Universidade Federal do Rio Grande do Sul
Faculdade de Medicina
Programa de Pós-Graduação em Ciências Pneumológicas

Ana Cláudia Coelho

Measurement of central and peripheral fatigue during whole body exercise: A new method

Porto Alegre, 2015

Ana Cláudia Coelho

Measurement of central and peripheral fatigue during whole body exercise: A new method

This thesis is submitted in accordance with the requirements for the degree of Doctor of Philosophy in Respiratory Sciences, at the "Programa de Pós-Graduação em Ciências Pneumológicas, Universidade Federal do Rio Grande do Sul".

Supervisors: Prof. Dr. Marli M. Knorst
Prof. Dr. Harry B. Rossiter

Porto Alegre, 2015

CIP - Catalogação na Publicação

Coelho, Ana Cláudia
Measurement of central and peripheral fatigue during whole body exercise: A new method / Ana Cláudia Coelho. -- 2015.
148 f.

Orientadora: Marli Maria Knorst.
Coorientador: Harry B. Rossiter.

Tese (Doutorado) -- Universidade Federal do Rio Grande do Sul, Faculdade de Medicina, Programa de Pós-Graduação em Ciências Pneumológicas, Porto Alegre, BR-RS, 2015.

1. isokinetic. 2. performance fatigue. 3. activation fatigue. 4. muscle fatigue. 5. electromyography. I. Knorst, Marli Maria, orient. II. Rossiter, Harry B., coorient. III. Título.

Elaborada pelo Sistema de Geração Automática de Ficha Catalográfica da UFRGS com os dados fornecidos pelo(a) autor(a).

DEDICATION

To my family and friends. For their love and understanding for all the moments that I was studying and not there for them. Thanks for supporting me on my journey until now.

ACKNOWLEDGEMENT

"*I am thankful for nights that turned into mornings, friends that turned into family, and dreams that turned into reality.*"
(Unknown author)

This thesis would not have been possible without support of countless people who helped me along the way.

I want to acknowledge my supervisor Prof. Dr. Marli Mari Knorst for her trusting and help to get a scholarship from "Coordenação de Aperfeiçoamento de Pessoal de Nível Superior" (CAPES) and for her support when I went back to Brazil.

I thank my team in the Rehabilitation Clinical Trials Center - Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, especially Dr. Daniel T. Cannon for his around-the-clock support in all areas of my research, and for his example as a researcher of the highest quality. I thank Dr. Richard Casaburi and Dr. Janos Porszasz for the opportunity to work with them and for make me fell as part of their family. Thanks go to Robert Cao for his help in finding volunteers for our research and for his professionalism. And I also thank Dr. Bahman Chavoshan, Dr. William Stringer, Dr. David Hsia and Dr. James Hansen for their support during the laboratory tests, Nancy Hsu for her help in part of the data analysis, Leticia Diaz, Carmen Lopez-Garcia, Rafi Kiledjian, Michelle Blum and Debora Cavanaugh for their support in the laboratory routine.

I am so thankful for the support of all my friends in Los Angeles and Porto Alegre during that period which was the best year of my life.

This study could not be done without the support of the Pulmonary Education & Research Foundation (PERF) and CAPES (3468/13-5). I thank the volunteers for their time and dedication.

SPECIAL ACKNOWLEDGEMENT

“If I have seen further it is by standing on the shoulders of giants.”
(Isaac Newton)

To my supervisor in US, Prof. Dr. Harry B. Rossiter; I thank him for his example, trust, support and the opportunities he gave me. I also want to thank him for inspiring me to make this dream become a reality.

“Simplicity is the ultimate sophistication.”

(Leonardo da Vinci)

ABSTRACT

Background: This thesis sought to establish a new method for instantaneous measurement of central and peripheral fatigue during whole-body exercise up to maximal aerobic capacity in humans. Until now, measurement of central and peripheral fatigue has been limited to isolated muscle tasks or to time points after exercise where the physiological conditions that brought about the limiting symptoms for exercise have subsided. Thus, development of a method to overcome this would allow the first demonstration of the relative contributions of central and peripheral fatigue to limiting exercise that elicited maximal strain of the combined neuromuscular and cardiopulmonary systems.

Objective: To develop and validate a method for quantifying peripheral muscle fatigue (MF, defined as the power produced for a given muscle stimulation), activation fatigue (AF, defined as the maximal evocable muscle activity), their sum, performance fatigue (PF, defined as the decline in maximal voluntary isokinetic power compared to the fresh, baseline, state) during cycling exercise at maximal aerobic capacity. In addition, this thesis aimed to determine the rate with which MF, AF and PF recovered to baseline after intolerance during whole-body exercise in humans.

Methods: To quantify fatigue during whole-body exercise, a method was developed to allow a rapid switch from standard cycling (where the relationship between power and cadence is hyperbolic) to isokinetic cycling (where power is independent of cadence, and cadence is fixed) to be implemented. By asking the participant to give a maximal isokinetic effort at any point during exercise or recovery, allowed the velocity-specific decline in maximal isokinetic power (P_{ISO}) to be measured. The difference in P_{ISO} between baseline and exercise quantified PF. It was tested whether the baseline relationship between P_{ISO} and electromyographic power in 5 leg muscles (RMS EMG) was velocity dependent, linear and reproducible, such that the relative contributions to PF could be isolated from: 1) the decline in muscle activation (AF); and 2) the decline in P_{ISO} at a given activation (MF).

Results: Healthy participants (n=13, 29 to 72 years old, ranging in aerobic capacity from 23.5 to 62.4 ml/min/kg) completed short (5 s) variable-effort isokinetic bouts at 50, 70, and 100 rpm to characterize the baseline relationship between RMS EMG and isokinetic power. Individual baseline EMG-P_{ISO} relationships were linear (r^2 = 0.95 ± 0.04) and velocity dependent (analysis of covariance). Subsequently, repeated ramp incremental exercise tests were performed on a cycle ergometer and breath-by-breath gas exchange and ventilation was measured. Exercise was terminated with a maximal isokinetic effort (5 s) at 70 rpm. P_{ISO} at intolerance (two legs, 335 ± 88 W) was ~45% less than baseline (630 ± 156 W, $p < 0.05$). Following intolerance, P_{ISO} recovered within 3 minutes ($p < 0.05$). AF and MF (measured in one leg) were 97 ± 55 and 60 ± 50 W, respectively. Mean bias (± limits of agreement) for reproducibility were as follows: P_{ISO} at baseline 1 ± 30 W; P_{ISO} at 0-min recovery 3 ± 35 W; and EMG at P_{ISO} 3 ± 14%.

Conclusions: The baseline EMG-P_{ISO} relationship was well modelled by a linear function, which was reproducible day-to-day. The variability of the individual EMG-P_{ISO} measurements between ~25% and 100% effort, around the linear model, was sufficiently tight that the baseline linear relationship allowed for a precise quantification of AF and MF at the limit of tolerance and in recovery from a maximal aerobic exercise task. It was also demonstrated that the EMG-P_{ISO} relationship was velocity dependent, as expected from the parabolic nature power-velocity curve. As such, this provides a valuable new method to identify the contributions of central and peripheral fatigue to limiting whole-body exercise in humans.

Keywords: isokinetic; performance fatigue; activation fatigue; muscle fatigue; electromyography; exercise testing.

RESUMO

Contexto: Esta tese procurou estabelecer um novo método de mensuração instantânea de fadiga central e periférica durante o exercício de corpo inteiro até a capacidade aeróbica máxima em seres humanos. Até agora, a mensuração da fadiga central e periférica tem sido limitada a tarefas musculares isoladas ou a momentos específicos após o exercício, nos quais as condições fisiológicas que levaram aos sintomas limitantes do exercício já estão abrandadas. Assim, desenvolver um método que supere estas limitações permitiria demonstrar pela primeira vez as contribuições relativas da fadiga central e periférica na limitação ao exercício, no qual haja estimulação máxima dos sistemas neuromuscular e cardiovascular.

Objetivo: Desenvolver e validar um método para quantificar a fadiga muscular periférica (MF, definida como a potência produzida para uma determinada estimulação muscular), fadiga de ativação (AF, definida como a atividade muscular evocável máxima), sua soma, fadiga de desempenho (PF, definida como a perda de potência isocinética voluntária máxima em comparação com a basal) durante o exercício realizado no cicloergômetro em capacidade aeróbica máxima. Além disso, esta tese teve como objetivo determinar as taxas de recuperação nas quais MF, AF e PF retornaram à linha de base após a intolerância durante o exercício de corpo inteiro em seres humanos.

Métodos: Para quantificar a fadiga durante o exercício de corpo inteiro, foi desenvolvido um método para permitir uma rápida transição do ciclismo padrão (em que a relação entre potência e cadência é hiperbólica) para o ciclismo isocinético (em que a potência é independente da cadência, e a cadência é fixa). Assim, ao pedir para o participante realizar um esforço isocinético máximo em qualquer ponto durante o exercício ou na fase de recuperação, permitiu-se quantificar o declínio velocidade-específica da potência isocinética máxima (P_{ISO}). A diferença na P_{ISO} entre a linha de base e o exercício quantifica a PF. Foi testado se a relação de base entre P_{ISO} e

potência eletromiográfica em 5 músculos da perna (RMS EMG) era velocidade dependente, linear e reprodutível, de tal modo que as contribuições relativas para PF pudessem ser isoladas a partir de: 1) a diminuição da ativação muscular (AF) ; e 2) o declínio na P_{ISO} num dado grau de ativação (MF).

Resultados: Participantes saudáveis (n=13, 29-72 anos, variando em capacidade aeróbica de 23,5 até 62,4 ml/min/kg) completaram tiros isocinéticos esforço-variável de curta duração (5 s) a 50, 70 e 100 rpm para caracterizar a relação basal entre EMG RMS e potência isocinética. As correlações entre EMG-P_{iso} basais foram lineares (r^2= 0,95 ± 0,04) e velocidade dependente (análise de covariância). Posteriormente, testes de exercício incrementais repetidos foram realizados em uma bicicleta ergométrica e as trocas gasosas e a ventilação foram mensuradas respiração a respiração. O exercício encerrava com um esforço isocinético máximo (5 s) a 70 rpm. Na intolerância, P_{ISO} (duas pernas, 335 ± 88 W) foi ~ de 45% menos do que na linha de base (630 ± 156 W, p <0,05). Após a intolerância, houve recuperação da P_{ISO} em 3 minutos (p <0,05). AF e MF (medido em uma perna) foram de 97 ± 55 e 60 ± 50 W, respectivamente. As médias de viés (± limites de concordância) para a reprodutibilidade foram as seguintes: P_{ISO} na linha de base 1 ± 30 W; P_{ISO} na recuperação 0-min 3 ± 35 W; e EMG em P_{ISO} 3 ± 14%.

Conclusões: A relação basal EMG-P_{ISO} foi bem modelada por uma função linear, que foi reprodutível no dia-a-dia. A variabilidade das mensurações EMG-P_{ISO} individuais entre ~ 25% e 100% de esforço, em torno do modelo linear, foi suficientemente forte de modo que a relação linear basal permitiu uma quantificação precisa de AF e MF no limite de tolerância e na recuperação do exercício aeróbico máximo. Foi também demonstrado que a relação EMG-P_{ISO} foi velocidade dependente, como esperado a partir da curva parabólica de potência-velocidade. Assim, esta tese apresenta um novo método útil para identificar as contribuições da fadiga central e periférica na limitação do exercício de corpo inteiro em seres humanos.

Palavras-chave: isocinética; fadiga de desempenho; fadiga de ativação; fadiga muscular; eletromiografia; teste de exercício.

LIST OF ABBREVIATIONS

ACh - neurotransmitter acetylcholine

ADP - adenosine diphosphate

AF - muscle activation fatigue

ATP - adenosine triphosphate

BR - breathing reserve

$C(a\text{-}v)O_2$ - arteriovenous oxygen concentration difference

C_aO_2 - arterial oxygen concentration

CK - creatine kinase

CNS - central nervous system

CO - cardiac output

CO_2 - carbon dioxide

COPD - chronic obstructive lung disease

CP - critical power

Cr - creatine

DH - dynamic hyperinflation

EELV - end expiratory lung volume

EFL - expiratory flow limitation

EILV - end inspiratory lung volume

EMG - Electromyography

FEV_1 - forced expiratory volume in 1 second

FFA - free fatty acids

FFR - fatigue resistant

FFS - fatigue sensitive

FG - fast-glycolytic

FOG - fast-oxidative-glycolytic

fr - breathing frequency

HR - heart rate

[Hb] - hemoglobin concentration

IC - inspiratory capacity

LT - lactate threshold

MET - metabolic equivalent of task

MF - muscle fatigue

MOAD - maximal accumulated oxygen deficit

MUAP - motor unit action potentials

MVC - maximal voluntary contraction

MVV - maximum voluntary ventilation

NADH - nicotine adenine dinucleotide, reduced

O_2 - oxygen

O_2Def - oxygen deficit

P - power output

P-t_{lim} - hyperbolic power-duration

P_aO_2 - arterial partial pressure of oxygen

PCr - phosphocreatine

PF - performance fatigue

P_i - inorganic phosphate

P_{ISO} - isokinetic power

PNS - peripheral nerve stimulation

RMS - EMG Root Mean Square

S_aO_2 - arterial oxygen saturation

SR - sarcoplasmic reticulum

ST or SO - slow twitch oxidative

SV - stoke volume

T-tubule - transverse tubule

T_E - expiratory time

T_I - inspiratory time

TMS - transcranial magnetic stimulation

TT - time trial

T_{TOT} - breathing cycle duration

VCO_2 - carbon dioxide output

V_D - physiologic deadspace volume

V_E - ventilation

V_{max} - maximum velocity

VO_2 - oxygen uptake

VO_{2max} - maximum oxygen uptake

VO_{2peak} - peak oxygen uptake

VO_{2sc} - “slow component” of maximum oxygen uptake

VO_{2ss} - steady state of maximum oxygen uptake

VO_{2xs} - “excess" maximum oxygen uptake

V_{opt} - optimum velocity

V_T - tidal volume of each breath

W' - constant amount of work

τ - time constant

LIST OF FIGURES

LIST OF TABLES

TABLE OF CONTENTS

1. INTRODUCTION

1.1 Physical activity and health

Accumulating scientific evidence shows physical activity to have profound health benefits amenable to substantial public health gains (1, 2). Unfortunately, most adults do not adhere to physical activity recommendations (3) for health maintenance, and a decline in physical activity is observed in older adults. Knowledge of the determinants of physical activity (unstructured activity incorporated in daily life) and exercise (structured, planned and repetitive activities) is needed to effectively promote an active lifestyle (4). The determinants of physical inactivity are broad and complex and include at least physiological, behavioral, and socioeconomic factors. Nevertheless, it is clear that declining physiological function imposes limits on the capacity for physical activity, by constraining muscular strength, power or endurance. Declines in physiological functions are particularly prominent in the elderly or in patients with chronic diseases of the circulatory, pulmonary, renal, musculoskeletal, or neurological systems. This creates a downward spiral of inactivity and physical deconditioning that further exacerbates development of co-morbidities such as obesity and metabolic syndrome. Physical inactivity causes diseases as diverse as cancer, neurological disease, rheumatologic disease, stroke, kidney failure, cardiovascular disease and diabetes (2).

Thus, to maintain and or promote active living for healthy aging, the mechanisms limiting physiological function during exercise, which provide the capacity for physical activity, should be identified. It is well known that the ability to sustain muscular exercise is highly dependent on the body's capacity for oxidative metabolism to meet the energy demands of the task. This requires a highly integrated, tightly controlled, effective response among the cardiopulmonary and neuromuscular systems to transport and utilize oxygen to minimize the influence symptoms associated with exercise intolerance: muscle fatigue, dyspnea and pain. However, the mechanisms limiting exercise tolerance are poorly understood. This is in no small part due to the complexity

in quantifying these mechanisms at the limits of whole-body dynamic exercise normally encountered during every day living.

This thesis will review the integrated physiological systems that support the ability to sustain muscular exercise. It will then discuss the limits to current methods used to determine the degree to which peripheral limiting symptoms such as muscle fatigue, and central limiting symptoms such as dyspnea or pain contribute to limiting whole-body exercise. This thesis will build on this prior work to develop and validate a novel method for assessing the central and peripheral components of exercise intolerance suitable for use in adult participants across a wide range of ages.

Thus, through a better understanding of the precise mechanisms limiting exercise tolerance in individuals I aim to provide a method that may be used to personalize targeted therapies for these mechanisms. It is therefore hoped to providing a route to optimized exercise tolerance gains, which will underpin the capacity for physical activity maintenance across the lifespan.

1.2 Physiological systems responses during acute exercise: an overview

This section provides an overview of the physiological systems responses necessary to bring about and sustain human movement (figure 1). Movement begins with the planning and activation of willed movements through motor cortex, spinal cord and motor nerve activation. Intact muscular excitation-contraction coupling is required to bring about muscle cross-bridge formation, and force (or power) production. This process consumes adenosine triphosphate (ATP), which must be supplied by increased neuromuscular metabolism. However ATP is stored at low concentrations in the body's tissues and therefore for activity to be sustained it is necessary to recruit a sustainable source of energy provision. This is provided by the pulmonary, circulatory, and mitochondrial systems, which must be capable of transporting sufficient oxygen from the atmosphere, mobilizing oxidizable substrates from bodily stores

(carbohydrates and fatty acids), and supplying blood flow to active tissues to clear carbon dioxide and metabolic acids to allow an exercise task to be sustained.

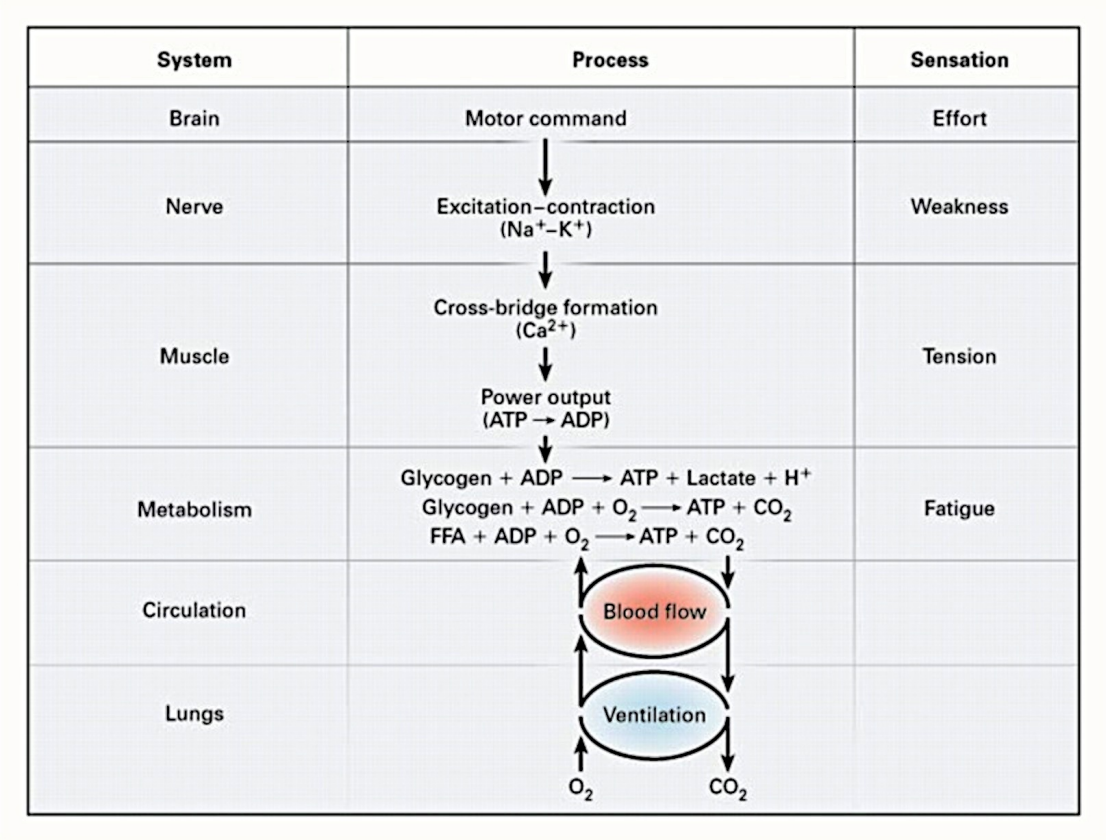

Figure 1. A schematic flow diagram showing the links between anatomic systems and physiological processes during exercise. The interactions among these processes provide the sources of perceived sensations that may contribute to exercise limitation. ADP denotes adenosine diphosphate, ATP adenosine triphosphate, and FFA free fatty acids (5).

