres (Infante *et al.*, 1965;Conjard & Pette, 1999). Whilst there were no significant differences in PCr content between the different temperatures there was a tendency for a lower PCr content in type I fibres in ET after 2 min of exercise, where there was also a decrease in mechanical efficiency. From this observation it is therefore tempting to assume that the decrease in 'global' mechanical efficiency at the onset of exercise is due to a decrease in the efficiency of the type I fibres which would support, in part, the hypothesis of Ferguson *et al* (2002). The present study is however limited since single fibre analysis was only performed in 2 subjects. This has the consequence that we are unable to subdivide the characterisation of hybrid fibres in greater detail and that due to the lower number of fibres than in chapter 5 statistically significant differences are not likely. For these reasons the analysis of further single muscle fibres is required to confirm the above assertion.

In conclusion the present study has demonstrated that passively increasing the temperature of the working muscle leads to an increase in anaerobic energy turnover and thus a decrease in mechanical efficiency during the first two minutes of heavy exercise. There was, however, no effect of elevating T_m on anaerobic energy turnover as exercise progressed or on $\dot{V}O_2$ at any point throughout the exercise period. It is hypothesised that the effect of T_m on mechanical efficiency is related to a greater PCr

degradation in type I fibres although further work is required in this area.

Chapter 7

THE EFFECT OF PRIOR HEAVY EXERCISE ON MECHANICAL EFFICIENCY DURING HEAVY EXERCISE AT DIFFERENT PEDAL RATES

7.1 INTRODUCTION

Whilst the previous experimental chapters have focused on the effect of passively increasing T_m on energy turnover and efficiency it is likely that, in the athletic world, T_m will in fact be raised through prior exercise or 'active warm-up'. It has been well established that a bout of prior heavy exercise results in an increase in the amplitude of the $\dot{V}O_2$ primary component and a decrease in the slow component (Burnley *et al.*, 2000;Burnley *et al.*, 2002a;Burnley *et al.*, 2002b;Koppo *et al.*, 2003;Carter *et al.*, 2004;Sahlin *et al.*, 2005;Endo *et al.*, 2005). More recently, these changes in $\dot{V}O_2$ after prior heavy exercise have been demonstrated to lead to a decrease in gross efficiency during heavy cycling exercise (Sahlin *et al.*, 2005). Moreover, prior exercise is known to result in, amongst other things, an elevated baseline blood lactate concentration e.g. (Burnley *et al.*, 2006), changes in muscle fibre recruitment patterns (Burnley *et al.*, 2002a;Krustrup *et al.*, 2004a) and an elevation in T_m (e.g. Robinson *et al.*, 1965;Saltin *et al.*, 1968), each of which has been hypothesised to contribute to the observed changes in efficiency and $\dot{V}O_2$ during a subsequent exercise bout.

When considering the efficiency of the musculature during exercise it is important to take into account the contraction frequency at which the muscle is working, since this will clearly alter its position on the efficiency/velocity relationship (for review see Sargeant, 1999). This is, however, an area which has received little attention, with the majority of studies being performed at relatively low or undisclosed cadences (60-95 $revs.min^{-1}$), whilst athletes tend to adopt a faster cadence during competition (Sargeant, 1994). Moreover, passive elevation of T_m has been shown to lead to a decrease in efficiency at 60 $revs.min^{-1}$ and an increase at 120 $revs.min^{-1}$ (Ferguson *et al.*, 2002), highlighting the considerable effect of contraction velocity on the muscular

response to exercise.

It is unknown, therefore, whether the physiological response to prior heavy exercise is affected by changing the contraction frequency at which the muscle is working. The purpose of the present study was to investigate whether the response to prior heavy exercise was affected by an increase in pedal rate (from 60-120 revs.min^{-1}). It was hypothesised that at 60 revs.min^{-1} there would be a decrease in efficiency, slow component and an increase in primary $\dot{V}O_2$ amplitude as observed previously at various pedal rates in this range. At 120 revs.min^{-1} it was hypothesised that prior exercise would also decrease efficiency and increase the primary $\dot{V}O_2$ amplitude, although this effect would be of a smaller magnitude due to the aforementioned effect of the rise in T_m.

7.2 METHODS

7.2.1 Subjects

Six healthy male subjects (age 25 ± 5 years, height 1.82 ± 0.09 m, body mass 79 ± 16 kg; means ± S.D.), with no history of muscle or metabolic disorders, volunteered for the study.

7.2.2 Pre-Experimental procedures

LT and $\dot{V}O_{2peak}$ were determined on an electromagnetically braked cycle ergometer at both 60 and 120 revs.min^{-1}, from which the external power output half way between LT and $\dot{V}O_{2peak}$ was calculated for the experimental trials (Δ50 %). A familiarisation trial was then carried out, ~7 d later, allowing the subjects to accustom themselves to the intensity of the prior and experimental exercise bouts and to breathing through the mouthpiece whilst exercising.

7.2.3 Experimental protocol

After habituation subjects attended the laboratory on four occasions, separated by a minimum of 7 d. In these trials subjects performed a 6 min cycling bout (Δ50 %) under control conditions (no prior exercise) at 60 (C60) and 120 revs.min^{-1} (C120) and preceded by the prior-exercise protocol, described in chapter 4, at 60 (P60) and 120 revs.min^{-1} (P120).

On arrival to the laboratory subjects inserted a rectal thermistor ~10 cm beyond the

anal sphincter and were prepared for venous cannulation. Following venous cannulation subjects lay at rest at normal room temperature (20-22 °C) for approximately 30 min. After this period subjects mounted the cycle ergometer and sat at rest for a further 2 min whilst a resting blood sample and $\dot{V}O_2$ measurements were taken. In the control trials T_m was also measured at this point. In P60 and P120 subjects then performed the prior exercise protocol and on completion remained seated on the ergometer for 2 min whilst T_m was measured and a blood sample was taken. In all trials subjects then cycled for 2 min at 20 W followed immediately by the 6 min cycling bout at the previously determined power output and designated pedal rate. Pulmonary gas exchange was continuously measured (breath-by-breath) throughout the exercise protocol and for 3 min post-exercise. Blood samples were also collected at 0.5, 1.5, 3, 5, 10, 20 and 30 minutes after the cessation of exercise. The $\dot{V}O_2$ data for each condition was averaged between two repeated trials, with blood samples obtained on only the first occasion.

7.2.4 Blood analysis

Blood samples were analysed for haematocrit, haemoglobin and lactate concentration as described in chapter 3.

7.2.5 Calculations

After the completion of the experimental trials kinetic parameters of the $\dot{V}O_2$ response, the slow component and O_{2def} were calculated as described in chapter 3.

Aerobic energy turnover was calculated through the measured $\dot{V}O_2$ and the RER (Weir, 1949), with net $\dot{V}O_2$ being the average $\dot{V}O_2$ over the last 3 min of exercise minus resting $\dot{V}O_2$. The small amount of oxygen released from myoglobin was not accounted for in the current study and so the "true" $\dot{V}O_2$ will be higher than that measured at the lung in this investigation.

The energy contribution from anaerobic sources was estimated from the blood lactate values, with net lactate accumulation being taken as the rise in lactate concentration from rest to peak-post exercise levels, on the assumption that for every 1 $mmol.l^{-1}$ of blood lactate accumulated during exercise 3 ml $O_2.kg^{-1}$ are yielded (for review see di Prampero & Ferretti, 1999). This value was calculated by plotting the maximal aerobic power and the accumulation of blood lactate, as a function of $\dot{V}O_{2peak}$, during cycling at various exercise intensities. Further regression analysis revealed the value of 3 ml $O_2.kg^{-1}$ for every 1 $mmol.l^{-1}$ of blood lactate accumulated.

Internal power output ($W.kg^{-1}$) was estimated as 0.153 $(frequency)^3$, where frequency was converted to Hz from the $revs.min^{-1}$, as described by Minetti *et al* (2001). A brief description of the derivation of this equation is detailed in chapter 4 (page 102). The external power output was automatically monitored, in a velocity dependent manner, on the cycle ergometer and this gave a value for external power output in further calculations. Mechanical efficiency (%) was defined as the ratio between total mechanical power output, accounting for the internal power output, and the rate of energy turnover (sum of aerobic and anaerobic energy turnover) above that at rest (for detailed calculations see, Gaesser & Brooks, 1975).