1.2.1 Central nervous system, motor unit recruitment and excitation-contraction coupling

Muscle contraction during exercise is initiated by a central command from the motor cortex of the brain that leads to activation of motor neurons, depolarization of motor end plates, propagation of muscle action potentials, calcium release, formation of cross-bridges, shortening of myofibrils and then the muscle contraction (5).

In 1954, two independent groups, Huxley & Niedergerke and Huxley & Hanson (6, 7), developed the *sliding filament theory* that describes a process used by muscles to contract. It is a cycle of repetitive events that cause a thin filament to slide over a thick filament and generate tension in the muscle (8). For this to occur, the central nervous system must first initiate a series of events, requiring an uninterrupted, intact, response from each, to bring about the planned action. The steps in this series are as follows (6-8):

1. An action potential originating in the central nervous system (CNS) depolarizes an α-motor neuron (or anterior motor neuron, that consists of a cell body, axon and dendrites), which transmits the action potential from the spinal cord to the motor end plate on the surface of the muscle.

2. The action potential propagates down the motor nerve by activating voltage-gated Na^+ channels along the axon toward the neuromuscular junction (or motor endplate), the interface between the end of a myelinated motor neuron and muscle fiber. When it reaches the junction, it causes a Ca^{2+} influx through the voltage-gated Na^+ channels.

3. The Ca^{2+} influx causes vesicles containing the neurotransmitter acetylcholine (ACh) to fuse with the plasma membrane, releasing ACh out into the extracellular space between the motor neuron terminal and the neuromuscular junction of the skeletal muscle fiber.

4. The ACh diffuses across the synapse and binds to, and activates, nicotinic ACh receptors on the neuromuscular junction. Activation of the nicotinic receptors opens its intrinsic Na^+/ K^+ channels, causing Na^+ to rush inwards and K^+ to trickle out. Because the channel is more permeable to Na^+, the charge difference between internal and external surfaces of the muscle fiber membrane becomes less negative, triggering an action potential.

5. The action potential spreads through the muscle fiber's network of T-tubules depolarizing the inner portion of the muscle fiber.

6. The depolarization activates L-type voltage-dependent calcium channels (dihydropyridine receptors) in the T tubule membrane, which are in close proximity to calcium-release channels (ryanodine receptors) in the adjacent sarcoplasmic reticulum.

7. Activated voltage-gated calcium channels physically interact with ryanodine receptors to activate them, causing the sarcoplasmic reticulum to release calcium into the sarcoplasm.

8. Sarcoplasmic calcium binds to troponin C, present on the actin-containing thin filaments of the myofibrils, which allosterically modulates tropomyosin complex. Under normal circumstances, the tropomyosin sterically obstructs the binding sites for myosin on the thin filament; however, once calcium binds to the troponin C, and causes an allosteric change in the troponin protein conformation, troponin T allows tropomyosin to move, unblocking the binding sites.

9. Myosin (which has ADP and inorganic phosphate bound to its nucleotide binding pocket and is in a ready state) binds rapidly to the newly uncovered binding sites on the thin filament (binding to the thin filament is very tightly coupled to the release of inorganic phosphate). Myosin is now bound to actin in the strong binding state. The release of ADP and inorganic phosphate are tightly coupled to the power stroke, where actin acts as a cofactor in the release of inorganic phosphate, expediting the release (Figure 2) (9). Attachment of myosin is a reversible process that gives stiffness to the muscle (i.e. it will resist if stretched), but will not itself generate force. This is sometimes referred to as the *low-force* state. The release of inorganic phosphate (P_i) from the actomyosin complex (step ii, in Figure 2) is thought to initiate the changes that result in force generation with the cross-bridge moving into the *high-force* state. Towards the ends of the rotation phase of the myosin head, ADP is released (step iii) and the actomyosin complex binds ATP (step iv). Having done so, the actin and myosin dissociate with the ATP still bound to myosin (step v). ATP is hydrolysed and the products remain bound to the protein (step

vi); this last process is thought to activate the myosin head, making it ready to bind to actin again (step i) (9). The cycling of the cross bridge pulls the Z-bands towards each other, thus shortening the sarcomere and the I-band, and resulting in force production in the muscle (Figure 3).

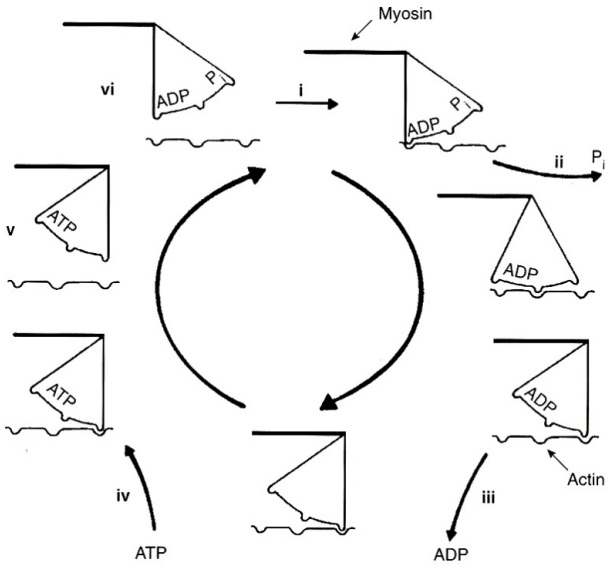

Figure 2. Stages in the cross-bridge cycle showing the relationship between mechanical and biochemical steps (9).

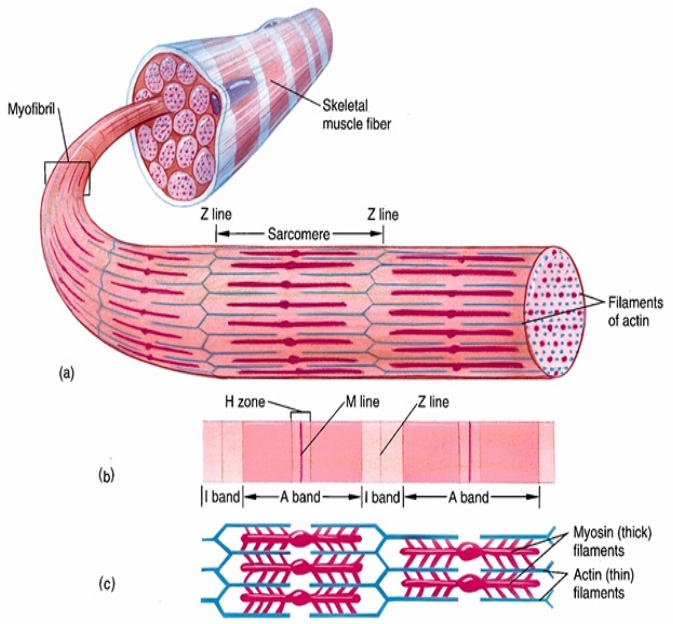

Figure 3. The microstructure of muscle: a skeletal muscle fiber contains numerous myofibrils, each consisting of units called sarcomeres (10).

10. Thus, as ATP binds to myosin, it releases from the actin molecule and cycles to a weak binding state. A lack of ATP makes this step (step v) impossible, and results in the rigor state characteristic of *rigor mortis*. The myosin then hydrolyzes the ATP, and uses the energy to move into a preparatory conformation, sometimes termed the “cocked back” position (in reference to the cocking of the hammer of a gun; step vi). In general, evidence (predicted and *in vivo*) indicates that each skeletal muscle myosin head moves approximately 10–12 nm for each power stroke cycle.

11. Steps 9 and 10 repeat as long as ATP is available and calcium is freely bound within the thin filaments.

12. While the above steps are occurring, calcium is actively pumped back into the sarcoplasmic reticulum. As sarcoplasmic calcium concentration falls, it is released from its relatively weak troponin binding, and thus the tropomyosin changes conformation back to its previous. Thus, the tropomyosin-troponin complex again covers the binding sites on the actin filaments and contraction ceases.

Under normal function of each of the steps above, the magnitude of the central motor command increases the recruitment of motor units, and thus force production.

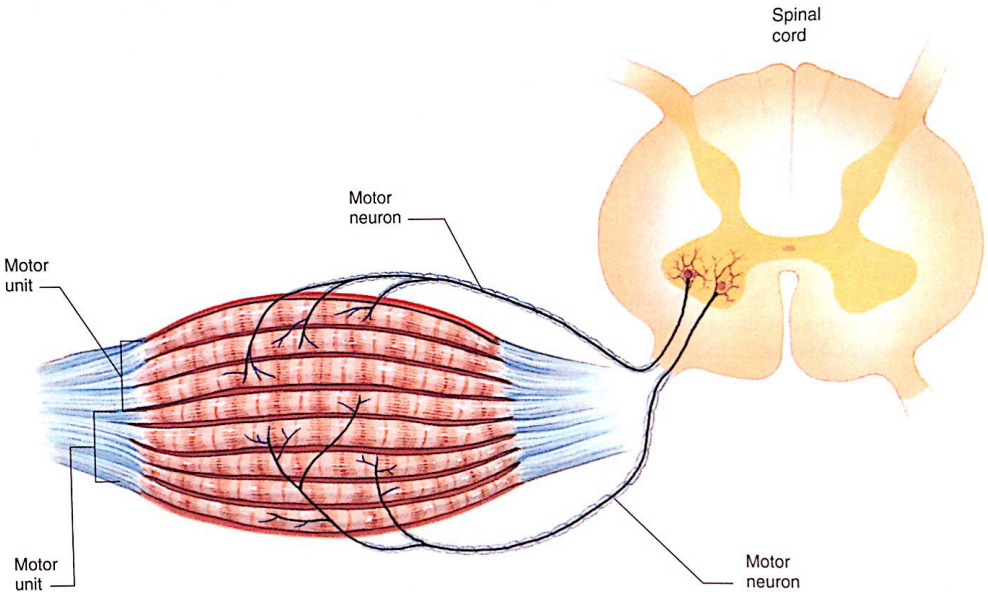

Figure 4. The motor unit. The motor unit is defined as a motor neuron and all the muscle fibers it innervates (10).

Figure 4 illustrates how muscle fibers are innervated by individual motor axons. Each motor axon innervates several muscle fibers, collectively termed the motor unit. Figure 5 shows schematically how a common central motor drive acts to increase firing rate and recruitment of motor units to generate force in the muscle. A maximal voluntary command is capable of activating virtually 100 percent of the motor units in a fresh muscle. However, fatigue acts to reduce force production through several processes, including but not limited to, reduction in central motor drive and the responsiveness of motor neurons, which may be decreased by central and peripheral factors (11) acting through reflexes in the spinal cord and by stimulation of receptors in the muscles. The relationship between the central motor drive and expected muscle force production determines the sense of effort. Effort and muscle fatigue are a major symptoms of exercise that can contribute to exercise limitation. The actions of fatigue on the motor system are discussed in detail below (Section 1.3 *Fatigue and exercise intolerance in whole-body exercise*).

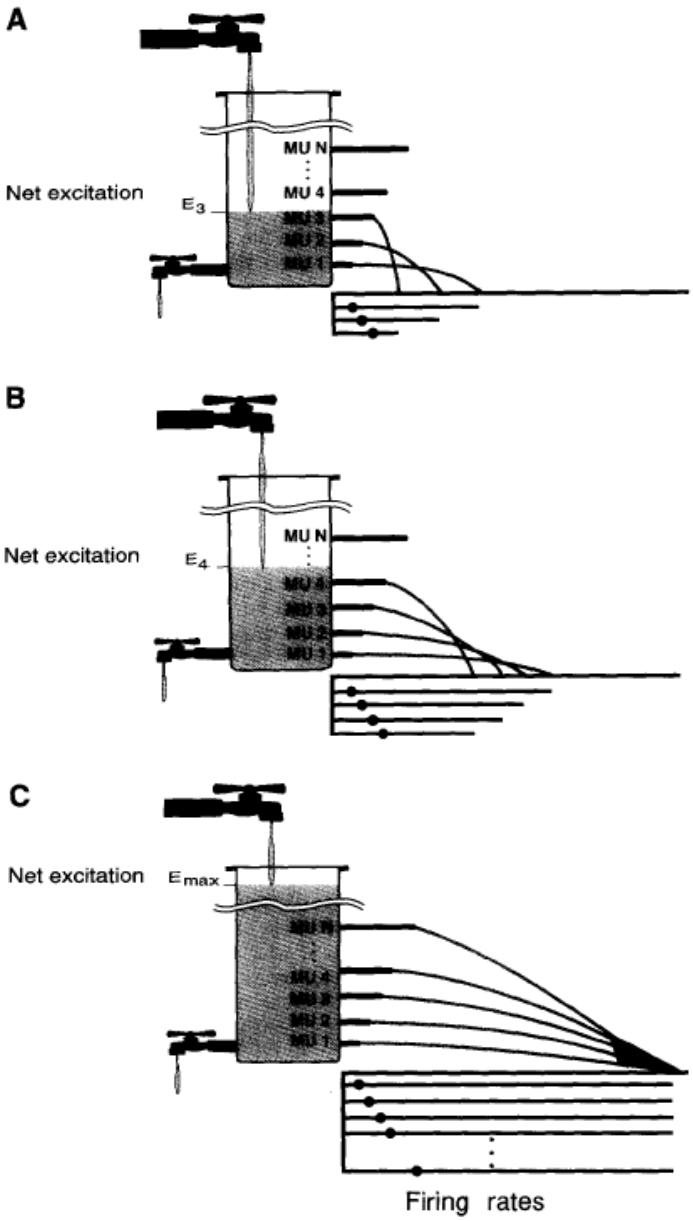

Figure 5. A model to summarize the regulation of motor units in muscle-force production. The water flow into the tank corresponds to the central neural drive into the motoneuron pool, while the outflow from the individual spouts, and the distance traveled, corresponds to the recruitment of a given motor unit and its firing rate. The length of each spout is representative of the initial motor unit firing rate, while the circle indicates the initial firing rate below which the motor unit cannot fire. The outlet valve (bottom left) represents motorneuron inhibition. Net water accumulation in the jar corresponds to the common drive to the motor system (excitation-inhibition). ***A*** shows the firing rates when the motor drive is only enough to recruit three motor units. ***B*** shows how an increase in the common drive will recruitment a new motor unit, and also the increase in the firing rates of already active motor units. ***C*** shows the convergence of the firing rates under a maximal common drive i.e. where the differences between individual 'spouts' become negligible compared with the water level (12).

1.2.2 Skeletal muscle structure and function

The role of skeletal muscle is to generate force (13) and, for that, the skeletal muscle exhibits a distinctive banding pattern when viewed under the microscope due to the arrangement of cytoskeletal elemental in the cytoplasm of the muscle fibers (Figure 3). As discussed, the principal cytoplasmic proteins are myosin and actin, also known as "thick" and "thin" filaments, respectively, which are arranged in a repeating unit called a sarcomere. The interaction of myosin and actin is responsible for muscle contraction (14).

The plasma membrane is called the sarcolemma with the cytoplasm known as the sarcoplasm. Within the sarcoplasm are the myofibrils. The myofibrils are long protein bundles about 1 micrometer in diameter each containing myofilaments. Pressed against the inside of the sarcolemma are flattened myonuclei, and myofibrils are interlaced with a mitochondrial network. Sub-sarcolemmal mitochondria surround the sarcoplasm and are well placed to receive substrates (oxygen, glucose, fatty acids) from the capillary network surrounding the muscle fibers. Intramyofibrillar mitochondria project into the deep myocellular space and are well placed to support oxidative ATP provision to the deeper tissues. While the muscle fiber does not have a smooth endoplasmic reticulum, it contains a sarcoplasmic reticulum that surrounds the myofibrils and holds a reserve of calcium ions needed to cause a muscle contraction. Periodically, it has dilated end sacs known as terminal cisternae. These cross the muscle fiber from one side to the other. In between two terminal cisternae there is a tubular infolding called a transverse (T) tubule, which is the pathways for action potentials to signal the sarcoplasmic reticulum to release calcium, causing a muscle contraction (15). This allows for myosin and actin ATP-dependent cross-bridge cycling and shortening of the muscle (14).

Muscle architecture refers to the arrangement of muscle fibers relative to the axis of force generation of the muscle. This axis is a hypothetical line from the muscle's origin to insertion. For some longitudinal muscles, such as the biceps

brachii, this is a relatively simple concept. For others, such as the rectus femoris or deltoid muscle, it becomes more complicated. While the muscle fibers of a fascicle lie parallel to one another, the fascicles themselves can vary in their relationship to one another and to their tendons (16).

Consideration of how human muscle meets the demands for different types of mechanical output must take account of the variation in the contractile and metabolic properties of the muscle fibers of which the whole muscle is composed, and their pattern of recruitment. On the basis of histochemistry of the predominant myosin ATPase expressed in serial sections, human muscle fibers are commonly divided into three major types: I, IIA and IIX. These types are more or less analogous to muscle fibers from animals, and can also be classified on the basis of their directly determined functional and metabolic properties as slow twitch oxidative (ST or SO), fast-oxidative-glycolytic (FOG; fatigue resistant, FFR) and fast-glycolytic (FG; fatigue sensitive, FFS), respectively (Table 1). It should be noted, however, that while conventional histochemical techniques are designed to differentiate muscle fibers into discrete types, and, although this categorization is convenient, it can be misleading since human muscle fibers resides within a continuum in most, if not all, metabolic and contractile properties among muscle fibers (Figure 5). The characteristics of muscle fiber size, force production capacity, metabolic profile, and fatigability form an approximate continuum that allows for exquisite control of motor activity.

In recent years it has become possible to show that there is a complex set of genes controlling the expression of the contractile proteins, and that these are closely linked to those determining metabolic properties. Of the contractile proteins, the isoform of the myosin heavy chain that is expressed seems to be the primary determinant of the maximum velocity of shortening of the fiber, and hence the capacity for power production (power = force x velocity).

One of the more important observations is that, whereas type-I and type-II myosin heavy-chain isoforms are not usually found in the same fiber, the majority of human type-II fibers have both the IIA and the faster IIX isoforms in

variable amounts. Indeed, it is notable that in normal healthy humans there are very few fibers that express purely the IIX isoform. The relatively large proportions of what have previously been defined by conventional histochemistry as IIB fibers presumably included many hybrid fibers which co-expressed significant amount of the IIA isoform (17-19).

Table 1. Principal systems of muscle fiber-type classification (13).

ATPase	**Contractile/metabolic**	**Physiological**
Type I	Slow, oxidative (SO)	ST (slow, fatigue resistant)
Type IIA	Fast, oxidative-glycolytic (FOG)	FFR (fast, fatigue resistant)
Type IIX	Fast, glycolytic (FG)	FFS (fast, fatigue sensitive)

Abbreviations: ST or SO, slow twitch oxidative; FOG, fast-oxidative-glycolytic; FFR, fatigue resistant; FG, fast-glycolytic; FFS, fatigue sensitive.

As might be expected, there is a close link between the expression of the contractile proteins, the metabolic properties of the fibers and the concentration of substrates. Thus the maximally Ca^{2+} activated myofibrillar ATPase activity is related to the myosin heavy-chain isoforms expressed in human fibers (20). In turn, this is reflected in the resting concentration of phosphocreatine (greater in IIA and IIX than in I), which provides a temporal and spatial buffering system for ATP turnover. In human muscle fibers, as the intrinsic speed of cross-bridge cycling increases with the proportion of the IIX isoform expressed, so does the resting concentration of phosphocreatine, to meet the increased rate of depletion during maximal exercise (19). Conversely, the maximal ATPase activity in type I fibers can be well supported by oxidative phosphorylation, a slow, but highly sustainable, process requiring delivery of O_2 and reducing equivalents to the electron transport chain in the mitochondrion for the production of ATP. As long as O_2 and substrate delivery (O_2 from the atmosphere, and glucose or fatty acids mobilized in the blood) can be maintained at the rate demanded by the ATP turnover of the exercise task then, exercise can be well sustained in type I and IIA muscle fibers by mitochondrial oxidative phosphorylation. Hence, type I and early-recruited IIA fibers have a

high expression of the mitochondrial network and a lower centration of phosphocreatine and glycolytic enzymes, whereas IIA/X and IIX fibers, mitochondrial expression is lower and glycolytic enzyme activity greater.

As proposed by Henneman and Mendel in 1981 (21), there is an orderly hierarchy of motor unit recruitment (e.g. Figure 5). This motor unit recruitment is largely based on size of the motor, which determines its threshold for firing. As such, at low levels of planned force, small motor units with thin axons and small, easily excitable motoneurons are recruited. On the other hand, as the planned requirement for force increases, there is a systematic recruitment of larger motor units so that the force steps caused by successive recruitment remain rather constant when expressed as a proportion of the prevailing force output. As a consequence of the pattern and duration of activation implicit in such a recruitment pattern, there is in general an association with increasing size and increasing fatigability and power output – this corresponds with a progression of recruitment from type I, to IIA, to IIA/X, to IIX (Figures 6 and 7).

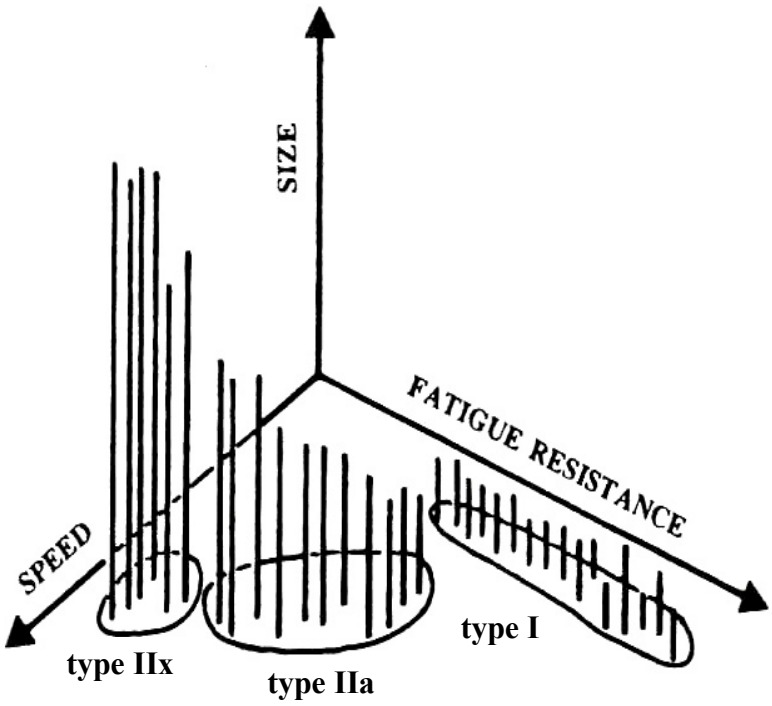

Figure 6. Motor unit variability in size, intrinsic speed and fatigue resistance. From Jones & Round (9).

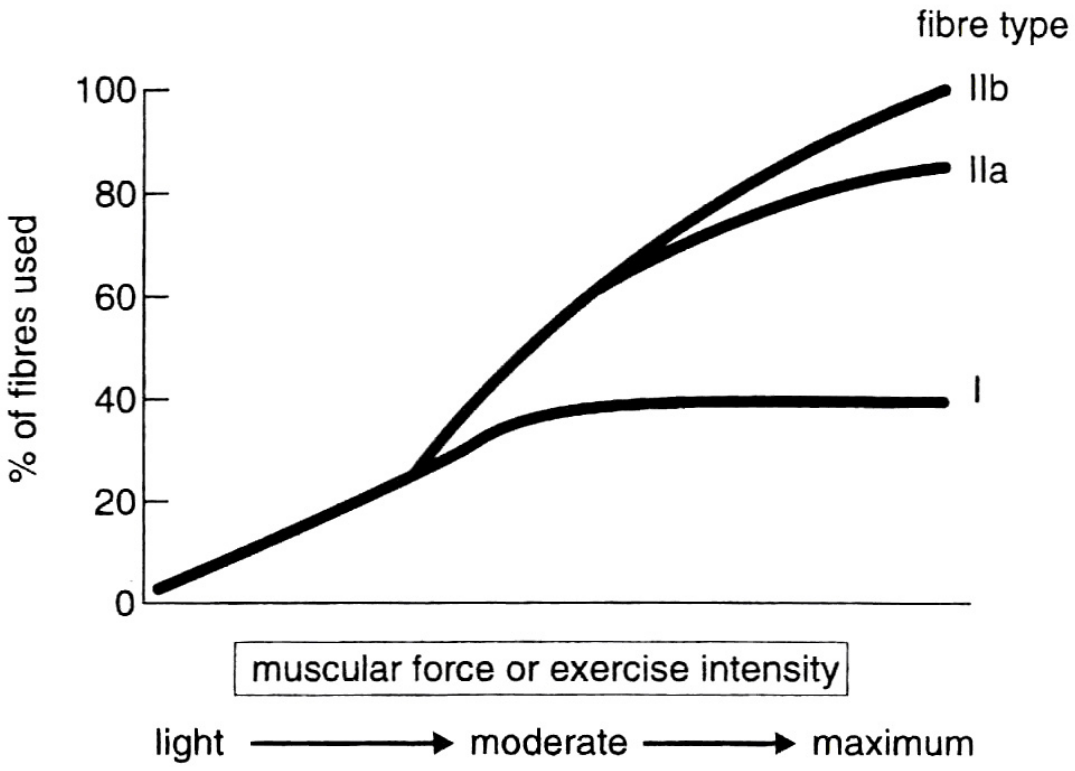

Figure 7. The pattern of muscle fiber recruitment across the intensity spectrum of muscle force or power (22).

1.2.3 Muscle function during static and dynamic contractions

To understand how skeletal muscle functions under static and dynamic contractions, it is necessary to explain the two fundamental relationships that determine the mechanical output of muscle: the muscle length-tension relationship and the force-velocity relationship.

1.2.3.1 *The muscle length-tension relationship*

It has been known since the 19th century that the active generation of isometric tension in muscle, the consequence of the formation of cross-bridges, occurs in the region of overlap of actin and myosin filaments within each sarcomere. In addition, at long muscle lengths there is an increasing element of passive tension as structural elements both outside and within the muscle fibers are

stretched. To obtain the active component of the length-tension relationship, the passive component is usually subtracted. The observed active length-tension relationship has been found to fit reasonably closely to that predicted from the degree of action and myosin filament overlap in accordance with the cross-bridge theory (Figure 8) (13).

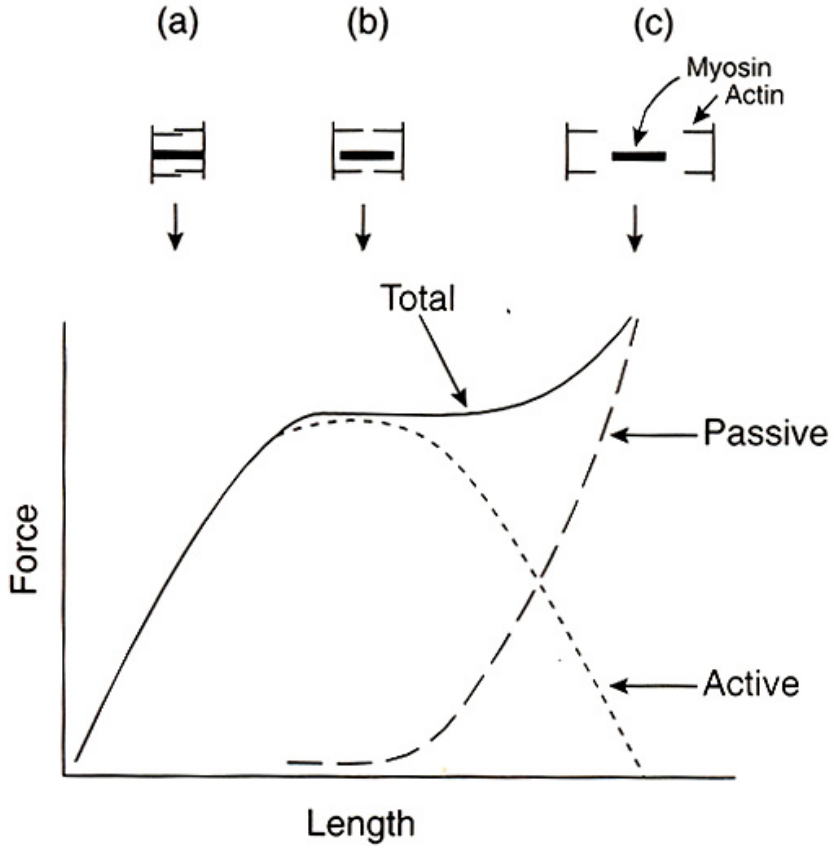

Figure 8. Relationship between muscle length and active (…) and passive (---) isometric tension. The amount of actin and myosin overlap which determines the active component is indicated for (a) a short length where actin filaments from opposite ends of the sarcomere overlap and force is reduced; (b) optimum length where the greatest active force is generated due to the maximum number of cross-bridges are formed (13).

1.2.3.2 *The force-velocity relationship*

Not only does force vary according to the degree of myofilament overlap, as described by the length-tension relationship, but it also varies with the velocity of shortening or lengthening. The force-velocity relationship of muscle was first

described in the 1920s and the basic description remains much the same, as shown in Figure 9. Thus, as velocity of shortening increases, the force that is generated falls in a hyperbolic fashion, eventually reaching zero at a velocity defined as the maximum velocity (V_{max}). During lengthening, the force generated by an active muscle in resisting that lengthening increases above that attained at zero velocity (isometric) before plateauing. The mathematical product of force and velocity is power, which has its own distinctive parabolic relationship with velocity, reaching a maximum at around an optimum (V_{opt}), which in isolated muscle preparations is around 30% of V_{max} (13). The power-velocity relationship strongly reflects the structure of the cross-bridges, and the kinetics of their attachment and detachment (23) and, thus the power producing capacity is highly dependent on the muscle ATPase isoform expressed (Figure 10).

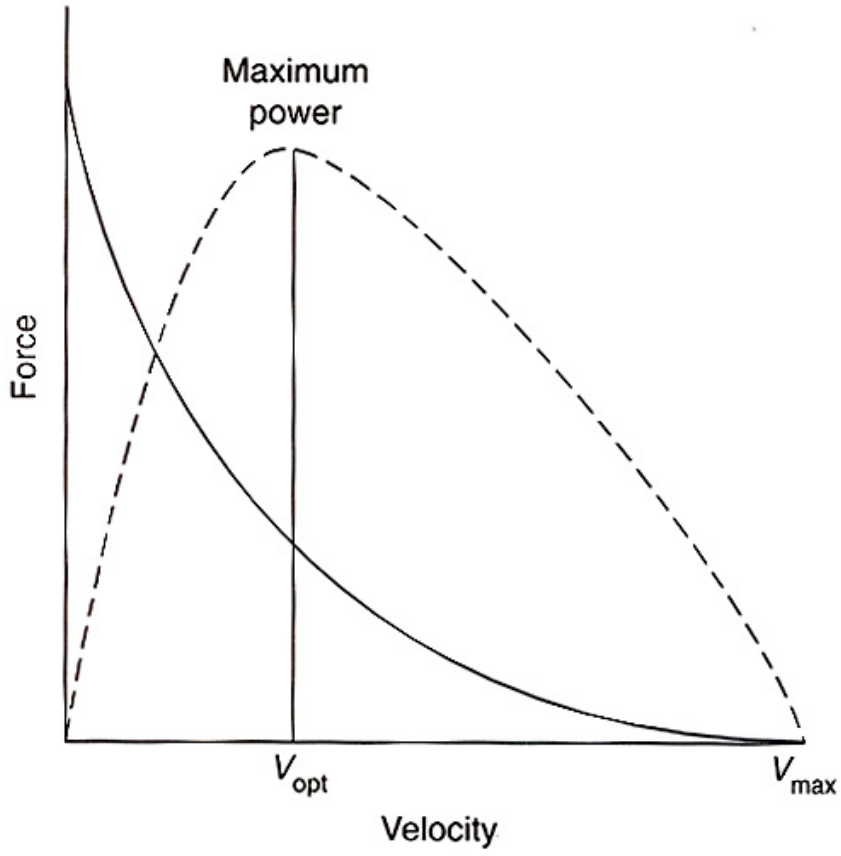

Figure 9. Force-velocity and power-velocity relationship of skeletal muscle. Maximum power is generated at the optimum velocity (V_{opt}), which is about 30% of the maximum velocity of shortening (V_{max}) when force is zero (13).

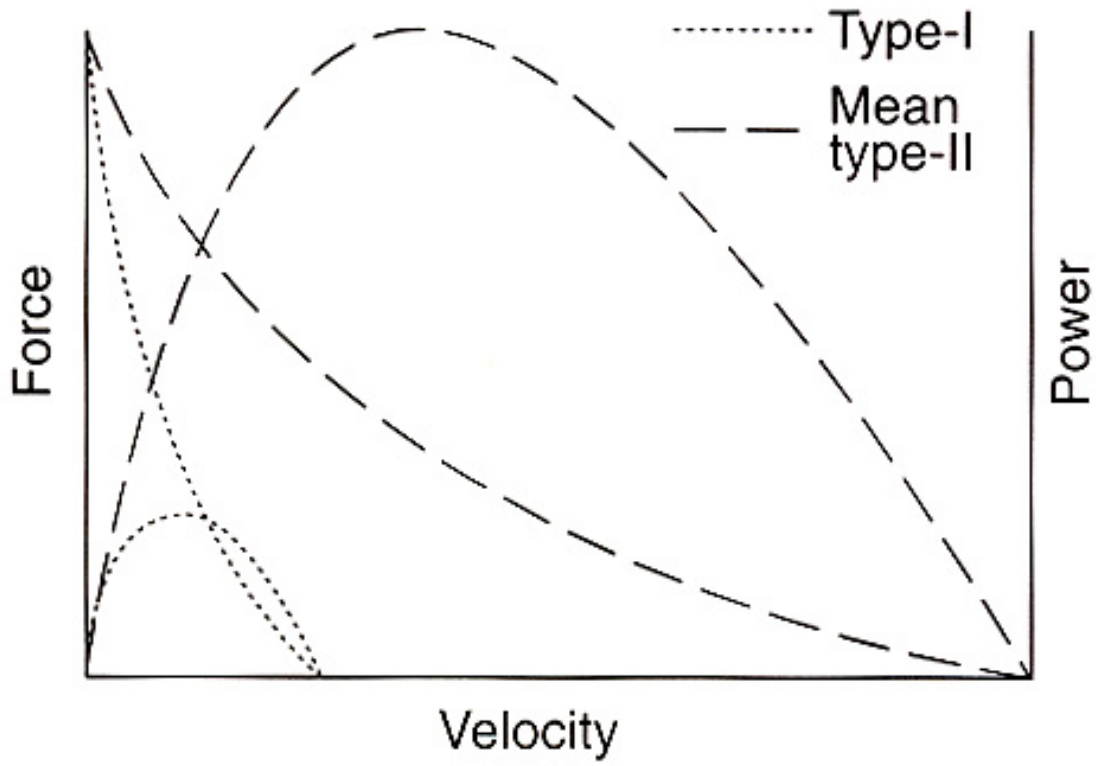

Figure 10. Force-velocity and power-velocity relationship of type I and type II skeletal muscle. The peak force is shown relative to the maximum force of each type of fiber. The differences in shorten velocity have a major impact on the power producing capacity of the two fibers types. (13).

1.2.3.3 *Isokinetic dynamometry for the study of muscle function*

The characteristics of the power-velocity curve determine that peak power production is dependent on the velocity at which it is assessed. This complicates the interpretation of changes in muscle function due to, for example, alterations in muscle activation or muscle fatigue. Specifically, for any given maximal muscle activation (maximal voluntary dynamic contraction), the expected power is dependent on the contractile velocity. Thus, in order to interpret a change in muscle power production during a maximal voluntary dynamic contraction the velocity of shortening must be experimentally constrained. Any decline or increase in power (due to some intervention) may then be appropriately interpreted. This type of muscle function testing is termed isokinetic dynamometry. Isokinetic dynamometry allows muscle force (typically,

torque, given the angular rotation of most human joints) or power to be measured at a velocity determined by the equipment. Most commonly isokinetic dynamometry is conducted using a single joint muscular movement (e.g. knee extension, elbow or hip flexion) with either isometric (static, zero velocity) or isokinetic (fixed velocity) muscle contractions.

With the appropriate equipment isokinetic dynamometry can also be used to assess muscle function during complex movement tasks requiring multiple degrees of freedom such as “whole-body” exercise. For example, in the late 1980s Sargeant, Jones and colleagues (24) developed a custom built cycle ergometer that controlled the angular rotation of the cycling (isokinetic), and measured the participant’s muscle force production by load cells mounted in the pedals. With this equipment they could measure maximal torque and power production during isokinetic cycling at different velocities. They showed that the parabolic nature of the power-velocity curve was retained during this complex movement task. They also demonstrated that exercise tasks resulting in fatigue (defined therein as the loss of maximal voluntary power production), were greater a high contractile velocities; consistent with a greater contribution to fatigue from fatigue-sensitive, type II muscle fibers (that possess a greater intrinsic contractile velocity), than fatigue-resistant type I muscle fibers (where contractile velocity is slower).

Thus, although specialized equipment is required for muscle function assessment by isokinetic dynamometry, the ability to constrain experimentally muscle velocity allows muscle function to be assessed under controlled conditions. This provides a reliable and accurate method for interpreting changes in muscle force or power production in for example, pathologies that limit muscle function or exercise tolerance, or following interventions such as exercise training to ameliorate exercise limitations.

1.2.3.4 *Electromyography (EMG) for the study of muscle function*

Electromyography (EMG) is used to measure patterns of muscle activation,

and has been extensively employed to interpret both dysfunctional and functional muscle recruitment patterns related to control of the motor system including cycling tasks (25).