7.2.6 Statistical analysis

Data were analyzed by two-way (trial and pedal rate) repeated measures ANOVA (including mechanical efficiency) and paired t-tests. Blood lactate data was analyzed by a three way (trial and pedal rate and time) repeated measures ANOVA. When a significant effect was located *post-hoc* bonferroni corrected *t*-tests were used to locate the differences. Significance was accepted at $P<0.05$. Data are presented as means ± S.D.

7.3 RESULTS

7.3.1 $\dot{V}O_{2peak}$ and LT

$\dot{V}O_{2peak}$ was not different ($P>0.05$) at the two pedal rates (3.9 ± 4.8 $l.min^{-1}$ at 60 $revs.min^{-1}$ and 3.8 ± 2.7 $l.min^{-1}$ at 120 $revs.min^{-1}$), with LT occurring at external power outputs of 198 ± 28 W at 60 $revs.min^{-1}$ and 145 ± 30 W at 120 $revs.min^{-1}$. During the experimental trials, therefore, the external power output set on the cycle ergometer was greater ($P<0.05$) at 60 $revs.min^{-1}$ (258 ± 29 W) compared to 120 $revs.min^{-1}$ (217 ± 22 W).

7.3.2 Rectal and muscle temperature

T_{rec} was 36.8 ± 0.2 and 36.9 ± 0.2 °C prior to exercise in C60 and C120, respectively. In P60 and P120 the effect of prior exercise was to increase ($P<0.05$) T_{rec} to 37.4 ± 0.4 and 37.6 ± 0.3 °C in P60 and P120, respectively. There was no difference in T_{rec} between the two pedal rates. Muscle temperature was higher ($P<0.05$) after the prior exercise compared to control values, at both pedal rates and at all depths (Fig 7.1). There was no difference in T_m between the three depths.

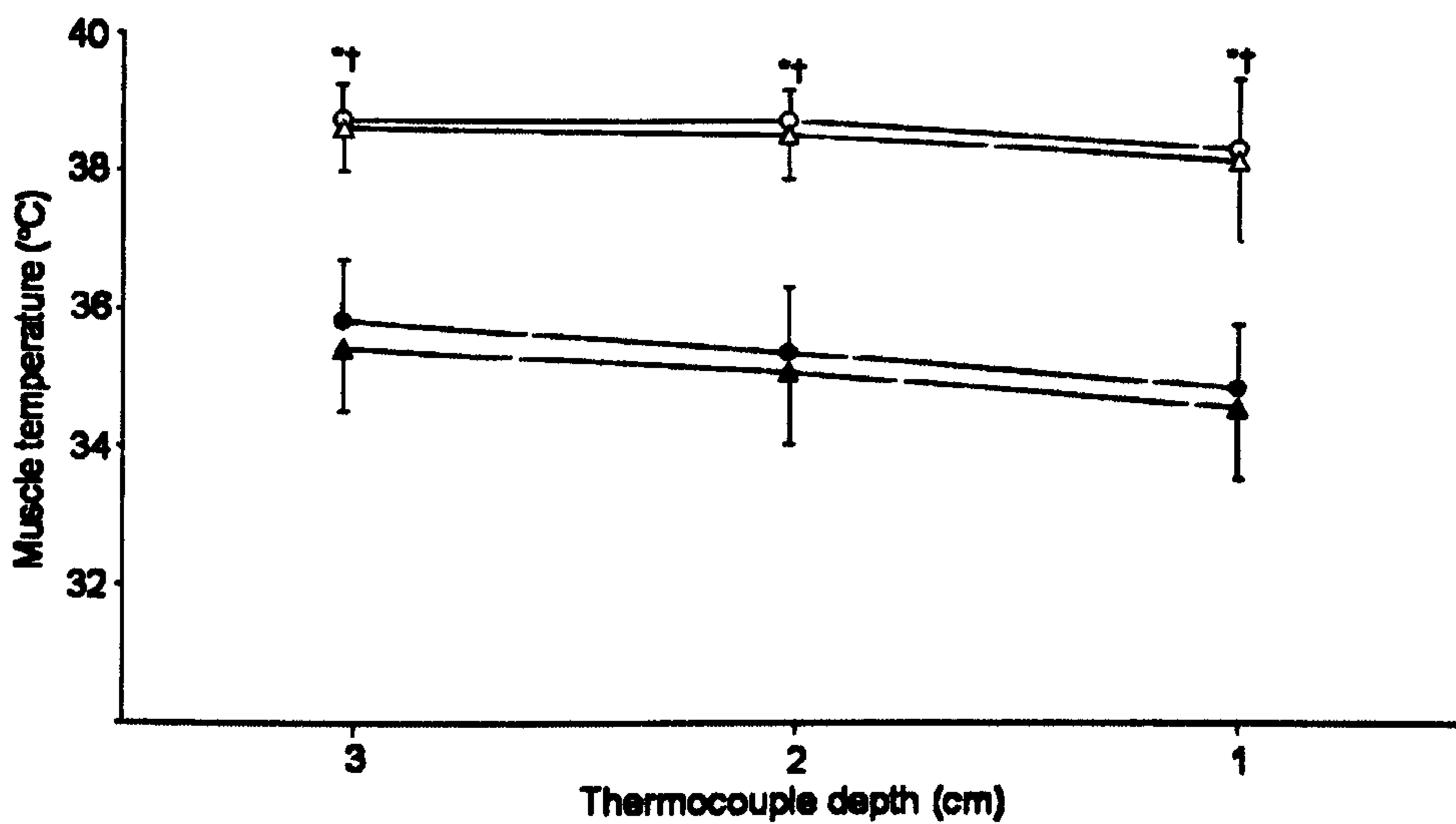


Fig 7.1. *Muscle temperature taken from the vastus lateralis at depths of 3, 2 and 1 cm during C60 (filled circles), C120 (filled triangles), P60 (open circles) and P120 (open triangles). Data are mean ± SD. * denotes a significant (P<0.05) difference between C60 and P60, with † denoting a significant (P<0.05) difference between C120 and P120. (N=6).*

7.3.3 $\dot{V}O_2$ kinetics, blood lactate and efficiency

Baseline $\dot{V}O_2$ was higher ($P<0.05$) after the prior exercise protocol. Prior heavy exercise had no effect on the time constant (τ) of the primary response or the primary $\dot{V}O_2$ amplitude at either pedal rate (Table 7.1). There was, however, a greater ($P<0.05$) primary amplitude in P120 compared to P60. The primary asymptote, or, the "absolute" primary amplitude (i.e. including baseline $\dot{V}O_2$), was greater ($P<0.05$) after prior exercise at both pedal rates, with the increase being greater at 120 revs.min^{-1}. Increasing the pedal rate also led to an increase ($P<0.05$) in the absolute primary amplitude when comparing C60 with C120, and, P60 with P120 (Table 7.1). The 95 % confidence intervals for the estimates of the primary τ and the primary $\dot{V}O_2$ amplitude over all 4 trials was 6.3 ± 2.0 s and 0.06 ± 0.02 l.min^{-1} respectively. The O_{2def} was similar ($P>0.05$) between all trials.

It can be seen from the group mean response in Fig 7.2 that the $\dot{V}O_2$ slow component was reduced ($P<0.05$) after the prior exercise regimen at both pedal rates (by 0.17 ± 0.19 l.min^{-1} at 60 revs.min^{-1}, and to a greater extent ($P<0.05$) by 0.36 ± 0.34 l.min^{-1} at 120 revs.min^{-1}). Moreover, the slow component was 0.10 ± 0.09 l.min^{-1} lower ($P<0.05$) in P120 compared to P60 (Table 7.1). End-exercise $\dot{V}O_2$ was not different ($P>0.05$) between all four trials (Table 7.1).

Table 7.1. *Oxygen uptake and efficiency in response to exercise with and without prior exercise, at 60 and 120 revs.min^{-1}.*

Parameter	C60	P60	C120	P120
Baseline $\dot{V}O_2$ (l.min^{-1})	0.87 ± 0.11	1.15 ± 0.17*	0.77 ± 0.16	1.15 ± 0.16*
Primary time constant (s)	22.2 ± 6.2	20.9 ± 5.6	21.2 ± 8.4	28.9 ± 10.5
Primary Amplitude (l.min^{-1})	2.25 ± 0.41	2.28 ± 0.22	2.47 ± 0.31	2.56 ± 0.10†
Absolute primary amplitude (l.min^{-1})	3.12 ± 0.42	3.43 ± 0.35*	3.25 ± 0.31†	3.71 ± 0.19*†
Oxygen deficit (l)	1.4 ± 0.7	1.3 ± 0.3	1.3 ± 0.7	1.0 ± 0.2
Slow component amplitude (l.min^{-1})	0.31 ± 0.19	0.14 ± 0.10*	0.39 ± 0.23	0.04 ± 0.15*†
End-exercise $\dot{V}O_2$ (l.min^{-1})	3.43 ± 0.40	3.51 ± 0.40	3.64 ± 0.23	3.75 ± 0.30
Aerobic Energy turnover (kj.min^{-1})	61.4 ± 8.4	66.4 ± 5.4*	65.0 ± 6.3	70.8 ± 5.2*
Anaerobic Energy turnover (kj.min^{-1})	6.7 ± 2.1	8.2 ± 2.9	10.1 ± 2.5	11.3 ± 0.9
Mechanical Efficiency (%)	24.0 ± 2.3	21.6 ± 1.8*	24.9 ± 1.2	22.6 ± 0.9*

Values are mean ± S.D. * denotes a significant difference from the control value at the same pedal rate. † from 60 revs.min^{-1} under the same condition. (N=6).

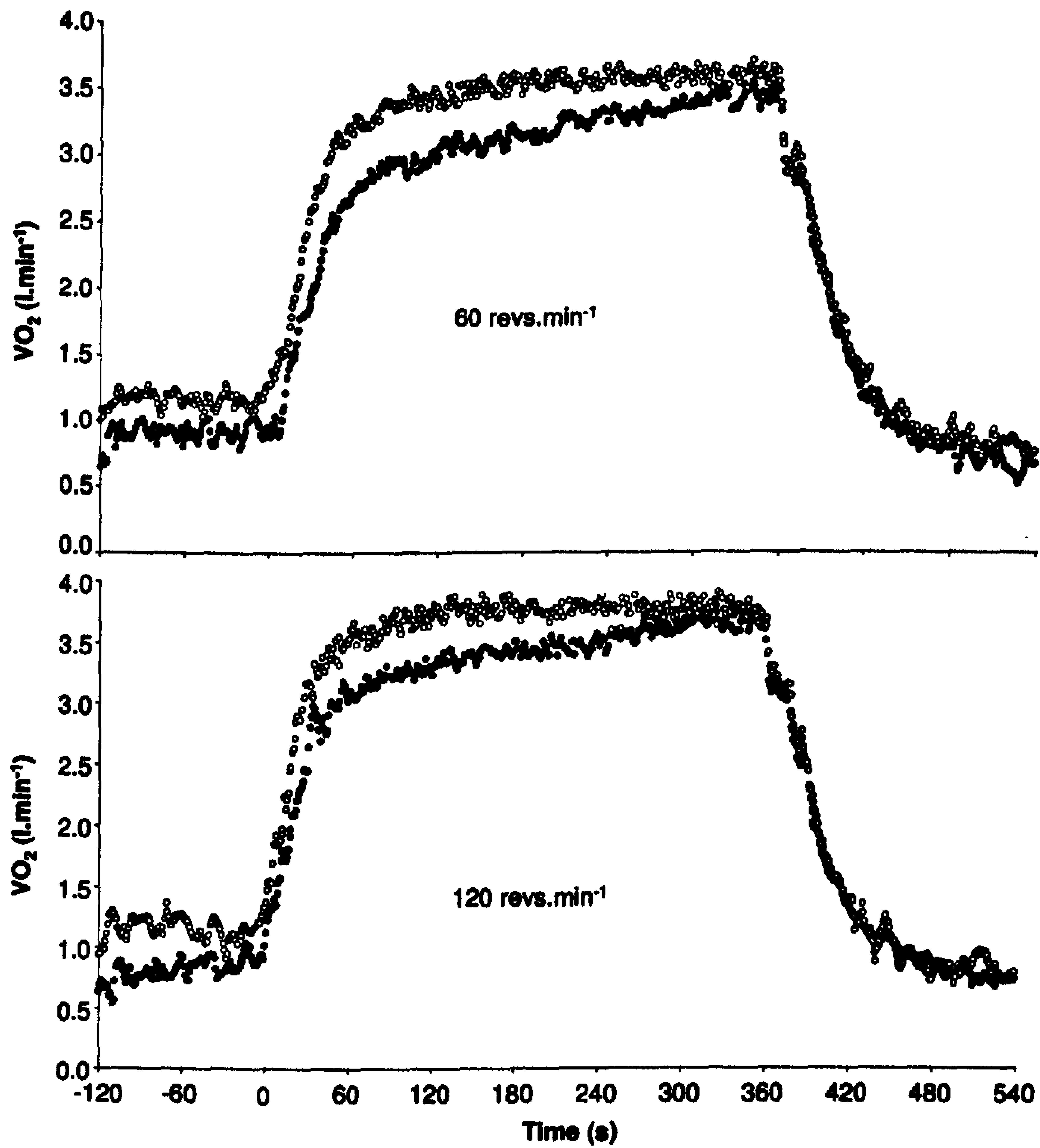


Fig 7.2. *Mean second-by-second* $\dot{V}O_2$ *response (after manual filtering, averaging and interpolating) to a 6 min of heavy exercise under control conditions (filled circles) and after prior exercise (closed circles) at 60 and 120 revs.min*$^{-1}$. *(N=6).*

There was no difference in plasma volume change between trials and resting lactate concentration was not different between trials (Fig 7.3). The effect of the prior exercise protocol was to increase ($P<0.05$) lactate concentration by approximately 7.6 $mmol.l^{-1}$ compared to the control trial (Fig 7.3). The six-minute exercise bout resulted in a further increase ($P<0.05$) in blood lactate in all trials. Thirty seconds after the completion of exercise the blood lactate concentration was greater ($P<0.05$) in both prior exercise trials compared to control trials (Fig 7.3). There was no effect of pedal rate on blood lactate concentration at any time point.

As a consequence of these changes in $\dot{V}O_2$ and blood lactate, estimates of mechanical efficiency were 2.4 ± 2.3 and 2.3 ± 1.4 % lower ($P<0.05$; from the ANOVA and post-hoc tests) after prior exercise at 60 and 120 $revs.min^{-1}$, respectively (Table 7.1). There were no differences in mechanical efficiency between the different pedal rates.