The EMG signal is the electrical manifestation of the neuromuscular activation associated with a contracting muscle. The signal represents the current generated by the ionic flow across the membrane of the muscle fibers that propagates through the intervening tissues to reach the detection surface of an electrode located in the environment. The anatomical and physiological properties of muscles and the control scheme of the nervous system as well as the characteristics of the instrumentation used to detect and observe it, affect the signal (26). During motor activity, motor unit action potentials (MUAP) of concurrently active motor units superimpose to form the surface EMG signal. As the excitation from the CNS increases to generate greater force in the muscle, a greater number of motor units are activated (or recruited) and the firing rates of all the active motor units increase (Figure 5) (27). A schematic representation of the genesis of summed MUAP are presented in Figure 11 (27).

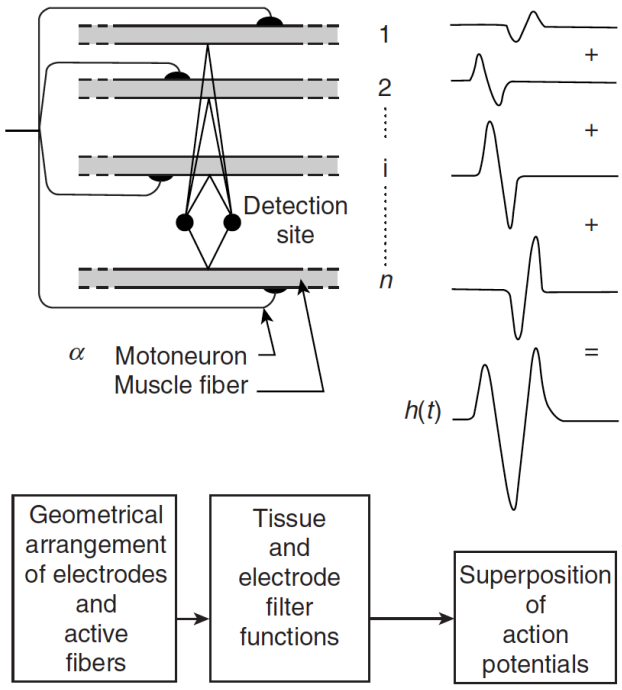

Figure 11. Schematic representation of the generation of the motor unit action potential (27).

It is important to note the many factors that influence the shape of the MUAP. Some of these are: (1) the relative geometrical relationship of the detection surfaces of the electrode and the muscles fibers of the motor unit in its vicinity; (2) the relative position of the detection surfaces to the innervation zone, that is, the region where the nerve branches contact the muscle fibers; (3) the size of the muscle fibers (because the amplitude of the individual action potential is proportional to the diameter of the fiber); and (4) the number of muscle fibers of an individual motor unit in the detectable vicinity of the electrode. The last two factors have particular importance in clinical applications. Considerable work has been performed to identify morphological modifications of the MUAP shape resulting from modifications in the morphology of the muscle fibers (e.g., hypertrophy and atrophy) or the motor unit (e.g., loss of muscle fibers and regeneration of axons). Although usage of MUAP shape analysis is common practice among neurologists, interpretation of the results is not always straightforward and relies heavily on the experience and disposition of the observer (27).

The electrical manifestation of a MUAP is accompanied by a contractile twitch of the muscle fibers. To sustain a muscle contraction, the motor units must be activated repeatedly. The resulting sequence of the MUAP is called a motor unit action potential train (MUAPT). The waveform of the MUAP within a MUAPT will remain constant if the geometric relationship between the electrode and the active muscle fibers remains constant, if the properties of the recording electrode do not change, and if there are no significant biochemical changes in the muscle tissue. Biochemical changes within the muscle can affect the conduction velocity of the muscle fiber and the filtering properties of the muscle tissue (27).

The EMG signal may be synthesized by linearly summing the multiple MUAPT signals. This approach is illustrated in Figure 12, where 25 mathematically generated MUAPTs were added to yield the summed EMG signal (shown at the bottom of Figure 12) (28). This composite signal bears striking similarity to the real EMG signal. From these measurements, it is possible to derive information about the relative intensity and frequency of activation of muscles

engaged (or not) in an exercise task.

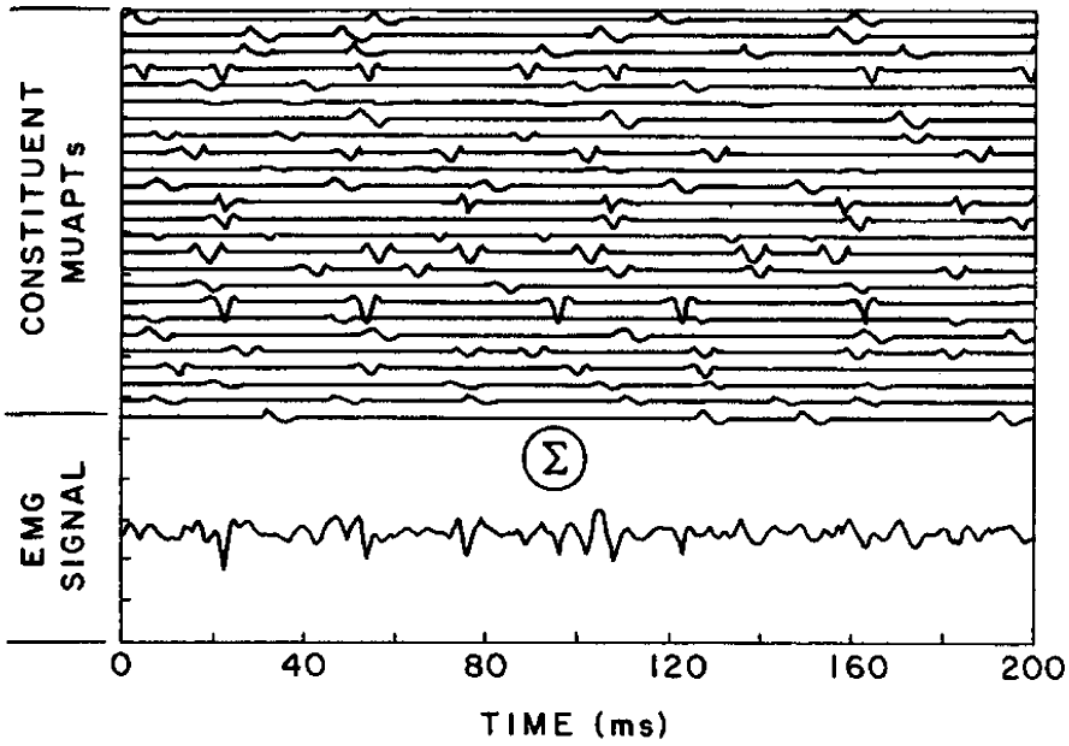

Figure 12. An EMG signal formed by adding (superimposing) mathematically generated MUAPTs (27, 28).

The EMG signal is dependent on time and intensity of muscle activation (among other variables), whose amplitude varies in a random nature above and below the zero value (dependent of whether the summed EMG MUATPs are moving towards or away from the recoding electrode). Thus, simple averaging of the EMG signal does not provide useful information (27). A simple method to overcome this limitation is to rectify the EMG signal before performing further interpretation. The process of rectification involves the concept of rendering only positive deflections of the signal. This may be accomplished either by eliminating the negative values (half-wave rectification) or by inverting the negative values (full-wave rectification). The latter is the preferred procedure because it retains all the energy of the signal (27).

Further signal processing is required to interpret EMG signals in terms of a

relative intensity of muscle activation. Mathematical derivations of time and force measurements indicate that the EMG Root Mean Square (RMS) value provides the most robust measurement of the energy of the EMG signals. The RMS measures the average energy (or power) of the surface electrical activity of the muscles under the electrode and provide a reliable estimate of muscle 'activation intensity'. Overall the use of RMS in electromyography, however, has been historically sparse in the past. Nevertheless, the use of RMS (as opposed to alternative EMG time and energy related variables) has increased the technical competence in EMG (27).

It should also be recognized that the quantitative RMS EMG signal provides only a subjective assessment of relative muscle activation during the motor task. This subjectivity is caused by, first, high levels of neural recruitment inter-subject variability and, second, the inaccuracy associated with using different surface electrode placement sites when conducting repeated trials (29). A common method to overcome these subjectivity problems in quantifying and interpreting the EMG signal, is to use a normalization technique. By comparing a specific EMG muscle activity measurements with a reference EMG value (commonly a maximal isometric or isokinetic effort in a baseline, or fresh state), and expressing the EMG activity as a percentage of this reference value allows an investigator to establish the relative intensity of muscle activity within a specified task. This enables the conversion of subjective EMG values into data points that have a significant interpretative meaning. EMG normalization allows for comparisons obtained over a variety of conditions. Between-subject comparisons can be made after normalizing the EMG activity, because the reference activity for a given subject is compared with the relative amount of activity for that subject, and is therefore dependent on each individuals' own maximal EMG activity. The investigator then has the ability to compare the relative EMG for a given workload across subjects. Furthermore, EMG normalization also controls for small differences introduced by, for example, electrode placement or skin impedance on different assessment days (29).

For EMG normalization to be effective, the reference activity has to be reliable and repeatable. Knuston et al. (30) compared dynamic with isometric leg

extension. Their results showed that the isometric leg extension tests had a higher intraclass correlation coefficient between trials compared with dynamic leg extension, therefore improving the reproducibility of the data.

This normalized RMS EMG provides a method by which motor activation of skeletal muscle may be assessed during specific exercise tasks. Coupling these measurements with force or power production by isometric or isokinetic dynamometry provides a reliable an accurate method to assess the functioning of the neural-mechanical coupling.

1.2.4 Muscle metabolism

ATP is the immediate source of energy for muscular contraction (31). Thus, sustained muscle contractions requires a continued supply of ATP to power the cross-bridge cycle and other energy-consuming processes associated with muscle contraction, such as calcium pumping. Intramuscular stores of ATP are low (8.2 mM), and without a biosynthetic supply of ATP, these stores could be completely depleted within a few seconds of the onset of an intense muscle contraction. Importantly, the intracellular ATP concentration is tightly regulated during muscular contractions, such that, in all but the most extreme conditions, [ATP] is unaltered. Thus, the ability to sustain muscular exercise depends in large part on the body's ability to support the regeneration of ATP at a rate commensurate with the energy demands of the task (Figure 13).

There are three processes by which [ATP] is produced within the muscle fiber (9) (Figure 3):

i. Phosphocreatine (PCr) breakdown: ADP is rapidly rephosphorylated from phosphocreatine via the enzyme creatine kinase, which is highly abundant in muscle. This equilibrium of the reaction strongly supports ATP formation, so that ATP concentration remains virtually constant as long as phosphocreatine remain has become almost exhausted. Phosphocreatine concentration is ~25-35 mM in skeletal muscle (greater

in type II fibers than type I).

ii. Anaerobic glycolysis: The catabolism of muscle glycogen (or glucose imported from the blood) results in pyruvate and lactate formation, producing ATP. Lactate production during exercise depends on the balance between glycolytic rate, which is controlled by the activity of intramuscular glycogen phosphorylase, and pyruvate oxidation, which is controlled by the activity of the pyruvate dehydrogenase complex and by enzymes of the tricarboxylic acid cycle, and is thus highly dependent on O_2 availability (32) (Figure 13). Glycogen phosphorylase and pyruvate dehydrogenase exist in active and inactive forms, and both are influenced by allosteric modulators as well as by substrate-product interactions (33). Also, both are activated by calcium, but glycogen phosphorylase is inhibited by decreased pH (34, 35), whereas the opposite is the case for pyruvate dehydrogenase (36). The balance in the activity of the two enzymes regulates the balance between fatty acids and glycogen oxidation, and determines lactate production (37, 38).

iii. Oxidative phosphorylation: Fatty acids and pyruvate (from the glycogen catabolism supply) provide oxidizable substrates for the tricarboxylic acid cycle within the mitochondrial matrix. Here acetate is oxidized to produce carbon dioxide (CO_2) and reducing equivalents (nicotine adenine dinucleotide, reduced - NADH) to supply the electron transport system to power oxidative phosphorylation; the coupled reduction of oxygen (O_2) to water producing ATP from ADP and Pi. This process is highly sustainable, as long as substrates (fatty acids, carbohydrates and O_2) can be readily supplied to the sites of their metabolism.

As the intensity of exercise increases, the concentration of intramuscular PCr decreases, and the concentrations of intramuscular ADP, adenosine monophosphate, and P_i increase. Increases in the intramuscular lactate concentration and decreases in the intramuscular potassium concentration contribute to a marked decline in muscle pH to below 6.5 (39-41). With prolonged submaximal exercise, the changes in intramuscular metabolite concentrations are less marked, but intramuscular glycogen is progressively

depleted. The body's fat stores represent a large reservoir of potential energy, but the rate at which fatty acids can be mobilized and oxidized is limited to approximately one quarter to one half of the rate at which glycogen can be oxidized. Thus, during high-intensity exercise the ability to sustain exercise depends strongly on the ability of the cardiopulmonary system to transport and utilized oxygen. Whereas the duration for which less intense, sub-maximal, exercise can be sustained is more dependent on the initial intramuscular glycogen concentration (42).

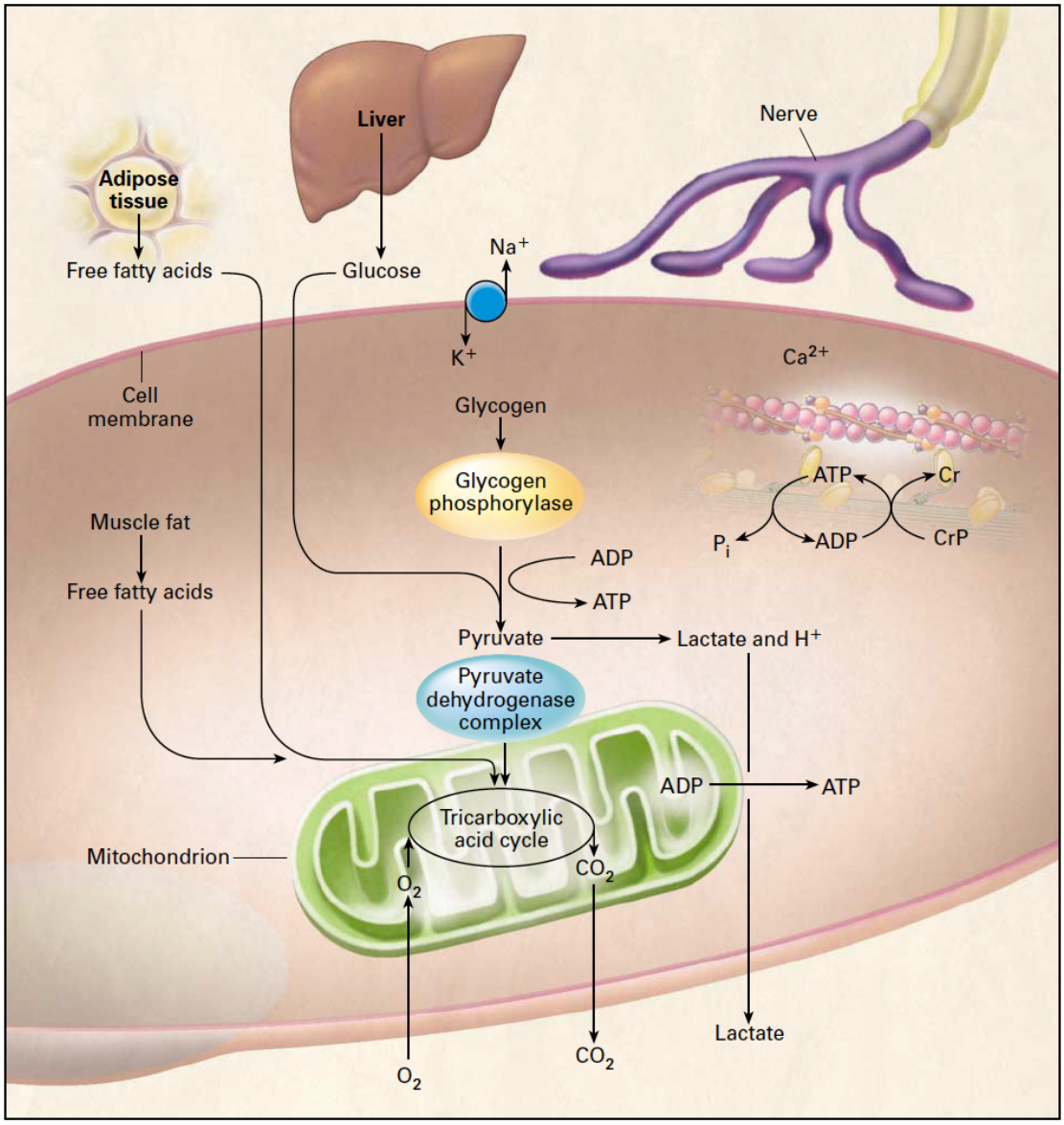

Figure 13. Major metabolic pathways during exercise. ADP denotes adenosine diphosphate, Cr creatine, CrP creatine phosphate, and P_i inorganic phosphate (5).

1.2.5 Circulation

Meeting the demands for delivery of O_2, glucose and fat free acids to active skeletal muscle, and clearance of carbon dioxide CO_2 and lactate requires an increase in microvascular blood flow. While the mechanisms that act to increase muscle blood flow during exercise are the result of complex integrated processes, the increase in oxygen delivery by the circulation is largely considered the primary event that enables exercise to be sustained. Poor oxygen delivery is associated with increased intramuscular lactate accumulation (5, 43-45), and premature exercise limitation.

During exercise lasting longer than a minute or two, the cardiac output (CO, where CO = SV x HR) increases both by increasing the volume of blood pumped from one ventricle of the heart with each beat (stoke volume; SV) and by increasing heart rate (HR). Each of these increase approximately linearly with the rate of oxygen utilized by the tissues for oxidative phosphorylation, i.e. muscle O_2 uptake (VO_2). During this adjustment, vasoconstriction occurs in non-active tissues (reduced hepatic, celiac, mesenteric and renal blood flow) and vasodilation in resistance vessels of the active muscles. This leads to an increase in the mean systemic arterial pressure and a large increase in blood flow to the muscles. Venous return is facilitated by the muscle pump, which in turn aids to increase cardiac output via the Frank-Starling effect (46-48).

Fick (49) recognized that virtually all of the heart's output passes through the lungs. Therefore, the law of conservation of mass can be applied to determine the relationship between CO and pulmonary VO_2: The product of the difference between the O_2 concentration of the blood entering and leaving the lung (the arteriovenous O_2 concentration difference, $C(a\text{-}v)O_2$) and the O_2 taken up from the alveoli, is directly proportional to the cardiac output (50):

$$CO = VO_2 / [C(a\text{-}v)O_2]$$

This relationship has a strongly predictable, approximately linear, pattern in response to upright cycle exercise in healthy humans (50-52):

$$CO = (5.7 \times VO_2) + 3.8 \text{ L/min}$$

The capacity to deliver O_2 to the periphery is therefore dependent on the maximum cardiac output and the O_2 carrying capacity of the blood. The latter is strongly dependent on the hemoglobin concentration [Hb] of the arterial blood:

$$C_aO_2 = ([Hb] \times 1.34 \times S_aO_2) + (0.003 \times P_aO_2)$$

Where C_aO_2 is the arterial O_2 concentration, 1.34 is a constant determined by to the mean oxygen binding capacity of hemoglobin, S_aO_2 is the percentage of the arterial hemoglobin that is bound to oxygen, and P_aO_2 is the arterial partial pressure of O_2. S_aO_2 and P_aO_2 are strongly dependent on effectiveness of pulmonary ventilation and perfusion: Ventilation to ensure that alveolar PO_2 is high (typically above 90 mHg) and perfusion that allows sufficient time for complete O_2 equilibration by diffusion between the alveolus and the pulmonary capillary.