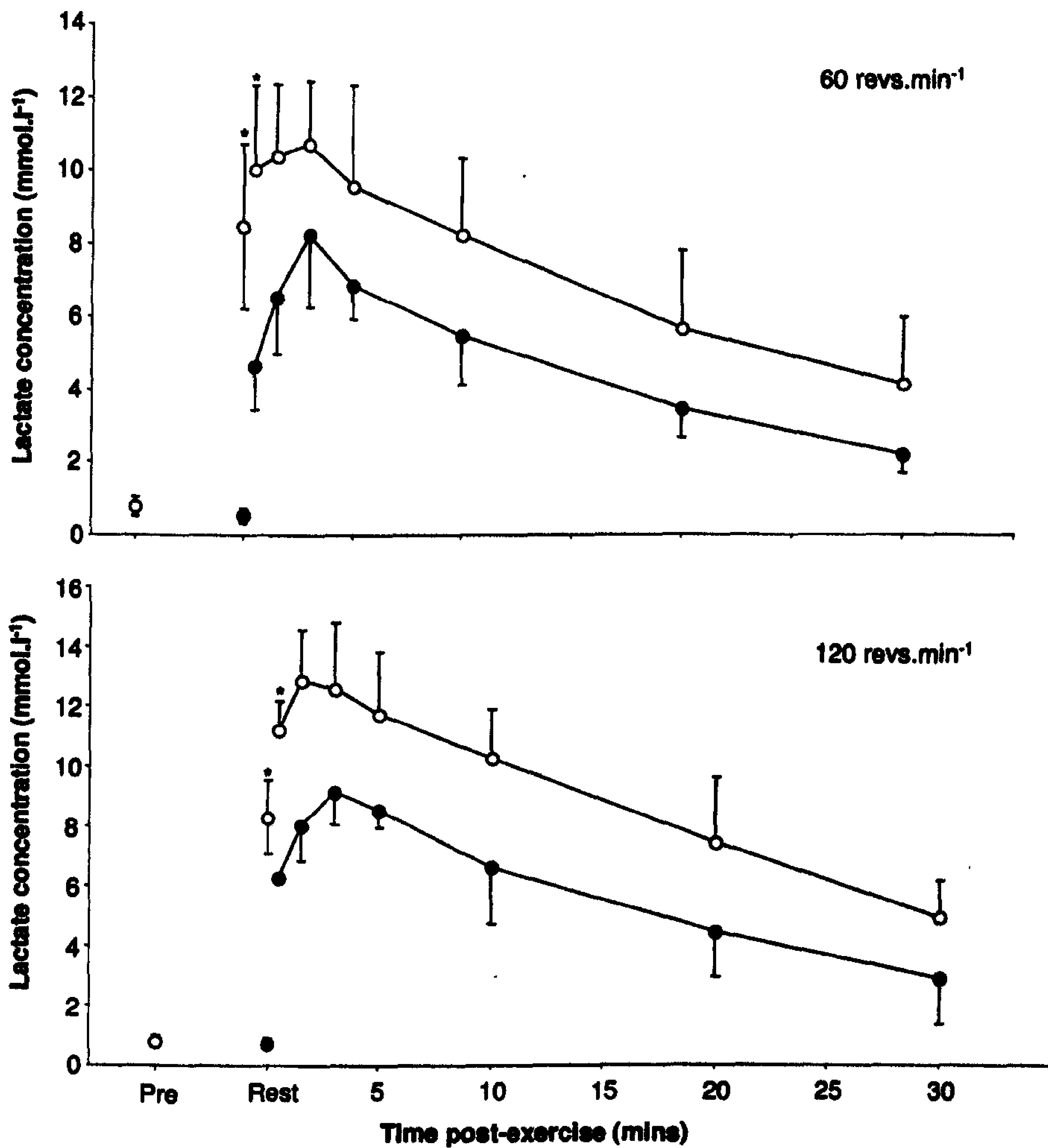


Figure 7.3. *Blood lactate concentration before prior exercise (pre), in the rest period before exercise and at various time point post-exercise at 60 and 120 revs.min^{-1} in control (closed circles) and prior exercise (open circles) trials. Values are mean ± SD. * denotes a significant difference between control and prior exercise trials. (N=6).*

7.4 DISCUSSION

The present study has demonstrated that prior heavy exercise to fatigue results in a decrease in mechanical efficiency at both 60 and 120 revs.min^{-1}. This decrease in efficiency occurred together with an increase in the "absolute" primary $\dot{V}O_2$ amplitude and a decrease in the slow component, at both pedal rates. Furthermore the changes in the "absolute" primary $\dot{V}O_2$ amplitude and slow component are greater at 120 compared to 60 revs.min^{-1}.

We originally hypothesised that efficiency would decrease at 60 revs.min^{-1} with the magnitude of this response being reduced at 120 revs.min^{-1}. The present data, however, clearly refutes this hypothesis. It is apparent, therefore, that the changes in efficiency and $\dot{V}O_2$ observed in the present study are not solely due to the elevation of T_m associated with the prior exercise, as suggested by Burnley *et al* (2002b). At this point it is worth noting that although baseline $\dot{V}O_2$ was higher after prior exercise similar changes in $\dot{V}O_2$ have been found when this is allowed to return to resting levels (Burnley *et al.*, 2006) meaning this is unlikely to have affected the values of efficiency after prior exercise.

The present data supports a T_m independent effect of prior exercise as a passive increase in T_m has been shown to decrease efficiency at 60 revs.min^{-1} and to increase efficiency at 120 revs.min^{-1} (Ferguson *et al.*, 2002). At 60 revs.min^{-1}, therefore, the decrease in efficiency observed after prior exercise may be partly attributed to the rise in T_m with the decrease in efficiency at 120 revs.min^{-1}, clearly the opposite of the T_m effect. Indeed at 120 revs.min^{-1} the effect of non-temperature related mechanisms must be greater than at 60 revs.min^{-1} to overcome the T_m related rise in efficiency

previously observed. At this point it is important to bear in mind that chapter 6 of this thesis showed that mechanical efficiency, when measured through changes in muscle and not blood metabolites, is only reduced in the first 2 min of exercise after passively increasing T_m. Similar work is, therefore, required at 120 revs.min^{-1} to confirm the increase in efficiency observed by Ferguson *et al* (2002) after a passive increase in T_m.

As muscle temperature is, therefore, not responsible for the decrease in efficiency after prior exercise, regardless of pedal rate, the other factors mentioned previously must be discussed as potential mechanisms. Regardless of pedal rate the prior exercise regimen employed in the present study led to an increase in baseline blood lactate concentration, which has previously been hypothesised to influence oxygen uptake and affect mechanical efficiency (Whipp & Wasserman, 1972). Several previous studies however have found that whilst lactate may act as a proxy for the changes in $\dot{V}O_2$ and efficiency, the lactate ion nor acidosis itself are responsible for these changes (Poole *et al.*, 1994;Sahlin *et al.*, 2005;Burnley *et al.*, 2006) making such a hypothesis unlikely.

Several other physiological factors are also altered by prior exercise. In fact non-contractile ATPase (Ca^{2+}ATP-ase) activity has been shown to increase after previous activity (Tupling *et al.*, 2003) with this increase in energy turnover contributing to the decrease in efficiency observed after prior exercise. This study by Tupling *et al*, however, employed a prolonged (16 h) prior exercise protocol, performed 36-48 h before the subsequent exercise bout, with the more immediate effects of prior exercise on non-contractile ATPase activity requiring further investigation.

It has also previously been shown that prior exercise leads to an increase in the aerobic contribution and decrease in the anaerobic contribution to energy turnover (Bangsbo *et al.*, 2001;Gray & Nimmo, 2001) and that aerobic energy pathways are less efficient than anaerobic energy pathways (Krustrup *et al.*, 2003). This change in energy source, after prior exercise, may therefore contribute to the decreased efficiency observed after prior exercise although the magnitude of such an effect would require further investigation.

One factor that has received a great deal of attention and which is altered by prior exercise is the muscle fibre recruitment patterns of the exercising muscle and how these relate to $\dot{V}O_2$ and efficiency. Prior heavy exercise is known to deplete the glycogen content of primarily type II fibres, although type I fibres are of course still recruited and glycogen depleted to a certain extent (Gollnick *et al.*, 1974;Thomson *et al.*, 1979;Vollestad *et al.*, 1992). It has been assumed, therefore, that glycogen depletion of predominantly one fibre type would lead to the greater recruitment of the other fibre type, which seems plausible. Research by Krustrup *et al* (2004a), however, found that not only did prior glycogen depletion of type I fibres lead to a greater recruitment of type II fibres, but there was also a greater recruitment, although to a lesser extent, of type I fibres. Further investigation by the same group during repeated knee extensor exercise, performed at 110 % maximum thigh O_2 consumption, found that there is a greater recruitment of all fibres, regardless of type, during the second exercise bout (Krustrup *et al.*, 2004c). It is likely therefore that the prior exercise protocol employed in the present study will have led to a greater recruitment of all fibre types, possibly favouring a greater increase in type I fibre recruitment, during the subsequent exercise bout.

This increased recruitment of muscle fibres, of all types, can account for a portion of the decreased efficiency and increased absolute primary amplitude, as observed in the present study and previously at various pedal rates (60 – 95 $rev.min^{-1}$) (Burnley *et al.*, 2000;Rossiter *et al.*, 2001;Scheuermann *et al.*, 2001;Burnley *et al.*, 2002a;Burnley *et al.*, 2002b;Koppo *et al.*, 2003;Carter *et al.*, 2004;Paterson *et al.*, 2005;Sahlin *et al.*, 2005;Endo *et al.*, 2005;Burnley *et al.*, 2006) since a greater number of contracting fibres will obviously require a greater energy turnover within the muscle. This greater number of fibres will be required after prior exercise since the fibres which have been fatigued by this exercise will have a decreased power contribution during subsequent exercise. Indeed, it has recently been demonstrated by Jones *et al* (2006) that there is a downward shift in the power-velocity relationship of the adductor pollicis muscle, working *in vivo*, after fatiguing exercise. This shift in the power-velocity relationship will mean that a greater number of fibres will have to be recruited in order to produce the same power output and will likely contribute to the decrease in mechanical efficiency observed at both pedal rates.

Whilst the present discussion has focussed on the effect of prior exercise on $\dot{V}O_2$ and efficiency another purpose of this study was to investigate the effect of different contraction frequencies. Indeed increasing the pedal rate from 60 to 120 $revs.min^{-1}$ after prior exercise resulted in a greater increase in the absolute primary amplitude and decrease in slow component. At this pedal rate the decrease in efficiency occurs even though, as discussed previously, the elevation in T_m will result in approximately a 1.3 % increase in efficiency (Ferguson *et al.*, 2002). This makes it likely, therefore, that the decrease in efficiency due to non-T_m related factors associated with prior exercise, e.g. increased fibre recruitment, at this pedal rate must be greater than at 60

rev.min^{-1} in order to counteract and indeed surpass the T_m effect. Previous work investigating the effect of pedal rate on fibre recruitment is limited, with the only study investigating this, at the same pedal rates as those employed in the present study, being somewhat inconclusive. Beelen *et al* (1993) found that an increase in pedal rate from 60 to 120 revs.min^{-1} led to an increase in glycogen depletion, used as a marker of fibre recruitment, of type IIX fibres, with a tendency for an increase in glycogen depletion of type I and IIA fibres also. The reason that the greater recruitment of type I and IIA fibres was not significant was probably due to the low subject numbers (N=4), making it likely that at higher pedal rates there is actually an increase in the recruitment of all fibre types, predominantly the fastest IIX fibres. This increased recruitment of muscle fibres at 120 revs.min^{-1} can help explain why there is a greater absolute primary amplitude in C120 compared to C60. Furthermore, the combination of prior exercise and the higher pedal rate, which both increase fibre recruitment, can explain the greater primary amplitude and the non-temperature related decrease in efficiency at 120 compared to 60 revs.min. Again it may be possible that at the faster pedal rate there is a greater non-contractile ATPase activity since there will be a greater number of contractions during the exercise period, although again there is limited work in this area.

Another factor investigated in this chapter was the slow component and how this was affected by prior exercise and pedal rate. In both control trials a progressive rise in $\dot{V}O_2$ was observed, beginning at around 2 min, as expected during exercise above the LT (Whipp, 1987). This slow component was reduced after prior exercise at both 60 and 120 revs.min^{-1}, with the decrease being greater at the faster pedal rate. Previous work has shown that the slow component is associated with an increase in fibre

recruitment, predominantly type II fibres (Krustrup *et al.*, 2004b). In the present study, therefore, the decrease in the slow component, after prior exercise, cannot be explained by changes in fibre recruitment as a greater recruitment is proposed to occur after prior exercise and at the higher pedal rate and would be hypothesised to increase the slow component. With this in mind Sahlin *et al* (2005) suggested that after prior exercise the slow component may have merged with the elevated amplitude and that both these events are related to the changes in fibre recruitment. On the other hand, it may be that after prior exercise the slow component is not related to fibre recruitment patterns, with these recruitment patterns setting the amplitude of $\dot{V}O_2$ with the slow component being related to another, as yet unidentified, factor. Further work where recruitment patterns and the metabolic response of single fibres is clearly required to further our understanding in this area and help to resolve these unanswered questions.

The present study has demonstrated that prior heavy exercise results in a decreased efficiency during heavy exercise at 60 and for the first time at 120 revs.min^{-1}, associated with an increase in the absolute primary amplitude and decreased amplitude of the slow component. At the higher pedal rate this was surprising since the elevated T_m associated with prior exercise will increase the efficiency of the muscle, an effect obviously superseded by other effects of prior exercise. In the world of sport and exercise this means that adopting a relatively fast cadence during cycling, as observed in world class athletes (Sargeant, 1994), is not necessarily as advantageous as previously suggested (Ferguson *et al.*, 2002) since it is prior exercise or 'active warm-up' that would be employed prior to competition and this results in a decrease in efficiency regardless of pedal rate. However, the role of this decrease in

efficiency on athletic performance was not measured in this investigation with further work required to determine the "physiological significance" of a 2.3 % decrease in efficiency.

Chapter 8

GENERAL DISCUSSION

8.1 INTRODUCTION

The main purpose of the present thesis was to investigate the effects of increasing T_m, passively or actively, on whole body energy turnover and efficiency as well as a homogenate and single muscle fibre level during various modes of exercise. The effect of a passive increase in T_m on the rate of energy turnover and single fibre metabolism was determined during the development of maximal power output and during sustained heavy exercise. The effect of increasing T_m through prior exercise on mechanical efficiency was also investigated at 60 and 120 revs.min^{-1}. A summary of the main findings are presented below:

- In chapter 4 passively increasing the temperature of the legs resulted in an increase in maximal power output, during a 6 s sprint on a friction-braked cycle ergometer. The increase in power output occurred alongside a greater rate of anaerobic ATP turnover and MFCV, with mechanical efficiency unaltered.

- A positive correlation between the Q_{10} for maximal power output and the percentage of MHC IIA present in the muscle, was also observed, indicating the possibility of a greater power contribution of fibres expressing predominantly the IIA MHC at higher T_m. Subsequently, it was demonstrated at a single fibre level that there was a greater ATP and PCr degradation, and thus activity, in type IIA fibres (Chapter 5), supporting the hypothesis that these fibres may provide a large portion of the greater power output at higher T_m, in the cadence range of 160-180 revs.min^{-1}.

- As the exercise duration is extended to 6 min and the power output is maintained at a constant heavy intensity, passively increasing T_m led to an increase in the rate of anaerobic ATP turnover in the first 2 min of exercise, at 60 revs.min^{-1}, with no effect of T_m during the remainder of the exercise period. Contrary to previous findings at this pedal rate there was no effect of temperature on $\dot{V}O_2$ and thus aerobic energy turnover. Mechanical efficiency was, therefore, reduced in the initial but not in the latter period of heavy exercise (chapter 6).

- In 2 subjects single fibre analysis revealed a trend (P=0.06) for a greater PCr degradation in type I fibres in the first two minutes of exercise, after the elevation of T_m. There was also a greater ATP degradation in type I fibres after 6 min of exercise during the heated trial.

- When T_m was elevated by prior exercise mechanical efficiency was reduced to a similar extent over the 6 min exercise period at both 60 and 120 revs.min^{-1} (chapter 7).

8.2 ANAEROBIC ENERGY TURNOVER AND SINGLE FIBRE METABOLISM

Passively increasing the temperature of the legs by approximately 3 °C resulted in an increase in maximal power output that was probably due to a greater rate of anaerobic ATP turnover, particularly the contribution from PCr hydrolysis, and MFCV. Whilst it was necessary in chapter 4, in order to investigate the mechanisms behind the greater power output, to have different amounts of 'work' carried out, between

conditions, it could be suggested that this increase in anaerobic ATP turnover was actually the result of the increased work itself and not due specifically to the increase in T_m. The effect of a passive increase in T_m on anaerobic ATP turnover was therefore investigated in chapter 6 during more sustained heavy exercise where the external power output was maintained constant between T_m conditions. This investigation found that there was a greater rate of anaerobic ATP turnover, after passively elevating T_m, between 0-2 min of cycling but not between 2-6 min, which suggests that the greater anaerobic ATP turnover observed in chapter 4 was a T_m-effect and not simply the result of a greater work carried out by the muscle. The enhanced rate of anaerobic ATP turnover from 0-2 min is also in agreement with previous work (Edwards *et al.*, 1972;Febbraio *et al.*, 1996).

The reason why a similar observation was not made between 2-6 min may be due to the fact that at this time point T_m will be approaching, but still significantly different from, the levels reached in the heated trials (Koga *et al.*, 1997;Ferguson *et al.*, 2006). It is possible that this reduced T_m difference between trials is not great enough to exert any influence on anaerobic metabolism during this period. Another possibility, maybe in combination with the narrowing T_m range, is that temperature has its greatest effect when anaerobic energy production is the predominant energy source. Since anaerobic pathways have a greater energy contribution in the initial period of exercise increasing T_m has a clear effect during this period, and a large effect during the development of maximal power output (chapter 4), but has a lesser or no effect as exercise progresses and the contribution of anaerobic metabolism lessens (Starkie *et al.*, 1999;Ferguson *et al.*, 2006).