At the start of exercise the pulmonary vascular bed dilates, in concert with the increase in the right ventricular output. This dilatation results in the perfusion of previously unperfused and underperfused regions of the lung in the normal pulmonary vascular bed, accounting for the fact that there is only a small increase in pulmonary artery pressure as pulmonary blood flow increases in the normal lung. A low pulmonary vascular resistance is essential for the normal exercise response of the left ventricle. Without it, the weakly-muscled right ventricle could not pump the venous blood through the pulmonary circulation to the left side of the heart at a fast enough rate to achieve the increase in cardiac output needed to support cellular respiration during exercise (48). In healthy humans these processes respond appropriately during exercise and the arterial blood remains essentially fully saturated (S_aO_2 >95 %) in all but the very fittest of people. Thus, oxygen delivery, the product of CO and C_aO_2, becomes highly dependent on CO because C_aO_2 varies little among healthy individuals and among exercise conditions.

The exercise increase in cardiac output is distributed preferentially towards active tissues. Despite this, the proportional increase in O_2 delivery is always less than of muscle VO_2, and thus the extraction of O_2 from (and addition of CO_2 to) the muscle capillary blood increases. Muscle blood flow increases in

rough proportion to its metabolic activity (53). Because of the falling capillary PO_2 and the Bohr effect (shifting the O_2 dissociation curve to the right, favoring O_2 unloading to the tissues), it is possible to extract up to ~85% of the O_2 delivered to the capillary bed of maximally working muscle. The oxygen supply to the muscle cells is dependent on five factors: cardiac output, distribution of perfusion to the tissue in need of O_2, partial pressure profile of O_2 in the capillary blood, hemoglobin concentration and hemoglobin's affinity for O_2 (48).

The transport of O_2 from blood to mitochondria is dependent on maintaining an adequate diffusion gradient for O_2 as the blood travels through the contracting muscle. The PO_2 gradient between blood and cells is high at the arterial end of the capillary but decreases as the blood approaches the venous end of the capillary. Thus, the ratio of the O_2 delivery ($CO \times C_aO_2$) to the O_2 consumption by the tissue (VO_2) will determine whether an adequate driving pressure for O_2 diffusion is maintained all the way down the muscle capillary. As muscle VO_2 increases during exercise, the ability of the circulation to maintain an adequate O_2 delivery rate is progressively challenged. In all humans, healthy or otherwise, mean capillary PO_2 reaches ~15-20 mm Hg at submaximal a power output. This PO_2 is associated with a sudden increase in lactate production (48), and the lowest metabolic rate that results in a progressive lactate accumulation is been termed the 'lactate threshold' (LT). Thus in pathological states that result in poor peripheral perfusion, such as heart failure or deconditioning associated with muscle capillary rarefaction, are associated with an early onset of metabolic acidosis in exercise. One adaptation of exercise training is to increase of muscle capillarity and mitochondria in skeletal muscle resulting in increased perfusion and greater ability to extract O_2 for oxidative phosphorylation (48).

1.2.6 Ventilation

Purpose of the lung is to "alveolarize the blood". This means that, in the ideal situation, all the blood leaving the lung would be exposed to an alveolar

capillary for a sufficient duration for the arterial blood gases (mainly O_2 and CO_2) to completely equilibrate with the partial pressures of the gases in the alveoli. The process of gas exchange between alveoli and capillary is dependent on diffusion. Thus, to maintain a high alveolar-capillary pressure difference for diffusion, the alveoli need to be ventilated regularly in order to refresh their gases with the atmospheric air. Other functions of the lung include the metabolism of some compounds, filtering of unwanted materials from the circulation, and acting as a reservoir for blood (47).

Ventilation is the movement of air between the environment and the lungs via inhalation and exhalation. The contraction of the respiratory muscles changes the dimension of the thoracic cavity, changing the pressure within, and consequently causing the air movement (inspiration and expiration). The most important respiratory muscles are the diaphragm, the external and the internal intercostal. The diaphragm is responsible for 45% of work done to draw air into the lungs during quiet breathing. When the diaphragm relaxes, gas is exhaled predominantly by elastic recoil of the lung and the tissues lining the thoracic cavity. During exercise both inhalation and exhalation become progressively more activate and accessory muscles of the abdomen and neck are recruited to generate the high pressures and gas flows required (54).

The total ventilation (V_E) is determined by the tidal volume of each breath (V_T) and the breathing frequency (fr) (47). Each tidal breath can be considered to contain:

i. A fraction that is not exposed to a pulmonary capillary and thus does not contribute to eliminating CO_2. This is termed the physiologic dead space volume (V_D), and consists of the volume of the conducting airways and alveolar spaces that are not perfused. At rest the pulmonary perfusion pressure is low, and therefore a large proportion of apical lung does is not perfused.
ii. A fraction that reaches perfused alveoli and thus is able to contributes to gas exchange. This is termed the alveolar volume.

The precise matching of alveolar ventilation to metabolic rate during exercise is achieved by increasing V_E. This increase is accomplished by increases in both V_T and fr. The increased V_T slightly increases airway dead space, due to tethering effects of the lung parenchyma on airway lumen size. However, the relative tidal volume increase exceeds this effect. The increase in V_T is achieved by reducing the end-expiratory lung volume (EELV) below the functional residual capacity (achieved by activating expiratory muscles) and increasing the end-inspiratory lung volume (EILV) (55). At lower exercise intensities, increases in ventilation are mostly achieved through tidal volume changes, rather than just increasing breathing frequency, which would increase dead space ventilation and compromise effective alveolar ventilation. To minimize the work of breathing during heavier exercise, tidal volume increases only to ~70% of the vital capacity (56), the lung compliance decreases markedly and the respiratory pressure production required for a given change in volume is very large, leading to exaggerated respiratory discomfort (i.e., dyspnea) (57).

The efficiency of the ventilation can be expressed in terms of the proportion of the tidal breath that continues to gas exchange, or the V_D/V_T ratio. Because the V_D contains no CO_2 (atmospheric air can be considered to be free of CO_2 and there is no gas exchange in the dead space, and thus CO_2 is not added), the V_D/V_T can be determined by the measuring the difference between partial pressures of the CO_2 in the mixed expired gas and the arterial blood. In a highly-efficient lung in a healthy participant, V_D/V_T is low at rest (~0.35) and falls after exercise onset (becomes more efficient) and either plateaus or continues to fall up to a values of ~0.20. During moderate intensity exercise, both tidal volume and breathing frequency increase roughly in proportion to exercise intensity, whereas at higher intensities, tidal volume reaches a plateau and further increases in ventilation are accomplished by increases in breathing frequency alone (58). At high exercise intensities, hyperventilation occurs to counteract the metabolic acidosis. This is achieved by an increase in breathing frequency, by reducing both the inspiratory (T_I) and expiratory times (T_E). However, the ratio of T_I to total breathing cycle duration (T_{TOT}), the duty cycle

(T_I/T_{TOT}), increases only slightly during exercise (~0.40 at rest to ~0.50 during high-intensity exercise).

In a poorly efficient lung, such as in a patient with emphysema or with pulmonary hypertension, the V_D/V_T ratio is high at rest and may remain high or even increase during exercise (59, 60). In addition, circulatory derangements in heart failure also reduce ventilator efficiency at rest and during exercise (61). Low ventilatory efficiency greatly increases the demands for ventilation and respiratory muscle work during exercise, as well as their associated sensation, breathlessness. Breathlessness is a major symptom of exercise that can induce exercise limitation.

Thus, O_2 and CO_2 move between alveolar gas and blood by simple diffusion. Fick's law of diffusion states that the rate of transfer of a gas through a sheet of tissue is proportional to the surface area and the difference in gas partial pressure between the two sides, and inversely proportional to the tissue thickness. The area of the blood-gas barrier in the lung is enormous (50 to 100 m^2), and the thickness is only 0.3 μm in many places, so the dimensions of the barrier are ideal for diffusion. In addition, the rate of transfer is proportional to a diffusion constant, which depends on the properties of the tissue and the particular gas. The constant is proportional to the solubility of the gas and inversely proportional to the square root of the molecular weight. This means that CO_2 diffuses about 20 times more rapidly than does O_2 through tissue sheets because it has a much higher solubility but not a very different molecular weight (47).

By the time the O_2 reaches the alveoli, the PO_2 falls to about one-third. This is because the PO_2 of alveolar gas is determined by a balance between two processes: the removal of O_2 by pulmonary capillary blood on the one hand and its continual replenishment by alveolar ventilation on the other. The rate of removal O_2 from the lung is governed by the O_2 consumption of the tissues and varies little under resting conditions. In practice, therefore, the alveolar PO_2 is largely determined by the level of alveolar ventilation. The same applies to the

alveolar PCO_2 (47). At rest, and during much of exercise, the arterial PCO_2 is tightly regulated, to ~40 mm Hg. This means that there is a very strong linear relationship between the rate of CO_2 exchange (VCO_2) and alveolar ventilation (and, when accounting for V_D/V_T, V_E) (62). Thus in health, the normal response from rest to moderate exercise is an increase in ventilation that is commensurate with CO_2 production (termed exercise hyperpnea).

Through much of the rage of work rates spanning the aerobic capacity, V_E is strongly coupled to VCO_2. During sustained exercise above LT, the associated metabolic acidosis stimulates V_E (via H^+ stimulation of the carotid body) and results in a compensatory hyperventilation. Here, P_aCO_2 typically falls to 30-35 mmHg at peak exercise. A peak P_aCO_2 of 35-38 mmHg indicates a less effective alveolar hyperventilatory compensation for the metabolic acidosis, while an absent hyperventilation is indicated by a P_aCO_2 in excess of 38 mmHg at peak exercise (63). Thus, $PaCO_2$ values obtained with incremental exercise allow for the determination of the adequacy or appropriateness of ventilation during exercise.

The role of this hyperventilation is to reduce alveolar PCO_2, increase VCO_2 and reduce the metabolic acidosis via the Henderson-Hasselbalch relationship:

$$[H^+] \times [HCO_3^-] \text{ <---> } [H_2CO_3] \text{ <---> } [CO_2] \times [H_2O]$$

In this relationship reducing $[CO_2]$ from the arterial blood acts to reduce $[H^+]$ (increase pH) in attempt to maintain blood-acid bases status at close to resting values. This comes at the expense of increased ventilatory requirements, increased V_D/V_T, and an enhanced sensation of dyspnea.

In healthy individuals, the maximal aerobic capacity is reached prior to reaching the maximum capacity for pulmonary ventilation (termed maximum voluntary ventilation, MVV) (64, 65). MVV is difficult to measure with accuracy and therefore it is often predicted based on forced expiratory volume in 1 second (FEV_1) multiplied by 35 or 40 (65, 66). The difference between V_E at VO_{2max} and MVV, or breathing reserve (BR), is large in young subjects but is reduced in trained individuals (where V_E at VO_{2max} is greater) on in subjects with lung disease (where MVV is reduced). Endurance exercise training reduces the

demands for ventilation predominantly via peripheral muscle adaptations (muscle capillarity and mitochondrial volume) that reduce the metabolic acidosis for any given work rate; thereby reducing the V_E necessary for ventilatory compensation. Thus, the breathing reserve depends on two main factors: ventilatory demand and ventilatory capacity. Ventilatory demand is dependent on metabolic demand, body weight, mode of testing, dead space ventilation, as well as neuroregulatory and behavioral factors. Ventilatory capacity is affected by mechanical factors such as airflow limitation and operating lung volumes, ventilatory muscle function, genetic endowment, aging, and disease. Ventilatory capacity can also be affected by bronchoconstriction or bronchodilation (65). In addition, a decline in maximal ventilation with time may represent inspiratory-muscle fatigue, with an accompanying intense respiratory distress, which could limit exercise (67).

While differences in ventilatory mechanics play an important role in determining the work of breathing and dyspnegenesis (and thus exercise limitation) amongst different individuals, as a general rule ventilatory capacity is sufficient to supply the requirements for gas exchange during exercise in young, moderately active, healthy individuals. To evaluate the degree of ventilatory constraint during exercise, the degree of expiratory flow limitation (EFL) can be examined by plotting the exercise flow-volume loop relative to the maximal flow (64). This relationship can provide information about the degree of expiratory flow limitation, operating lung volumes, as well as breathing strategies used in response to exercise. The degree of EFL during exercise has been previously expressed as a percent of V_T that meets or exceeds the expiratory boundary (65, 68, 69). The presence of EFL promotes dynamic hyperinflation and intrinsic positive end-expiratory pressure with increased work of breathing, functional impairment of inspiratory muscle strength, increased sensations of dyspnea, and adverse effects on hemodynamics (70, 71). When the degree of expiratory flow limitation becomes significant (>40–50 % V_T), EELV typically increases (65, 68, 69, 72).

With EFL, expiratory flow rates are independent of expiratory muscle effort and are determined by the static lung recoil pressure and the resistance of the

airways upstream from the flow-limited segment (73-75). In flow-limited patients, the mechanical time constant for lung emptying is increased in many alveolar units, but the expiratory time available is often insufficient to allow EELV to return to its original values, resulting in gas accumulation and retention (i.e., gas trapping). The increased CO_2 production with exercise necessitates an increase in alveolar ventilation by increasing V_T and breathing frequency to maintain P_aCO_2. However, the increased V_T in combination with diminished expiratory time due to increased breathing frequency can cause dynamic hyperinflation (DH) in patients with EFL (73). Thus, the main consequence of expiratory flow limitation during exercise is the development of DH (73, 76). DH in early exercise may be a compensatory mechanism to increase V_E with limited (or minimal) respiratory discomfort (77); however, with increasing exercise a threshold is reached (around an inspiratory reserve volume of 0.5L, or within 10% of total lung capacity), where V_T plateaus. At this point the breathing occurs at the least compliant portion of the respiratory system's pressure volume curve; the diaphragm muscle fibers are maximally shortened, and dyspnea develops at an extremely accelerated rate because of the disparity between the inspiratory effort and tidal volume response (73, 77). Recent work has shown that below this V_T inflection (or plateau), dyspnea increases linearly with workload; however once the inspiratory capacity (IC) drops below a critical value, dyspnea increases abruptly and becomes the most frequently selected reason for exercise termination regardless of exercise protocol (78).

1.2.7 Exercise intensity

The maximal rate at which the body can take in oxygen is termed maximum oxygen uptake (VO_{2max}) or aerobic capacity. Because VO_{2max} is determined by the product of the maximum CO and maximum $C(a\text{-}v)O_2$ difference it represents the intersection of maximum oxygen delivery to, and oxygen utilization in, peripheral tissues. Thus, it is evident that the factors that influence VO_{2max} during exercise include cardiac function, the oxygen carrying capacity

and oxygenation in the arterial blood, and the ability of the muscles to extract oxygen (58).

In healthy subjects, of the variables involved in oxygen delivery, it is the limitations of the cardiovascular system that are most responsible for limiting VO_{2max} (79). Ventilation and gas exchange are usually sufficient to maintain P_aO_2 and S_aO_2 up to maximal work rate (80). Numerous studies have shown that VO_{2max} can be increased through exercise training (81, 82). The major adaptions that facilitate this response are both increased peripheral O_2 extraction (83) and an increase in cardiac output secondary to an augmented stroke volume response to exercise (84). Indeed, many studies have shown positive cardiac adaptation with exercise training (85-89). The increased stroke volume response with exercise results in a reduced submaximal heart rate with exercise training; however, peak heart rate is generally unaffected, or slightly reduced, in trained individuals (84). As VO_2 increases with incremental exercise, the variables in the Fick equation will eventually reach their upper limits, and as a result, a plateau of the VO_2 will occur. The plateau in oxygen consumption despite an increase in workload is defined as VO_{2max}. However, many subjects, particularly clinical patients, do not demonstrate this plateau in VO_2 (90), for a variety of reasons which may include intolerable symptoms of breathing discomfort (dyspnea), muscular fatigue, chest pain, and so forth (90, 91). If a plateau is not seen, then the highest VO_2 achieved, termed the VO_{2peak}, is used as an estimate of VO_{2max} (90, 92).

However, during daily activities, individual rarely achieve or sustain VO_{2max}. Therefore, while aerobic capacity sets with upper limit for the body's aerobic energy transfer, individually usually reside within sub-maximal range of the aerobic capacity. For this reason exercise can be usefully grouped into domains characterized by common physiological strain. The exercise domain schema proposed by Whipp (93) considers a term for each exercise intensity (moderate, heavy, very-heavy and severe) that align closely with the sensory perceptions of effort (94), and are defined by the metabolic responses to exercise (Figure 14).

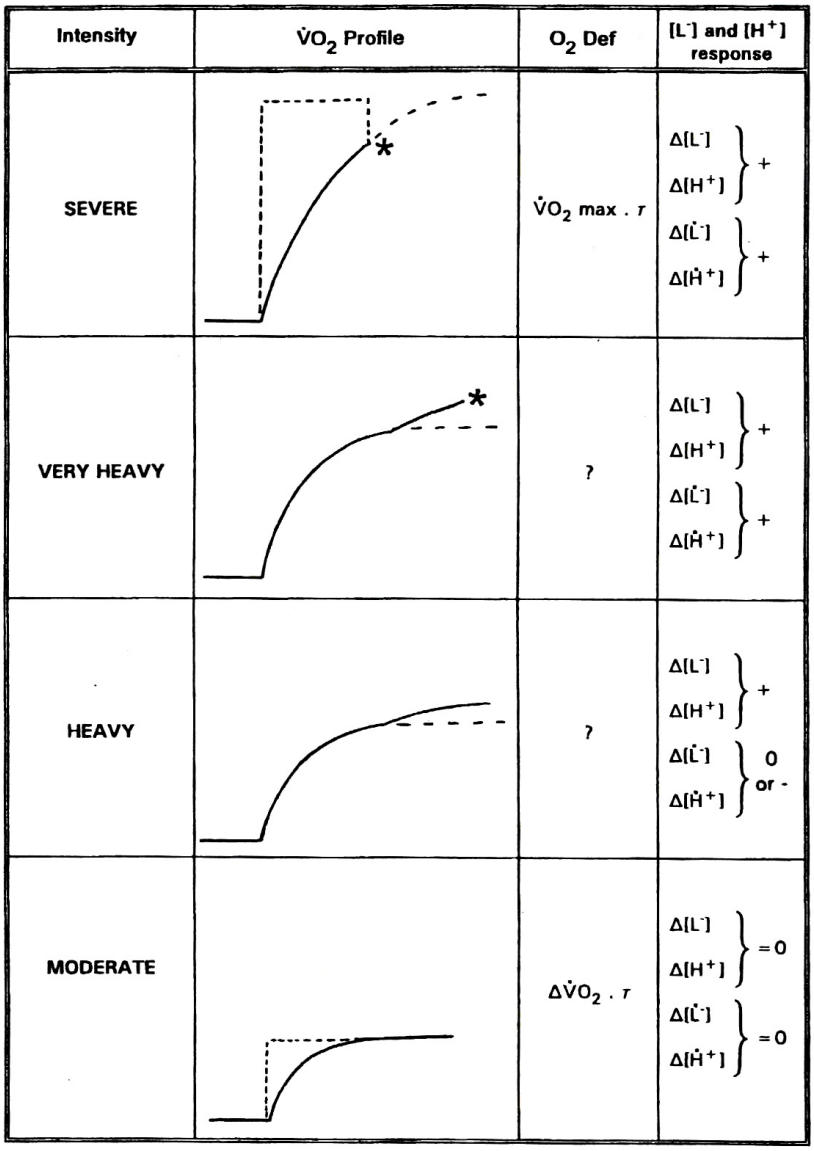

Figure 14. Schematic representation of the temporal response of O_2 uptake VO_2 to constant-load exercise at different work intensities. *Moderate*: below lactate threshold (LT); there are no sustained increases in [lactate] or $[H^+]$ (i.e. both the absolute increments above baseline, $\Delta[L^-]$ and $\Delta[H^+]$, and their rates of change, $\Delta[\dot{L}^-]$ and $\Delta[\dot{H}^+]$, equal zero. *Heavy*: above LT, with VO_2 reaching a steady state but with a delayed time course; there are now sustained increases in [lactate] or $[H^+]$ (i.e. $\Delta[L^-]$ and $\Delta[H^+]$ are positive) but $\Delta[\dot{L}^-]$ and $\Delta[\dot{H}^+]$ eventually decline back to zero or even become negative. *Very Heavy*: above LT, but with a component of "excess" VO_2 to attain the VO_{2max}; note that there are sustained increases in both $[L^-]$ and $[H^+]$ and $\Delta[\dot{L}^-]$ and $\Delta[\dot{H}^+]$. *Severe*: a supra-maximal work rate where intolerance occurs so rapidly that the excess VO_2 components has not had time to develop discernibly; again, $\Delta[L^-]$, $\Delta[H^+]$, $\Delta[\dot{L}^-]$ and $\Delta[\dot{H}^+]$ are positive. O_2Def represents the calculable O_2 deficit, depicted by the area enclosed by short dashes; * indicates VO_{2max} (93).