Whilst passively increasing T_m has been shown to have a major effect in homogenate

muscle it is important to investigate whether these observations are due to a fibre type specific effect of T_m. It has previously been shown that during short term maximal exercise all muscle fibre types (type I, IIA, and IIX) will be active (Karatzaferi *et al.*, 2001b) and that both type I and II fibres being active to some extent during sustained heavy exercise (Krustrup *et al.*, 2004b). This means that each fibre type will be active during the exercise undertaken in the present thesis with *in vitro* work showing a velocity specific effect of T_m on different fibre types (He *et al.*, 2000).

During short-term maximal exercise it has previously been suggested that type I fibres are more sensitive to an increase in T_m at pedal rates between 60 and 140 revs.min^{-1}, and may result in the increase in power output (Sargeant & Rademaker, 1996). This suggestion, however, was made from the observation of a positive correlation between the percentage of type I fibres in biopsy samples and the Q_{10} values for power output. Further work also suggested a preferential effect of T_m on type I fibres during heavy exercise (Ferguson *et al*, 2002). On the other hand the present thesis found a positive correlation between the Q_{10} for power output and the percentage of MHC IIA from biopsy samples during maximal cycling at pedal rates between 160-180 revs.min^{-1}. This difference again highlights the velocity-specific effect of temperature although these findings have previously not been corroborated with a direct measure of single fibre metabolism.

In order to further investigate the hypothesised velocity specific effect of passively increasing T_m on single fibre metabolism single fibre fragments were characterised according to their MHC composition and analysed for ATP and PCr content from samples obtained during the development of maximal power output (160-180 revs.min^{-1}) and during sustained heavy exercise (60 revs.min^{-1}). Single fibre PCr

content is useful in these studies as it provides a measure of the activity of a muscle fibre since it splits immediately upon contraction (Infante *et al.*, 1965;Conjard & Pette, 1999). Using this method the investigations of the present thesis (chapter 5 and 6) found that during the development of maximal power output there was a greater PCr and ATP degradation in type IIA fibres, at the higher T_m, whilst during more sustained heavy exercise there was a trend for a greater PCr degradation in type I fibres in the first 2 min of exercise at the higher T_m. Taking these findings together it appears that at lower pedal rates the type I fibres are affected to greater extent whilst at higher pedal rates it is the faster type IIA fibres which are more sensitive to the rise in T_m. It must be noted that whilst it is tempting to assume such a suggestion further work is clearly required, as only 2 subjects were studied in chapter 6. From these findings the question arises as to why does the contraction velocity have such a profound effect on which fibre type is affected by temperature?

It was shown, in chapter 5, that a greater PCr degradation in type IIA fibres results in an increase in power output. As mentioned previously the greater power output at higher T_m was originally thought to be due to an increase in the activity of type I and not type IIA fibres (Sargeant & Rademaker, 1996) when maximal power output was achieved in the range of 60-140 revs.min^{-1}. At 160-180 revs.min^{-1}, however, the muscles will clearly be working at a different point on their power velocity relationship, which is in turn different for each fibre type (Figure 2.7, Bottinelli *et al.*, 1996) and can lead to a different metabolic response to changes in T_m (Ferguson *et al.*, 2002). Understanding the effect of T_m on these individual power-velocity relationships is complicated during whole body exercise as one cannot directly equate the findings during *in vitro* work to the cadences achieved during cycling although

reasonable estimates can be made.

This hypothetical diagram (Fig 8.1) is constructed based on the previous work by Sargeant (1994). It was extended to include subdivisions of type II fibres using the relationship between the V_{max} values of type I, IIA and IIX fibres (Bottinelli *et al.*, 1996) and a V_{opt}, which does not change with temperature, at 1/3rd V_{max} (Bottinelli *et al.*, 1996;He *et al.*, 2000). From this diagram the explanation for the conflicting results of Sargeant and Rademaker (1996) and the present thesis becomes apparent. As proposed by Sargeant and Rademaker (1996), at the lower pedal rates used by these investigators the difference in power between the control and heated conditions is greatest in type I fibres meaning that the increasing power contribution of type I fibres will likely be responsible for the greater power output observed. This will also help to explain the possible increase PCr degradation in type I fibres observed in chapter 6 of the present thesis.

As the pedal rate increases to the values achieved in chapters 4 and 5 it can be seen that the V_{max} of the type I fibres is almost exceeded in the control condition, with only a small increase in power contribution. On the other hand the type IIA fibres increase in power contribution is large and at these velocities accounts for the majority of the increase in global power output, reflected by the increased activity of these fibres in chapter 5. There is also a slight increase in the power production of the IIX fibres at the pedal rates achieved in the present study, with this becoming larger as the velocity of contraction increases.

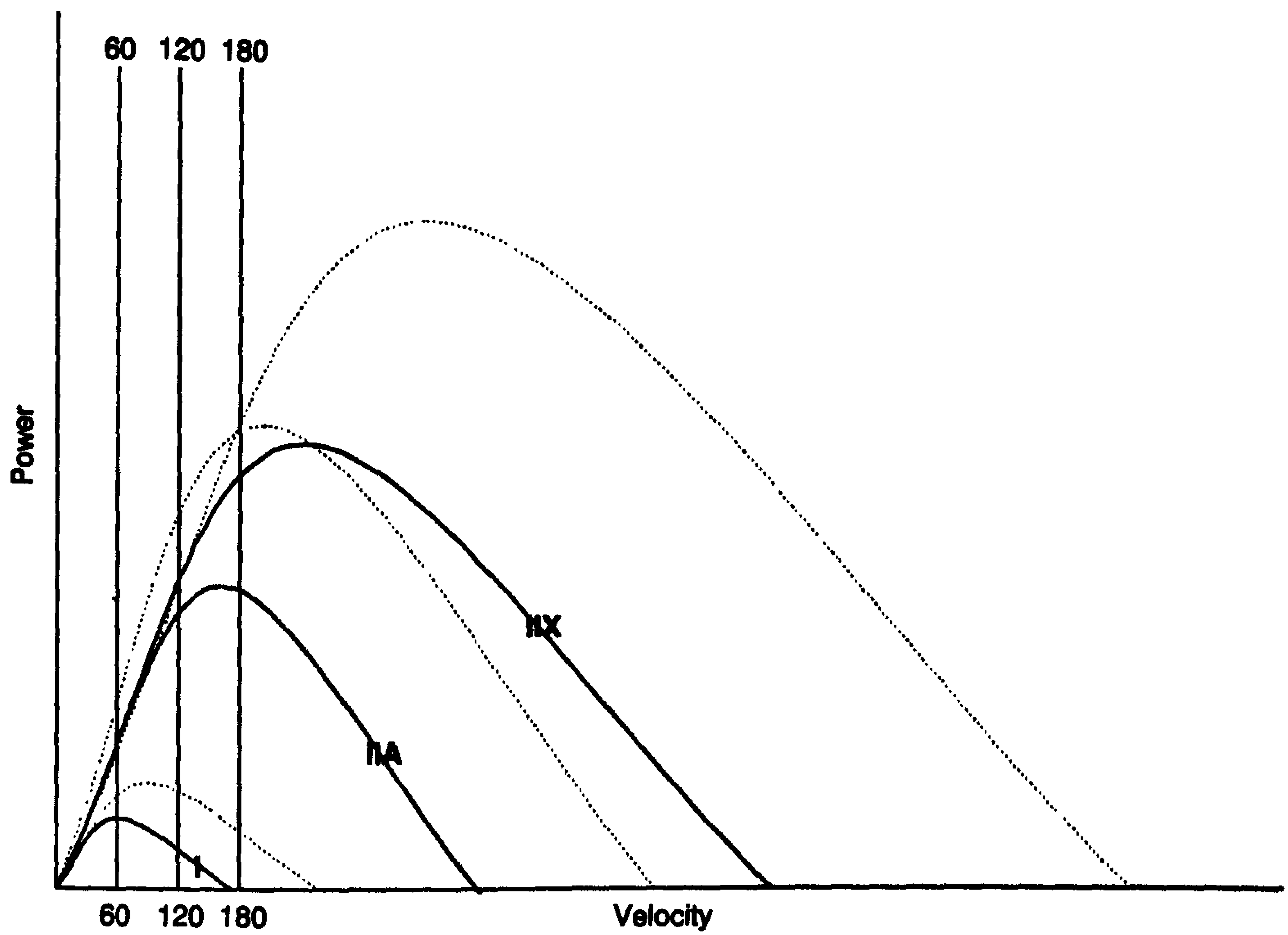


Figure 8.1. *Hypothetical power-velocity relationship of control (solid lines) and heated (dashed lines) type I, IIA and IIX fibres. The three vertical lines represent 60, 120 and 180 revs.min^{-1}. These lines represent a mean of each fibre type, since there will actually be a continuum of power-velocity relationships within each group.*