The moderate intensity exercise domain encompasses the power output (P) range for which steady state in VO_2 is attained without a sustained metabolic acidosis, i.e. <LT (93, 95). In the heavy intensity domain, i.e. power outputs between LT and critical power (CP; see below), achievement of a VO_2 steady state is delayed by up to ~20 min. The corresponding increases in arterial [lactate] and [H^+] eventually stabilize, but are accompanied by a reduced work efficiency relative to moderate exercise (96, 97). In the very-heavy intensity domain (i.e. >CP), VO_2, arterial [lactate] and [H^+], muscle acidity, [inorganic phosphate] and phosphocreatine breakdown each continue progressively, driving VO_2 up to with VO_{2max}; once VO_{2max} is attained exercise intolerance occurs shortly thereafter (98, 99). Power outputs for which the initial whole-body ATP turnover rate exceeds that at VO_{2max} are classified as severe intensity (100) in the Whipp schema (93).

1.2.7.1 Moderate intensity

Moderate exercise spans the range of work rates from rest to LT (Figure 14, *moderate*). In this domain there is little or no sustained metabolic acidemia or increase in arterial blood or muscle lactate concentration. The rate with which metabolism increases at the onset of a new power output, reflected in the VO_2 kinetics, may be is considered to be the fundamental response in the moderate intensity domain. Thus the moderate intensity VO_2 response is a useful for reference the altered profiles that occurs at higher intensities (93).

The steady state increment in VO_2 equals that of the mean rate of muscle O_2 utilization, however this is not the case for the non-steady state. It is important to recognize that muscle VO_2 is not capable of increasing to its new steady state immediately following exercise onset. Rather it responds with an approximately exponential time course. The time constant (τ) of this response reflects intracellular VO_2 control by the turnover dynamics of the intramuscular high-energy phosphate pool (101-103). The expression of this muscle VO_2 response profile at the lung, however, is delayed as a result of the vascular

transit delay between the exercising muscles and the pulmonary capillaries (104). While the VO_2 response kinetics do not immediately reach a steady-state, the muscle efficiency is thought to be constant. Thus, the ATP demands of the exercise that are not supplied by oxidative phosphorylation (i.e., VO_2) must be provided by alternative sources; phosphocreatine or lactate production. The magnitude of this substrate level phosphorylation has an O_2 equivalent, termed the O_2 deficit (O_2Def).

The oxygen deficit is calculated from the product of the exercise-induced VO_2 increment (i.e., the steady-state of VO_2 - ΔVO_{2ss}) and the τ (105) or "mean response time" (106) of the VO_2 response. The ΔVO_{2ss} for a give work rate does not vary appreciably amongst individuals differing in gender, age, state of healthy or state of training: it is ~ 10 (±1) ml/min/W. As such, differences in τ amongst these populations determine the magnitude of the O_2Def to a given work rate. Because the capacity for phosphocreatine breakdown is limited, as is the tolerance for accumulation of a metabolic acidosis, the kinetics of the VO_2 response determining the O_2Def are very strongly related to exercise tolerance (107).

The τ does not vary appreciably between work rates of different amplitudes in this intensity domain (105, 108-110), and the early transient rise in blood lactate (111), which is not uncommon at these work rates, does not seem to influence the response discernibly. Furthermore, the off-transient VO_2 time constant is not appreciably different from that at the on-transient (109, 112), despite lactate having typically returned to resting levels before the start of the recovery phase.

1.2.7.2 Heavy intensity

In the heavy intensity domain, above LT, the VO_2 response becomes considerably more complex (96, 106, 113, 114), with both time-and amplitude-based nonlinearities of response. The ΔVO_{2ss} increases as a linear function of

work rate (W) with a slope of ~10 ml/min/W for exercise below LT. However, at work rates above LT, a steady state of VO_2 is delayed or unattainable. When VO_2 does stabilize, its asymptotic value is markedly increased; the increase being a function of both the supra-threshold work rate and time. Values of 12-14 ml/min/W are not uncommon during tests of 10-15 min duration (96, 115, 116) (Figure 14, *heavy*).

Whipp & Mahler (23) showed the difference between the “expected” steady-state value (i.e., projected from the sub-LT VO_2-W relationship) and the actual achieved. This difference is positive for the range of work rates above LT at which the steady-state projection is less than the subject’s VO_{2max}. This additional increment in VO_2 has been termed "excess" VO_2 (VO_{2xs}). This VO_{2xs} is the result of a “slow component” (thus, it is also termed the VO_{2sc}) adding to the fundamental VO_2 kinetics seen during moderate intensity exercise. Moreover, this superimposed component is of delayed onset, beginning some minutes into the test (106, 110, 113, 114). Both Paterson & Whipp (114) and Molé (2) demonstrated that not only was this slow component of delayed onset but the early component of the kinetics remains exponential and projects to a steady-state that gives the same gain (i.e. ΔVO_{2ss}/W) for sub-LT exercise.

The presence of the VO_{2sc} component during high-intensity exercise appears therefore to undermine three fundamental assumptions of models of human pulmonary gas exchange. First, the gain term is not constant; that is ΔVO_2 is not a linear function of the work rate (117). Secondly, the power-duration curve being attributable to a single aerobic term (having a rapid τ of only 10-20 s) is not justified (118). And, finally, the assumptions inherent in the conventional means of computing the O_2 deficit under these conditions are not valid. Thus, for any work rates above the LT the O_2Def cannot be known without measuring the muscle phosphocreatine and lactate production rates directly. Nevertheless, the supplementary and delayed VO_{2sc}, to the exponential asymptotic VO_2 that is predicted by sub-LT exercise (114), may prove important in elucidating the mechanism of VO_{2sc}. For example, it seems to rule out a significant influence of either the O_2 cost of cardiac and respiratory work or of the temperature.

1.2.7.3 Very heavy intensity

There is a range of supra-LT work rates in which a delayed steady state may eventually be reached (96, 98, 116, 119), but a higher works rates still, VO_2 arterial [lactate] and $[H^+]$ continue to increase as long as exercise is continued until the VO_{2peak} or VO_{2max} is attained (96, 98, 119) (Figure 14, *very heavy*). The work rate that demarks the heavy and very heavy intensity domains correlates closely with the asymptote of subject's power-duration curve (98) (Figure 15).

1.2.7.3.1 The power-duration relationship

Above this CP output, the more rapidly the VO_{2sc} projects VO_2 towards its maximum, the shorter will be the tolerable duration of the exercise. Above CP, therefore, it is not possible for a subject to perform a constant power exercise task that provides a particular percentage of the VO_{2max}. Any $\%VO_{2max}$ can only be attained fleetingly, as VO_2 continues to rise. The highest work rate at which a steady state of VO_2 can be attained (and hence a sustainable $\%VO_{2max}$) appears to coincide with the highest work rate at which blood [lactate] and $[H^+]$ and VO_2 do not continue to rise throughout the test (96, 98, 120) (Figures 14 and 15).

The hyperbolic power-duration ($P\text{-}t_{lim}$) relationship was first described by Monod and Scherrer for a single muscle group in 1965, and has since been extended to whole-body exercise (98). This model characterizes a CP output that, once exceeded, will lead to exhaustion in a duration predicted by the completion of a constant amount of work (W') (121):

$$W' = (P - CP) \times t_{lim}$$

The robust nature of the $P\text{-}t_{lim}$ relationship is demonstrated in its ability to characterize exercise tolerance for a wide range of exercise modalities, including cycling, running, swimming, kayaking, rowing and knee-extension

over durations of ~2-30 min (121), for different subject populations, ranging from adolescents to the elderly, and from elite athletes to patients with chronic heart or lung diseases, and for different species like humans, lungless salamanders, ghost crabs, mice, horses and also rats (122).

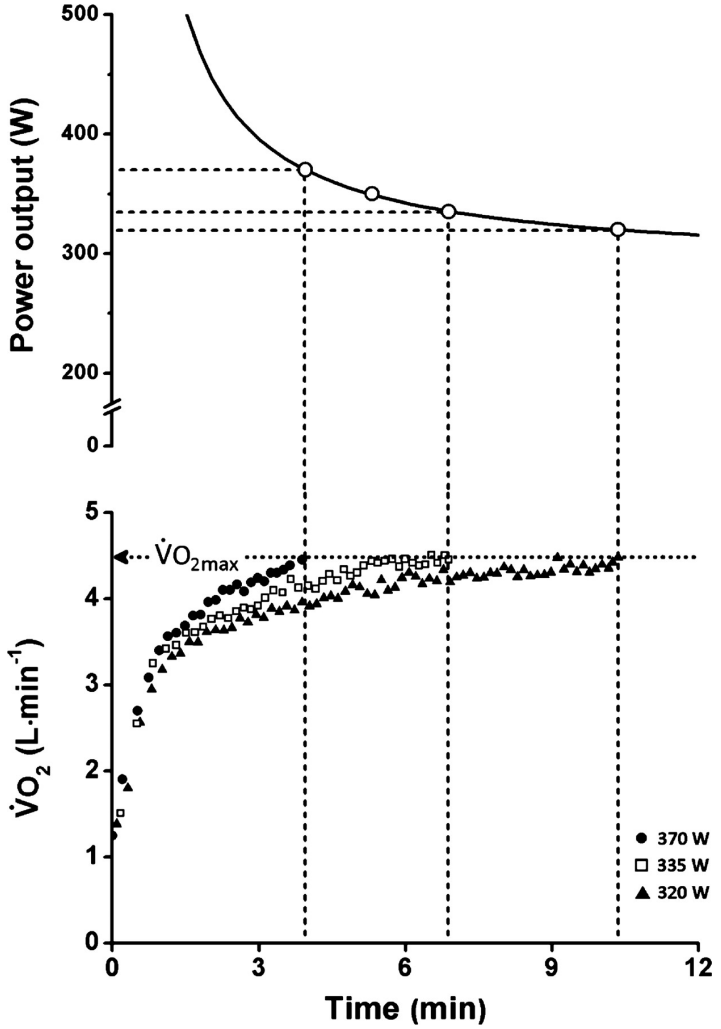

Figure 15. The hyperbolic relationship between power output and time (*top*) in a typical subject, established from four different constant-power (P) tests (○) each performed to the limit of tolerance. VO_2 responses for the constant-P tests (*bottom*) illustrate that intolerance was achieved at VO_{2max} in each case (examples from only 3 tests are shown for clarity), with the combined fundamental and slow component VO_2 kinetics determine the time to achieve VO_{2max} (107).

CP, which lies between the LT and VO_{2max} (on average CP resides at ~50% of the difference between LT and VO_{2max}, but this percentage is high variable

among individual subjects), reflects an intrinsic threshold of aerobic energy provision that defines the greatest constant power output for which steady states in ventilation, gas exchange (e.g., VO_2, VCO_2) and metabolic (e.g., muscle and blood acid-base status) variables can be achieved (98). That CP reflects a parameter of aerobic function is supported by the fact that it is sensitive to interventions affecting oxygen transport and utilization, such as breathing hypoxic gas mixtures or endurance exercise training. In addition, while CP is typically measured as an external power output, it actually reflects an intrinsic metabolic rate, that once exceeded puts into effect a cascade of event that precipitate exercise intolerance (107, 123). Intolerance occurs once an amount of work (reflected in the mathematical constant, W') is completed. Because of this, the W' has been likened to a (predominantly) anaerobic energy source (124), potentially comprising stores of intramuscular glycogen, high-energy phosphates and oxygen, consequently, W' has also been considered to be equivalent to the O_2Def (125) or the subject's "anaerobic work capacity" (126-129).

Thus, the metabolic demands above CP necessitate continued energetic contributions from O_2 deficit-related mechanisms (i.e., muscle PCr, stored O_2, and glycolysis/glycogenolysis with associated lactate and H^+ production), resulting in the accumulation of metabolites such as intramuscular Pi, H^+, and interstitial K^+; each of which has been implicated in skeletal muscle fatigue (130). As such, CP appears to represent a rate of aerobic metabolism that, once exceeded, leads to the progressive depletion of stored energy resources and accumulation of associated metabolites (98, 99, 131) (132); the critical limits of which determine W' and exercise tolerance. Notionally, W' may be utilized rapidly by exercising at high power outputs, or may be sustained for longer durations by exercising at lower work rates (133).

In either case exercise above CP increases the O_2 cost (and ATP cost) of exercise, setting VO_2 on a trajectory towards its maximum (98, 100). Thus, as reflected in the VO_{2sc}, exercise above CP is necessarily accompanies by a progressive work inefficiency (increasing metabolic requirement for energy

production for a given constant power output). The mechanisms determining the VO_{2sc} itself remain controversial but, since the majority of the VO_{2sc} has been shown to derive from the active locomotor muscles, rather than e.g. additional cardiac or respiratory muscle work, increased temperature or energy of thermoregulation, it has been proposed to be the result of a cascade of events related to muscle fatigue (134, 135):

i. Supra-CP exercise causes a large breakdown of muscle PCr, muscle glycogen and utilization of stored O_2 (99, 132, 136).
ii. This results in the accumulation of metabolites associated with muscle fatigue, such as Pi, H^+ and ADP (99, 132, 136)
iii. The high energy demands of the exercise reduce the free energy of ATP breakdown, causing derangements in handling of muscle ions, such as slowed Ca^{2+} re-uptake to and release from the sarcoplasmic reticulum, accumulation of extracellular K^+ and intracellular Na^+, each of which contribute to muscle fatigue: a reduction in the force or power production for a given muscle stimulation.
iv. This muscle fatigue causes an increase in the ATP requirements for a given power output via a range of putative mechanisms including additional recruitment of type II, poorly efficient, muscle fibers to maintain power output (137-140), or direct influence of fatigue on cellular efficiency (141, 142).
v. Increased ATP requirements demand further breakdown of PCr and glycogen to lactate and increased stimulation of oxidative phosphorylation, increasing VO_2.

Since the accumulation of fatigue-inducing metabolites may therefore be causal in the progressive reduction in muscle efficiency, it has been proposed that W' should not be considered as a *depletion* of muscular energy stores, but rather an *accumulation* of fatigue related metabolites that drive the symptoms associated in exercise limitation to some maximal level (131, 135). In accordance with this notion, small muscle mass exercise (handgrip or single leg exercise) in healthy participants is associated with near-complete depletion of energy stores within the active muscle, reflecting the fact that in this exercise

mode the source of the exercise limitation is likely at the level of the ability of the muscle to produce power at the required rate (143). Alternatively, during whole body exercise the major source of the exercise limitation may vary depending on the environmental conditions or state of the health of the subjects. For example, during exercise at 5,500m altitude in unacclimatized participants both CP and W' are reduced (144). Here the reducing in CP is expected because of the effects of altitude on O_2 delivery and utilization. The reduction is W', however, appears somewhat of a paradox in this situation because, were W' to be dependent on intramuscular energy stores becoming depleted, then the W' value should not change. That is it reduced by ~50% reflects the fact that at altitude the locus of the exercise limitation is likely shifted towards central factors in the circulation and ventilation. The limiting symptoms of whole-body exercise at altitude therefore are likely reached before complete depletion of muscular PCr for example.

This phenomenon is clearly illustrated in patients with chronic obstructive pulmonary disease (COPD) (145). COPD patients manifest reduced elastic lung recoil and obstruction of forced expiratory flow. Thus, during exercise it is common for these patients to experience expiratory flow limitation and dynamic hyperinflation, which above a certain level, dramatically drives the sensations of dyspnea. COPD patients have a greatly reduced W': ~25-50% of the value for healthy young or age-matched controls (146). The predominant mechanism limiting exercise tolerance in severe COPD patients is thus, thought to be reaching the limits of MVV. Unlike in healthy participants, in COPD patients, the attainment of VO_{2peak} does not coincide with complete depletion of intramuscular energy stores, rather it is thought that there is an intramuscular energy reserve remaining at the tolerable limit of walking or cycling (although this is yet to be proven in whole-body exercise). Thus in these patients, W' does not represent a depletion of an energy stores (while COPD patients do have small, weak leg muscles, and therefore smaller volumes of stored energy, these muscles are not 25-50% smaller than controls). Instead Neder et al. (145) demonstrated that W' in severe COPD is dependent on the attainment of MVV, whereas in controls is was dependent on reaching VO_{2max} (Figure 16).

This suggests that the W' is only partly related to muscle metabolism, most likely the role of muscle metabolism in driving the ventilation to is capacity, and that W' is low in these patients because of accumulation of metabolic by-products of the exercise (CO_2, H^+) rather than depletion of sources of stored energy (PCr, glycogen, O_2).

Together these findings emphasize that exercise tolerance in the very heavy intensity domain is dependent on the rate within which limiting symptoms are attained, and while these symptoms are likely to be strongly linked to the induction of muscle fatigue, they may not be dependent on depletion of energy stores or muscle fatigue per se. As such, increasing CP (for example by aerobic exercise training) and increasing W' (for example by strength training to increase muscle mass or by endurance training to reduce metabolic acidosis that increases ventilatory demands) are viable therapeutic targets in patient populations where exercise intolerance is a primary symptom.

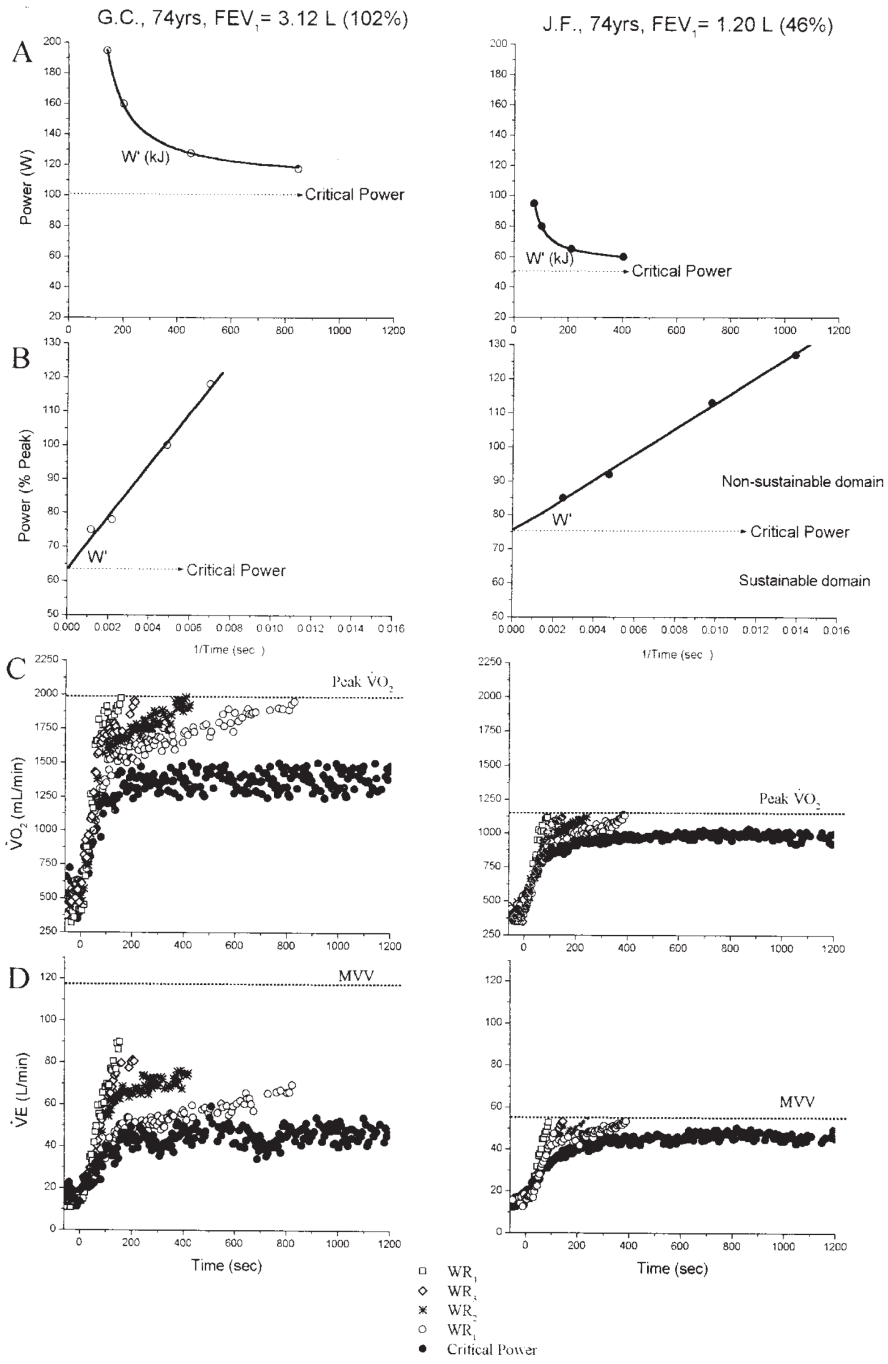

Figure 16. The P–t_{lim} relationship in response to four progressively intense (WR1 to WR4) exercise tests in a healthy control (left panels) and a patient with COPD (right panels), matched by age. (A) A hyperbolic relationship was found in both subjects. (B) The linearized P-t_{lim}^{-1} response as a function of percentage peak work rate. (C) Oxygen uptake (VO_2) at t_{lim} in each power output did not differ from the VO_2 at the maximum ramp-incremental test (peak) in both subjects. (D) Patients' ventilation (V_E) at t_{lim} was not significantly different from the maximum voluntary ventilation (MVV), whereas V_E was an inverse function of t_{lim} in the control subject. Note that a 20 min test at the subject's critical power (solid circles, panels C and D) was successfully sustained at relatively higher metabolic and ventilatory stress in the COPD patient (145).

1.2.7.4 Severe intensity

Severe intensity exercise is defined by Whipp (120) as power outputs that from exercise onset demand a rate of ATP production that exceeds VO_{2max}. Thus, the main characteristic of severe-intensity domain exercise is that the achievement of VO_{2max} occurs without the supplementary action of the VO_{2sc} (147) (Figure 14, *severe*). In this domain maximum rate of pulmonary O_2 exchange is achieved only a few minutes after exercise onset [~2-4 min in healthy young active subjects (148)] and is manifest with continual increases in blood [lactate] and $[H^+]$ throughout the exercise. Under these conditions, the O_2Def becomes independent of the work rate, and has therefore been termed the "maximal accumulated oxygen deficit" (MOAD). It can be calculated as the product of $\%VO_{2max}$ and τ (120). Severe intensity exercise is, by definition, limited to short durations (typically less than 3-4 minutes), and defines the upper limit for endurance exercise.

Thus, LT, CP and VO_{2max} emerge as physiologically justifiable criteria for establishing relative exercise intensity. Power outputs below LT may be characterized as *moderate*; those between LT and CP, as *heavy*, those above CP but below VO_{2max} as *very heavy*, and those above VO_{2max} as *severe* (Figure 14). Importantly, the pattern of physiological response in a given intensity domain is similar in subjects of different fitness (regardless of the particular range of power output), and exercise tolerance within each of these domains is expected to be limited by different but distinct physiological events or processes. For example, in normal individuals LT occurs at approximately 50% of VO_{2max}, but the distribution is very large, with the normal range extending from 35% to at least 80%. Consequently, if the exercise intensity is assigned to a particular percentage of VO_{2max}, then one subject could be exercising at a sub-LT power and be "comfortable" whereas for another it could be greater than CP causing that subject to become limited within a few minutes. Thus, it is important to recognize that subjects exercising at the same $\%VO_{2max}$ or metabolic equivalent of task (MET) level, can meet the energy demands of the task with markedly different metabolic responses, and thus these intensity

“normalization” methods do not stand up to scrutiny.

Investigation of the limiting factors of exercise, therefore, should account for these differences in intensity domain ranges among individuals, with exercise normalization, or prescription, being better based on the physiologic intensity domains described above (120).

1.3 Fatigue and exercise intolerance in whole-body exercise

1.3.1 Definition of fatigue

The term fatigue has many definitions. It can be variously interpreted as malaise, exhaustion, tiredness, muscle pain, loss of muscle force or power, or an increased muscular effort. Thus it is vital to define an appropriate lexicon of terms when discussing the role of ‘fatigue’ in exercise. When applied to muscular exercise, the term fatigue can refer to “failure to maintain the required or expected force or power” (149) or “failure in the ability to continue working at a given exercise intensity” (150). Using this view, fatigue would occur suddenly, hence, the phrases to reach “the point” of fatigue or “at exhaustion”.

Although the absurdity of this position is obvious, it would follow that fatigue in muscles (and in the CNS supporting muscle activity) begins only at the point of task failure when a subject exercises at a set rate to “exhaustion.” In fact, the maximal force-generating capacity of muscles starts to decline almost immediately once exercise commences, so that fatigue really begins almost at the onset of the exercise and develops progressively before the integrated neuromuscular system fails to perform the required task. Hence, a more realistic definition of fatigue is “any exercise-induced reduction in the ability to exert muscle force or power, regardless of whether or not the task can be sustained” (151). Thus “fatigue” is distinct from the terms “task failure” or “exercise intolerance” (the latter being essentially synonymous, but with task failure better describing the response to isolated muscle tasks whereas

exercise intolerance better describes the response to whole body exercise). During exercise, fatigue is a progressive phenomenon whereas exercise intolerance describes a single ‘point state’ of limitation.

Because of the potential clinical significance of fatigue of respiratory muscles, a meeting of physicians formally defined muscle fatigue as “a loss in the capacity for developing force and/or velocity of a muscle, resulting from muscle activity under load and which is reversible by rest” (152). Finally, this should be distinguished from usage of fatigue that refers not only to a physiological or pathological state in which muscles perform below their expected maximum, but to a symptom reported by subjects in whom there may be no obvious defect in muscle performance. Indeed, it is the most common symptom in medical and psychiatric practice (153).

Table 2 presents some key terms to understand the fatigue process (154). Because peripheral force-generating capacity usually declines early in exercise, and because CNS changes occur before muscles fail to perform a task, the most useful definition of muscle fatigue must encompass, as given above, “any exercise induced reduction in force or power generating capacity, for a given degree of stimulation.” Rest reverses it. This definition ignores competing intramuscular mechanisms that potentiate force during “fatiguing” exercise (155-158) and focuses on the net reduction in performance that ultimately develops.

With this construct, “voluntary activation” refers to a notional level of “drive” to muscle fibers and motoneurons. This term is used loosely, often without distinction between drive to the motoneuron pool and that to the muscle. These are not the same: the former recruits motoneurons and increases their firing, and the other relies on muscle fibers to translate the motoneuron firing into force. As applied to motoneurons, the term voluntary activation is not accurate because it does not specify the source of their excitation (from descending motor paths, reflex inputs, and from associated spinal circuitry). The “maximal evocable force” is that produced when the muscle is fully activated by volition or appropriate electrical stimulation and is the formal term for true maximal

force (154).

Table 2. Definition of key terms related to fatigue and exercise intolerance.

Definition of Components of Fatigue During Exercise	
Central fatigue	A progressive reduction in voluntary activation of muscle during exercise.
Supraspinal fatigue	Fatigue produced by failure to generate output from the motor cortex; a subset of central fatigue.
Activation fatigue	Fatigue resulting in a reduction in neuromuscular activity measured by electromyography. A subset of central fatigue, which includes supraspinal fatigue, spinal fatigue, fatigue of the neuromuscular junction, or effects of fatigue on muscle conduction.
Muscle fatigue or Peripheral fatigue	Any exercise-induced reduction in the ability of a muscle to generate force or power. This is limited to fatigue produced by changes at or distal to the muscle sarcolemma.
Performance fatigue	The sum of central and peripheral fatigue that act to reduce to the total force of power output under maximal voluntary effort.
Task failure or Exercise Intolerance	Cessation of a muscular task or bout of exercise. Task failure may be accompanied by peripheral fatigue, central fatigue, or both.
Definition of Measurement Variables Used to Quantify Fatigue	
Maximal evocable force	Maximal force that can be produced by a muscle or muscle group; it would occur when twitches interpolated during a maximal voluntary contraction add no more force.
Maximal voluntary contraction	A maximal contraction that a subject accepts as maximal and that is produced with appropriate continuous feedback of achievement.
Twitch interpolation	A method to measure voluntary activation in which one (or more) stimulus is delivered to the motor axons innervating the muscle during a voluntary effort.
Voluntary activation	Level of voluntary drive during an effort. Unless qualified, the term does not differentiate between drive to the motoneuron pool and that to the muscle. Maximal voluntary activation can be measured using twitch interpolation during a maximal voluntary contraction.

Modified from (154)

1.3.2 Central and peripheral fatigue

During voluntary contractions, muscles are activated by complex pathways starting in the cortex and leading to excitation of lower motor neurons in the spinal cord. The axon of the lower motor neuron carries the action potentials to the neuromuscular junction of the muscle. Typically, the processes inside and proximal to the spinal cord are defined as central, whereas the processes in the peripheral nerve, neuromuscular junction, and muscle are defined as peripheral. The precise point characterizing where “central” and “peripheral” are separated varies in the literature, with some suggesting that “central” should include all of the nervous system including the neuromuscular junction. However it is defined, clearly fatigue can potentially arise at any point within the pathway that links the motor cortex to the muscle cross-bridge (154).

The failure to maintain the initial maximal force during exercise depends both on “peripheral” fatigue occurring distal to the point of nerve stimulation and on “central” fatigue resulting from a failure to activate the muscle voluntarily. This arbitrarily includes branch-point failure and failure at the neuromuscular junction in the “peripheral” component. That component of overall muscle fatigue dependent on a progressive failure to drive motoneurons (and muscle fibers) voluntarily is termed “central fatigue” (159).

Early studies by Merton (160) suggested that in well-motivated individuals, the fatigue in a small muscle of the hand could be entirely peripheral. Later studies suggest that a small degree of central failure of activation often occurs during maximal activation of muscles and that during fatigue there is often a substantial central component (154).

The sufficiency of neural drive to muscles and other aspects of neuromuscular control clearly change in exercise. These include the sensory accompaniments to the fatiguing task, the increasing tremor, which can develop during exercise and persist afterwards, and the gradual recruitment of other muscles. These accompaniments are not contentious, but the mechanisms that produce them

are not necessarily well understood. When exercise is “open loop” (i.e., with no duration or distance etc., as a goal), the decision to terminate it is a voluntary act and thus can be influenced by cognitive processes (154).

1.3.3 Causes of fatigue

What causes central and peripheral fatigue during exercise? The mechanisms determining fatigue leading to exercise tolerance have been intensely investigated for over a century (161). Most of this research has been based on the assumption that, in highly motivated subjects, the tolerable duration of aerobic exercise is limited by central and/or peripheral muscle fatigue (130, 132, 162-165). In other words, it is assumed that aerobic exercise stops at the point commonly called exhaustion because fatigued subjects are no longer able to generate the power output required by the task despite their maximal voluntary effort. Indeed, task failure/exhaustion is often referred to as the “point of fatigue” (166).

Otherwise, it is very clear that fatigue arising within neuromuscular system can only indirectly explain why exercise is terminated (167). Muscle fatigue seems to cause task failure during isolated muscle tasks only when submaximal exercise requires intense muscle contractions (80% of the maximal voluntary contraction - MVC) (168). Examples of such activities would be resistance training or certain activities of daily living in sedentary old adults, such as stair climbing (169).

Investigations of the physiological factors determining exercise intolerance have focused on the cardiovascular, respiratory, metabolic, and neuromuscular mechanisms of muscle fatigue (161) those include limited oxygen delivery (132, 162), metabolic and ionic changes within the active muscles (170, 171), supraspinal reflex inhibition from muscle afferents sensitive to these changes (162), and altered cerebral blood flow and metabolism (164).

Exercise tolerance in highly motivated subjects is limited by perception of effort as postulated by the psychobiological model based on motivational intensity theory (172-175). Perception of effort and potential motivation are the key-determinants of exercise tolerance comes from experiments in which these two parameters have been manipulated independently from the cardiovascular, respiratory, metabolic and neuromuscular mechanisms of muscle fatigue thought to determine exercise tolerance (132, 161). In these experimental studies, mental fatigue (176), sleep deprivation (177), a psychostimulant (178), the presence of a competitor (179), and monetary reward had a significant effect on time to exhaustion.

1.3.3.1 *Mechanisms of central fatigue: Attenuation and inhibition of muscle recruitment during whole body exercise*

“Central fatigue” is a progressive reduction in voluntary activation or maximal evocable force during whole body exercise. Part of this central fatigue is “supraspinal fatigue”, because motor cortical output is reduced as very-heavy intensity exercise is progressed. Eventually, there is “intolerance” when the exercise can no longer be continued. This point is often termed “exhaustion” by exercise physiologists. Surprisingly, the neural mechanisms underlying task failure have received little attention from electrophysiologists who are capable of stimulating the neuromuscular apparatus to check that the exercise stops when effective muscle contraction is still possible (159). Some studies have confirmed this (180-182).

It is not possible to specify all the sites within the CNS at which contributions to voluntary activation, central fatigue, and supraspinal fatigue occur. The traditional model for considering muscle performance traces a causative “chain” from high levels within the CNS via descending paths to the motoneuron and then via motor axons to the neuromuscular junction, the sarcolemma, T tubules, and ultimately the actin and myosin interactions (Figure 17) (154).

A common assumption in such a model is that any change at a link in the chain will affect force production. To continue this analogy, the chain is as “weak” as

any of its links so that, theoretically, evolution might have ensured that all were equally strong. This does not hold because, for example, in normal subjects during voluntary tasks, events at the muscle cell and at supraspinal levels provide definite limits, while conduction block in motor axons and failure at the neuromuscular junction does not. Of course, diseases and lesions (e.g., myasthenia gravis or spinal cord injury) damage preferentially particular links in the chain (154).

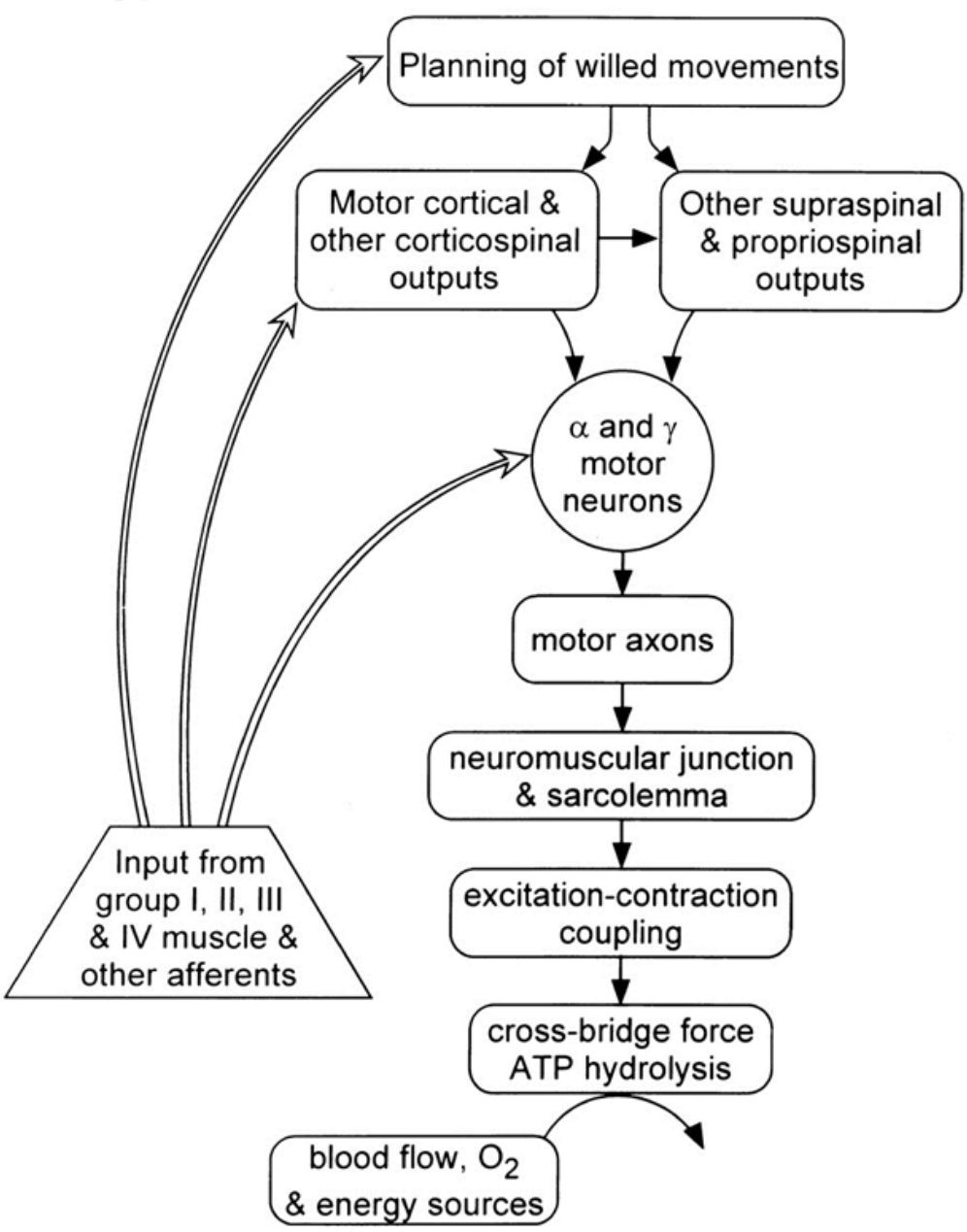

Figure 17. Diagrammatic representation of the “chain” involved in voluntary contractions. A major source of feedback that from the muscle, is shown acting at three levels in the central nervous system. Other sources of feedback that also act at these levels are not shown (154).