It must be pointed out that whilst this schematic gives the estimated power velocity-relationship for the three discrete fibre groups it is now well known that there is actually a continuum of fibre types due to co-expression of MHC (Larsson & Moss, 1993) meaning that there may also be an increase in power contribution of fibres expressing a large proportion of MHC IIA. In fact, although not significant, in chapter 5, there was a tendency for an increased activity of hybrid type IIAX25 fibres (75-99 % MHC IIA), meaning that there may also be a small increase in the activity of these fibres. This hypothesis could be extended to propose that increasing the pedal rate further, although this may not be biomechanically possibly, would preferentially effect type IIX fibres, although the present thesis cannot confirm this.

8.3 AEROBIC ENERGY TURNOVER AND $\dot{V}O_2$ KINETICS

Whilst an increase in T_m resulted in significant increases in the rate of anaerobic ATP turnover and single fibre metabolism it will be the aerobic system which provides the majority of energy during exercise beyond about 30 s (Parolin *et al.*, 1999). Previous work has, however, shown somewhat conflicting results.

In chapter 6 of the present thesis there was no effect of a passive increase in T_m on aerobic energy turnover or any of the kinetic parameters of the $\dot{V}O_2$ response. This finding is in agreement with the results of Burnley *et al* (2002b) where cycling was performed at a pedal rate of between 70-95 revs.min^{-1}, but is in contrast to the observation of a greater amplitude of $\dot{V}O_2$ at 60 revs.min^{-1} (Ferguson *et al.*, 2002) and a reduction in the slow component at an undisclosed, and likely slow, pedal rate (Koga *et al.*, 1997). The only major difference between the work of Ferguson *et al* (2002) and the methodology used in chapter 6 is the heating method employed, with

subjects in the former cycling immediately after the immersion of the legs in hot water, conditions likely to result in an elevated T_{rec} (although this was not measured) alongside the elevated T_m. Whilst the study of Ferguson *et al* (2002) attributed the changes in $\dot{V}O_2$ to the rise in T_m it is possible that these observations were, in fact, the result of the rise in T_{rec}, although further work is required in this area. The present thesis has therefore added to the previous evidence showing that a passive increase in T_m has no effect on aerobic energy production, which previous work has clearly shown is not the case after a bout of prior exercise.

When the temperature of the muscle was elevated by prior heavy exercise (chapter 7) the $\dot{V}O_2$ response is significantly different from those previously observed with passive elevation of T_m (Chapter 6, Ferguson *et al.*, 2002) and in line with the majority of previous studies at relatively slow pedal rates (Burnley *et al.*, 2000;Burnley *et al.*, 2002a;Burnley *et al.*, 2002b;Koppo *et al.*, 2003;Carter *et al.*, 2004;Sahlin *et al.*, 2005;Endo *et al.*, 2005;Burnley *et al.*, 2006). This would suggest that these responses i.e. an increase in the absolute primary amplitude of $\dot{V}O_2$ and a decrease in the slow component are not T_m-related, as demonstrated by the work of Gray and Nimmo (2001). Furthermore, there was no change in the speed of the rise in $\dot{V}O_2$ (tau) after prior exercise, which would support the previous suggestion that blood flow and delivery of oxygen to the muscles are sufficient at the onset of exercise (e.g. Bangsbo *et al*, 2000).

In chapter 7 the $\dot{V}O_2$ response to prior exercise was also investigated at 120 revs.min^{-1} a pedal rate nearer to that adopted by world-class cyclists (Sargeant, 1994) and where the muscles will be working at a different point on the efficiency-velocity relationship. At this pedal rate both the increase in the absolute primary amplitude and

the decrease in the slow component were greater than the response observed at 60 revs.min^{-1}. Although there was no measure of single fibre metabolism in this investigation it is likely that the increase in $\dot{V}O_2$, regardless of pedal rate, after prior exercise is due to an increase in fibre recruitment of all types (Krustrup *et al*, 2004a; Krustrup *et al*, 2004c)However there may also be several other contributing factors (e.g. non-contractile ATPase activity), as discussed in chapter 7. Furthermore, at the higher pedal rate (120 revs.min^{-1}) the further increase in $\dot{V}O_2$ will also likely be the result of an additional recruitment of all fibre types (Beelen *et al*, 1993), although further work is necessary to confirm these suggestions. Regardless of the mechanisms it is apparent that prior exercise results in quite drastic changes in $\dot{V}O_2$ during exercise, a response not observed after a passive increase in T_m. What is not clear from these changes and the aforementioned changes in anaerobic energy turnover and single fibre metabolism is how, if at all, an increase in T_m affects the mechanical efficiency of the muscle, a factor of great importance during the majority of athletic events.

8.4 MECHANICAL EFFICIENCY

Previous work and the present thesis has shown that passively increasing the temperature of the muscle will lead to an increase in anaerobic energy turnover (Edwards *et al*, 1972; Febbraio *et al*, 1996) and either an increase or no change in $\dot{V}O_2$ (Ferguson *et al*, 2002; Burnley *et al*, 2002). No study has combined both measurements to make an accurate characterisation of mechanical efficiency and how this is affected by changes in T_m during cycling exercise. Furthermore, although efficiency has been shown to decrease when T_m is elevated through prior intense exercise during cycling exercise at a cadence of 80 revs.min^{-1} (Sahlin *et al*, 2002b)

this has not been investigated as the cadence is increased.

The experiments of the present thesis have shown that when T_m is passively elevated prior to the development of maximal power output, where $\dot{V}O_2$ will have a minimal contribution (Parolin *et al*, 1999), mechanical efficiency is unaltered (chapter 4). This occurs since the greater rate of anaerobic ATP turnover, and type IIA fibre activity, is counteracted by the greater power output observed. Conversely, when the external power output is maintained at a constant level passively increasing the temperature of the muscles reduces mechanical efficiency in the first 2 min of exercise due to, as mentioned previously, a greater rate of anaerobic energy turnover and the possibility of a greater type I fibre metabolism.

The effect of increasing T_m, through prior exercise, on mechanical efficiency was investigated at 60 and 120 revs.min^{-1} (chapter 7). In these studies it was shown that, in contrast to the previous findings of Ferguson *et al* (2002), when T_m was passively elevated, mechanical efficiency was decreased to a similar extent (~2.4 %) regardless of pedal rate. This demonstrates that whilst T_m may reduce efficiency in the initial period of exercise (chapter 6) the majority of the reduction in efficiency, at 60 revs.min^{-1}, will be the result of non-T_m related mechanisms, likely the greater fibre recruitment discussed previously. At 120 revs.min^{-1} whilst the response is similar to that shown at 60 revs.min^{-1} the decrease in efficiency can be attributed to non-T_m related mechanisms (i.e. a greater fibre recruitment due to both prior exercise and the higher pedal rate) as previous work has found that an increase in T_m will actually increase mechanical efficiency at these pedal rates.

8.5 SUMMARY

Muscle temperature has previously been shown to alter the mechanical properties of skeletal muscle, with little data being available regarding the metabolic response within the muscle. From this thesis it is now known that, during short-term maximal exercise, at higher T_m a greater rate of anaerobic ATP turnover and an increased activity of type IIA fibres contribute to the greater maximal power output at the relatively fast pedal rates achieved (160-180 $revs.min^{-1}$). This contradicts the previous work suggesting that athletes with the most to gain from an increase in power output (i.e. sprinters, throwers etc) will have the least to gain from an increase in T_m, since type I fibres were originally suggested to be more sensitive to changes in T_m in the locomotory range (Sargeant & Rademaker, 1996;Rademaker, 1997). During more prolonged exercise at 60 $revs.min^{-1}$, however, passively increasing T_m leads to an increase in anaerobic ATP turnover and therefore a decrease in mechanical efficiency, but only during the initial period of exercise, which is associated with a trend for a greater PCr degradation in type I fibres. These differences in the effect of T_m on specific fibre types, in the current experiments, serve to highlight the velocity-specific nature of the T_m effect.

Previous work has shown that at 120 $revs.min^{-1}$ efficiency is increased after passive elevation of T_m and that this may explain why top class athletes adopt fast cadences during competition (Sargeant, 1994;Ferguson *et al.*, 2002). On the other hand, after prior exercise, which is the more likely method by which T_m would be elevated in the athletic world, mechanical efficiency is actually decreased to a similar extent at both 60 and 120 $revs.min^{-1}$. This means efficiency will be decreased after 'active-warm up' of this intensity regardless of the contraction frequency employed during the

subsequent exercise bout.

8.6 FUTURE RESEARCH

The findings of the present thesis have highlighted the need for further studies investigating the effect T_m on skeletal muscle metabolism *in vivo*.

- Determine the effect of passive elevation of T_m, and also pedal rate, on the activity of the non-contractile ATPases (Ca^{2+}-ATPase and Na^+-K^+-ATPase) and other key enzymes during both short-term and more prolonged exercise

- Carry out further single fibre analysis on the muscle samples obtained from subjects in chapter 6 to confirm the effect of passively elevating T_m on single fibre metabolism during heavy exercise

- Investigate the effect of passive elevation of T_m on $\dot{V}O_2$ and muscle metabolism (both homogenate and single fibre) during heavy exercise at 120 revs.min^{-1}, giving an accurate measure of mechanical efficiency and single fibre activity

- Study the effect of prior exercise on the non-contractile ATPase activity

- Investigate the effect of prior exercise on single fibre recruitment at 60 and 120 revs.min^{-1} and investigate how these relate to the changes in $\dot{V}O_2$ and mechanical efficiency (the anaerobic portion calculated through muscle analysis).

- Investigate, with greater subject numbers than previously carried out, how muscle fibre recruitment, through single fibre ATP and PCr concentration, differ between 60 and 120 revs.min^{-1}.

- Study the effect of prior exercise (<60 % $\dot{V}O_{2peak}$) on maximal power output and investigate the mechanisms behind the contractile changes using similar methods employed in chapter 4 and 5.

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APPENDICES

APPENDIX 1

CONSENT FORM – NAME OF STUDY

PLEASE READ THE FOLLOWING CAREFULLY AND COMPLETE AS REQUIRED

Name: .... Age: .... Date of Birth:..................

Do you exercise at least 3 times for 30mins a week? YES/NO
If NO, can you provide a successful certification of ECG profiling? YES/NO

Are you currently in good health? YES/NO
If NO, please specify:

Have you suffered from any serious illness or accident? YES/NO
If YES please specify:

Are you currently taking any medication either over the counter or on prescription? YES/NO
If YES, please specify:

Are you currently attending your G.P. for any condition? YES/NO
If YES, please give particulars:

Are you participating in any other laboratory experiment or testing at present? YES/NO

Are there any other personal reasons that you would like to discuss relating to your participation in this experiment? YES/NO

PLEASE READ THE FOLLOWING CAREFULLY:

Persons may be considered unfit to participate in this experiment if they:
Have an infectious disease, have a fever, suffer from fainting spells or dizziness, have a known history of medical disorders such as high blood pressure, sweating disorder, heart or lung disease.
If on the day of testing you feel unwell please inform the experimenters who will take appropriate action.

Name of study

My replies to the above questions are correct to the best of my knowledge and I understand that this information will be treated with the strictest confidence. It has been explained to my satisfaction and understanding the purpose of this experiment and the possible risks involved.

I understand that I may withdraw from the experiment at any point and that I am under no obligation to give reasons for my withdrawal. I undertake to obey the testing regulations and the instructions of the experimenters regarding safety, subject only to my right to withdraw as described above.

Experimental Period:..................................

Signature of Participant:...
Date: ....

Signature of Experimenter:...
Date: ....

APPENDIX 2

MEMORANDUM

To: Dr R Ferguson, Applied Physiology

From: Mrs Gwen McArthur, Head of Court Office – [Secretary to the Group]

copy: *Ms L Frew, Research & Consultancy Services*
Ms Z Wilson, Finance Office

Date: 17th March 2005

PROTOCOL APPROVAL

Project No : UEC0405/28 (previously EC35 : 01/02)

Project Title : The influence of temperature on skeletal muscle metabolism during sustained cycle exercise at different contraction frequencies

Investigators: Dr R Ferguson, Supervisor
Dr G De Vito; Mr S Gray, Postgrad student, Applied Physiology

Location: Department of Applied Physiology

Insurance: University Policies confirmed

I can confirm that the University Ethics Committee has approved the above protocol and has approved the addition of Mr Stuart Gray, Postgraduate students, to the named investigators for this study.

I would remind you that if there are any changes made to the protocol the Committee must be informed of these and given the opportunity to consider them.

I would draw to your attention that the Committee would expect you to report back on the outcome of the project with an account of anything which may prompt ethical questions for any similar future project, and with anything else that you feel the Committee should know about.

On behalf of the Committee I wish you success with this project.

GMcA

MEMORANDUM

To:	Dr R Ferguson, Applied Physiology
copy:	*Ms L Frew, Research & Consultancy Services* *Ms Z Wilson, Finance Office*
From:	Dr Fiona Campbell, Assistant Planning/Governance Officer
Date:	22nd June 2006

APPROVAL OF AMENDED PROTOCOL

Project No : UEC0405/50

Project Title : The effect of prior heavy exercise on mechanical efficiency during cycling in humans at different contraction frequencies

I am pleased to inform you that the Convener of the University Ethics Committee has taken action and approved the extension to the above protocol and the addition of Mr Stuart Gray, to the list of investigators.

I would remind you that if there are any changes made to the protocol the Committee must be informed of these and given the opportunity to consider them.

I would draw to your attention that the Committee would expect you to report back on the outcome of the project with an account of anything which may prompt ethical questions for any similar future project, and with anything else that you feel the Committee should know about.

On behalf of the Committee I wish you success with this project.

Fiona Campbell.

FC

UNIVERSITY OF STRATHCLYDE

DEPARTMENT OF APPLIED PHYSIOLOGY

MEMORANDUM

To:	**Mrs Gwen McArthur** **Senior Assistant Registrar (Court), Secretary to University Ethics Committee**
Copy:	**Lynda Frew** **Contracts Officer, Research and Consultancy Services** **Mr Stuart Gray** **Postgraduate student, Department of Applied Physiology**
From:	**Dr. Richard Ferguson** **Lecturer, Department of Applied Physiology**
Subject:	**Ethics Application**
Date:	**8th June 2006**

UEC0405/50 The effect of prior heavy exercise on mechanical efficiency during cycling in humans at different contraction frequencies

Dear Gwen,

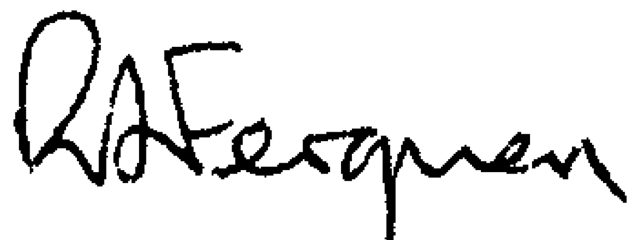
The above protocol was performed between the period of October 2005 and May 2006. However, the undergraduate students (Gareth Thomas and Ross Cannon), who will now graduate in July 2006, were only able to successfully complete 4 out of the required 8 participants.

In light of this I would like to make the following requests.

- the protocol continue to be conducted between the period of June 2006 and October 2006 in order to complete the further 4 participants.
- the committee add Mr Stuart Gray (Postgraduate student, matric no: 200453634) to the list of investigators. Mr Gray is fully trained in all the relevant procedures.

I can confirm that all the procedures have been previously approved by the committee and factors such as prevention of infection (UECM 225.2) are still appropriate.

With kind regards



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APPENDIX 3

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