1.3.3.2 *Mechanisms of peripheral fatigue: Impairment of muscle force producing capacity during whole body exercise*

The muscle is typically considered to be a major locus for fatigue during exercise, with depletion of energy resources and increase in fatigue-inducing metabolites being contributory. As described in sections 1.2.2, 1.2.3 and 1.2.4, peripheral muscle fatigue is a complex process that mainly includes the increase of Pi, Ca^{2+} sensitivity, and limiting O_2 supply and/or utilizing capacity.

The exchange of phosphate between ATP and PCr is catalyzed by creatine kinase (CK) according to the following reaction:

$$PCr + ADP + H^+ \rightarrow Cr \text{ (creatine)} + ATP$$

During periods of high-energy demand, the ATP concentration initially remains almost constant while PCr is broken down to Cr and Pi. While Cr has little effect on contractile function (183), Pi may cause a marked decrease of myofibrillar force production and Ca^{2+} sensitivity as well as sarcoplasmic reticulum (SR) Ca^{2+} release. Early in exercise sarcoplasmic [Pi] increases rapidly, in almost a 1:1 ratio to PCr breakdown. The initiation of the 'power stroke' in muscle contraction requires that Pi is released from the actomyosin head (Figure 2). An increased sarcoplasmic concentration of Pi inhibits this release and thus is thought to be a major determinant of the reduced ability for muscle to produce force or power. The largest accumulation of Pi occurs early in exercise and corresponds to when the greatest reduction in muscle fatigue is manifest. In animal models that lack creatine kinase, and therefore do not accumulate myoplasmic Pi during on muscle contractions, muscles are highly fatigue resistant (130). However, early investigations clearly showed in isolated muscle preparations that although [Pi] accumulation from ~2 mM (i.e. at rest) to ~10 mM (i.e. equivalent to ~ 3 minutes of high intensity exercise) correlated strongly with muscle fatigue, further increases in [Pi] had little additional fatiguing influence. This may be partially explained *in vivo* by Pi uptake in glycolysis (Pi is a substrate for the glycolytic enzyme glucose-6-phosphatase), which may contribute to slowing the accumulation of myoplasmic Pi and slowing the onset of muscle fatigue. In addition, Pi is also sequestered into the sarcoplasmic

reticulum where it interferes with Ca^{2+} release. It is known that a failure of sarcoplasmic reticulum Ca^{2+} release contributes to fatigue, and that in fatigued muscles the store of releasable Ca^{2+} in the sarcoplasmic reticulum declines. This has been explained by the movement of Pi from the myoplasm into the sarcoplasmic reticulum, causing precipitation of calcium phosphate within the sarcoplasmic reticulum. Pi may therefore also contribute to fatigue by this CaPi precipitation, which reduces the amount of Ca^{2+} available for release, thus reducing force production (184). As such, increased Pi is considered to be a major cause of muscle fatigue *in vivo* (185).

In addition, stimulation of tendon organs and free nerve endings in the muscles during conditions of metabolic and mechanical strain, reduces the responsiveness of the motor neurons, whereas stimulation of muscle spindles increases their responsiveness (186). Stimulation of free nerve endings in muscle by increased concentrations of K^+ and H^+ in the interstitial fluid, and by increased temperature may lead to reflex inhibition of the motor neurons (187-189). Outward transport of K^+ across the sarcolemma reduces the intracellular $[K^+]$ and increases the extracellular $[K^+]$, leading to reductions in membrane charge and muscle action potentials. The responsiveness of the motor neurons and muscles is reduced at maximal exercise in normal subjects, and at lower power outputs in patients with impaired cardiovascular or respiratory function. In either case, the reductions in responsiveness lead to increases in the requirement for central motor command in order to maintain the exercise intensity demanded by the task (190) and eventually, to fatigue and cessation. Reduced responsiveness of the muscles is commonly associated with stimulation of the sympathetic nervous system (191).

Among the changes that accompany increasing exercise intensity is a large increase in the intramuscular $[H^+]$ concentration from 100 nmol per liter (pH 7.0) at rest to 400 nmol per liter (pH 6.4) or more at intolerance. This may inhibit of excitation-contraction coupling and thus reduce the responsiveness of the muscle to stimulation of motor units (192). However, the role of intracellular $[H^+]$ in fatigue is more equivocal (130), and, here, large increases in intramuscular concentrations of Pi inhibiting cross-bridge cycling are generally

accepted as the main culprit for reducing muscle force or power for a given magnitude of Ca^{2+} release, lead to muscle fatigue (193).

On the other hand, in intact muscles the supply of O_2 depends on blood flow and the diffusion of O_2 from capillary blood across the interstitial space and into the muscle fiber, consequently, the supply of O_2 from capillaries to muscle fibers provides one limit to muscle performance during prolonged aerobic exercise. A limiting O_2 supply (or utilization capacity) leads to a greater accumulation of lactate and H^+ as well as an accentuated PCr breakdown accumulating Pi. In the extreme, intramuscular [ATP] may fall, leading to an enhanced accumulation of [ADP], which itself limits cross-bridge cycling. While lactate has been long implicated in causal in fatigue, recent evidence suggests that it may actually be protective of fatigue (130, 194). Nevertheless, lactate accumulation is necessarily accompanied by intra and extracellular acidosis, which may contribute to each of central and/or peripheral fatigue.

Thus, it has been a long debated issue whether O_2 supply through this pathway is adequate or whether there are conditions in which O_2 supply is limited, and that this contributes to reduced muscle performance and more rapid fatigue (195). It has already been noted that blood flow ceases in a continuous maximal contraction (196), and under these circumstances, failure of O_2 supply will presumably contribute to the rapid fatigue. During intermittent contraction, blood flow recovers between contractions, and it might be assumed that auto-regulation of the blood supply would match the blood flow to the overall metabolic needs of the muscle (197). Some athletes show a mild arterial hypoxemia during high-intensity exercise, and this is associated with a reduced performance (198). This performance decline is partly caused by peripheral fatigue and can be reduced by an increased inspired [O_2] and exacerbated by reduced inspired [O_2] (199, 200). Thus, O_2 delivery is intimately related to peripheral muscle fatigue, but fatigue induction by this mechanism likely works through increased intramuscular [Pi] and/or changes in extracellular [K^+] affecting excitability.

1.3.4 Functional consequences of fatigue

Central and peripheral fatigue contribute to limiting the activation of, and force production by, skeletal muscles. Consequently, fatigue can cause exercise intolerance directly by limiting the rate of force production once the maximal evocable force declines to the level required by the task (Figure 18). However, this model of fatigue and exercise intolerance is largely derived from isolated muscle preparations in animal models, or from isometric contractions in humans. These models do not necessarily relate to dynamic contractions in large muscle mass in humans where power (rather than force) is the limiting variable and where central fatigue (rather than peripheral fatigue) is thought to play a much larger role in determining the point of exercise intolerance.

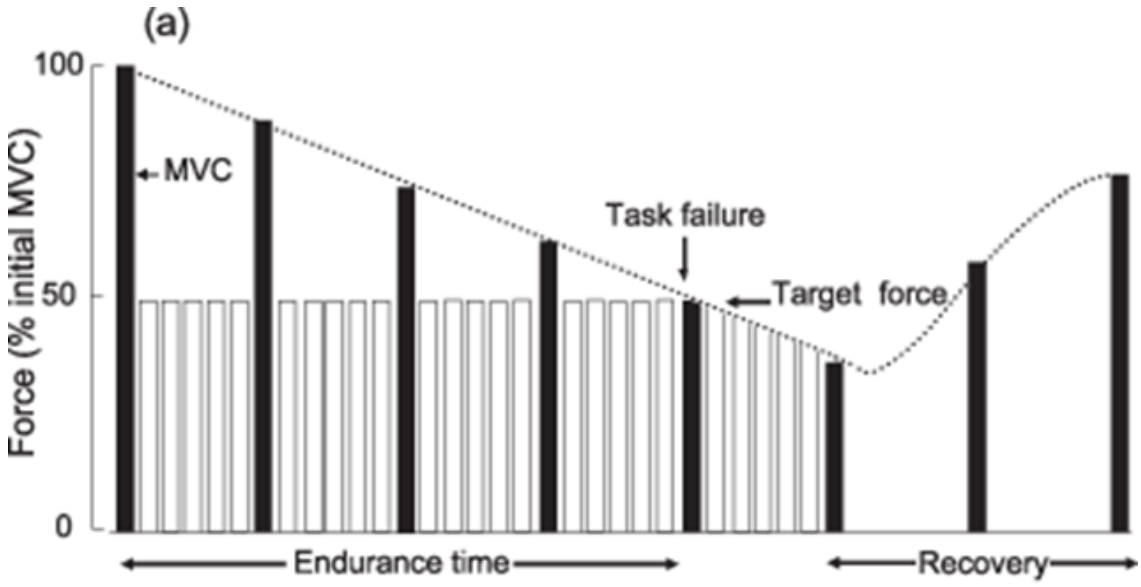

Figure 18. A proposed model of how fatigue leads to task failure, derived from isolated muscle preparations and isometric contractions in humans (201). MVC, maximal voluntary contraction.

Probably the most significant functional change during fatigue that impairs dynamic muscle contractions is in the shape of the power-velocity curve. During fatigue both the maximum isometric force and the maximum shortening

velocity are reduced. However, the reduction in shortening velocity is probably more significant in impacting power production (Figure 19) (202). Fatigue in power producing capacity is therefore much greater at the higher the velocities of shortening. Therefore, the optimum velocity for maximum power output is reduced in the fatigued state, as are both the maximum power itself and the power at any given velocity of shortening.

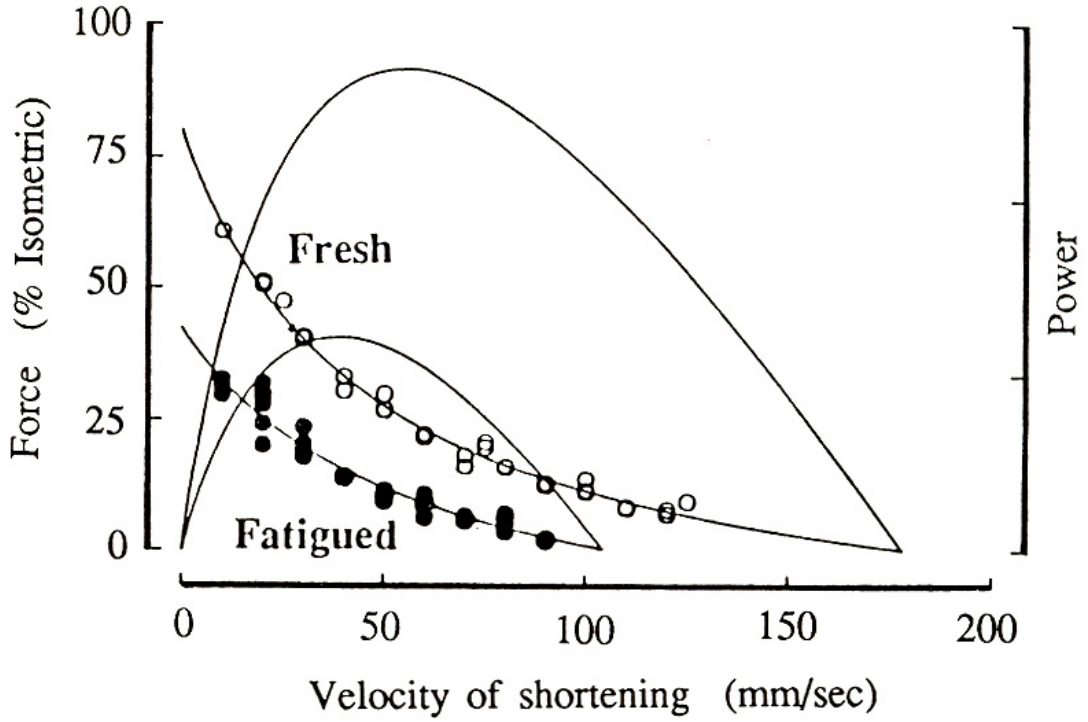

Figure 19. Force-velocity and power-velocity relationships for fresh and fatigued muscles. Rat gastrocnemius muscle. Data for fresh muscles (open symbols) and muscles after a 15s fatiguing tetanus (filled symbols) (202).

1.3.5 Quantifying fatigue *in vivo*

The profound changes in the power-velocity curve during fatigue pose a significant problem for the assessment of fatigue during exercise in humans. Evidence of fatigue may be assessed from reductions in maximal evocable force or power during a task, from an increase in RMS EMG for a given sub-maximal contraction, or a decline in RMS EMG in maximal voluntary contractions.

The most conventional approach is to assess the maximal voluntary force production. However, this is technically limited to contractions in isolated muscle groups, such as during a handgrip or knee-extension task. In this system the most common model of assessment would be the measurement of the decline of isometric force production. This is because measuring isometric force overcomes the complexity of the velocity-dependence of force production. That is, were dynamic contractions to be used, then the velocity of muscle contractions would have to constrained in order to ensure the decline in force (or torque) measured was not due to differences in the velocity of shortening. Modern equipment, such as the isokinetic dynamometer has provided an opportunity to investigate in humans the changes in the force-velocity and power-velocity relationships with fatiguing exercise. Nevertheless, this important technological advance is limited by the equipment to the investigation of single muscle groups (such as the knee-extensors), and thus the dynamics of the functional consequences of fatigue in limiting everyday whole-body activities such as walking remain largely unexplored.

In addition, the use of voluntary contractions does not allow the locus of the fatigue mechanism to be distinguished: i.e. whether fatigue is central (e.g. inhibition of motor activity in the cortex or spinal cord) or peripheral (e.g. reduction in the ability of the muscle to release Ca^{2+} or for the cross-bridge to produce force). The most conventional approach to assess central fatigue occurring during exercise is estimated by twitch interpolation (160) (Figure 20). Twitch interpolation consists of supramaximal potentials that are added to the muscle or motor nerves during a maximal voluntary contraction. These can be made using surface stimulation of the muscle (electrodes placed directly on the skin over the surface of the muscle) or magnetic stimulation of the motor nerves in distal or proximal to the spinal cord. Using this method, if lower motoneurons are not completely recruited during by maximal volition, or are not firing fast enough, then the evoked stimulus will generate additional force, termed a ‘superimposed twitch’. These external potentiated, or tetanic, stimulations can identify with additional force is produced by the muscle when additional ‘drive’ is provided, thus identifying the presence of central fatigue

(Figure 20a) (11). A decline in voluntary activation during or after sustained contractions implies a reduced motor drive that originates at or above the stimulation site on the axons of the lower motoneurons (i.e., at the spinal and/or supra-spinal levels) (202). For sustained maximum voluntary contraction of the *quadriceps*, central fatigue has been shown to be a factor leading to loss of force in many subjects towards the end of a 60 s isometric contraction, but with similar contractions of the *adductor pollicis* of the hand there is little or no evidence of central failure. This suggests that the muscle mass engaged in the exercise has an important influence on the magnitude of central fatigue, and thus central fatigue is likely to be more pronounced during whole-body exercise tasks (203). Naturally, by the nature of the technique, twitch interpolation has only been examined during relatively simple forms of exercise involving one muscle group. It is not known if it is possible to fully activate several muscle groups as they fatigue, especially if they are involved in a complex series of movements as a whole body exercise (e.g. walking or cycling) (202).

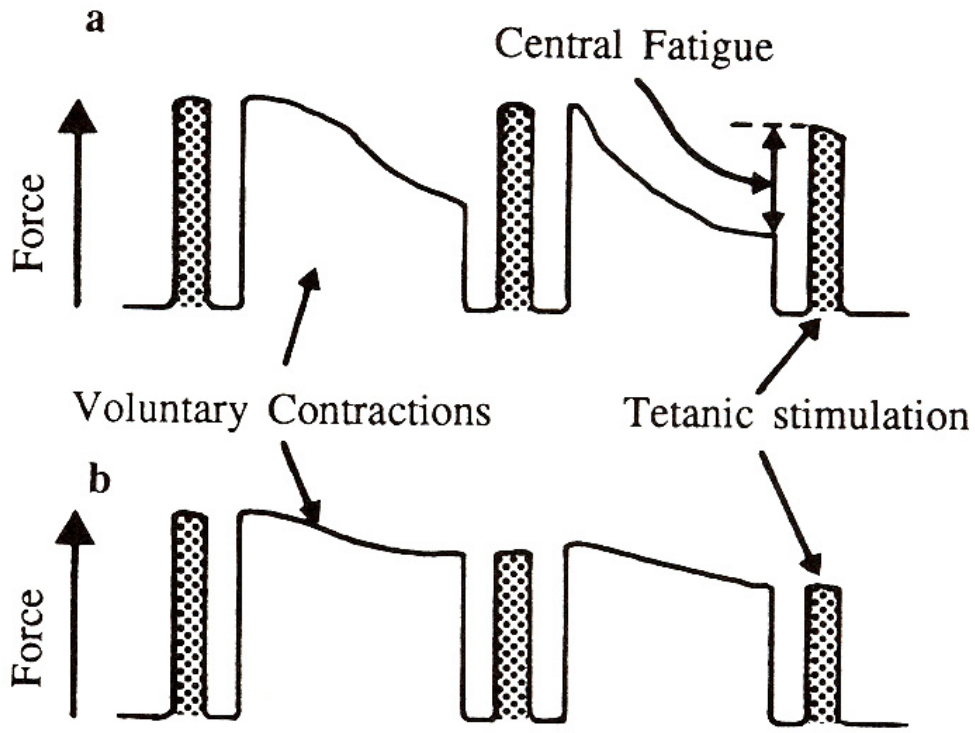

Figure 20. Voluntary contractions with interposed tetanic stimulation. a, fatigue partly due to central failure; note the greater fall in voluntary force compared to tetanic force. b, voluntary and tetanic force decline in parallel showing no central fatigue (202).

To address the technical complexity of assessing changes in the power-velocity curve during fatiguing whole-body exercise, Cannon et al. (204) developed a method to measure maximal evocable isokinetic power production instantaneously during and following standard cycling ergometer exercise. They built on previous work using an isokinetic cycle ergometry (205, 206), to develop a method that allowed a standard cycle ergometer to be instantaneously switched between normal cycling (where the relationship between force and cadence is hyperbolic) to isokinetic mode, where the cadence is fixed by the investigator and the force on pedals is measured. By requiring the participants to produce a maximal voluntary isokinetic effort at various points during normal cycling exercise, they were able to measure the decline in velocity-specific power in response to a whole-body exercise task.

Using this method they showed that muscle fatigue is pre-requisite for the reduction in work efficiency observed during heavy and very-heavy intensity exercise that occurs during sustained dynamic exercise in healthy subjects (Figure 21). This muscle fatigue causes an increase in the energy demands of the exercise (as measured by the VO_2 slow component). The VO_2 slow component itself has been shown to strongly relate to accelerated breakdown of intramuscular phosphocreatine and increased fatigue-inducing inorganic phosphate accumulation (207). Thus, intramuscular and 'whole system' fatigue-related processes occur early in constant power exercise above LT (as early as 3 minutes in Cannon *et al*. (200)), and necessitate progressive increases in muscle recruitment, muscle blood flow, cardiac output, and ventilation.

Cannon *et al.* (200) therefore provided a method by which fatigue during whole-body exercise may be investigated. However, this investigation remains limited to quantifying velocity-specific changes in maximum evocable power. In other words, in its current format, this method is not able to isolate whether the measured fatigue is attributable to central fatigue, peripheral fatigue, or both. Thus, there remains little information about how fatigue affects force or power production during movements, as opposed to during isometric contractions (202). Reports of fatigue during running and cycling indicate that dynamic performance decreases to a greater extent than does the isometric force, and

thus measurement of the processes involved in loss of force during isometric contractions may not well reflect the loss of power during dynamic movements which make up most of our everyday activities.

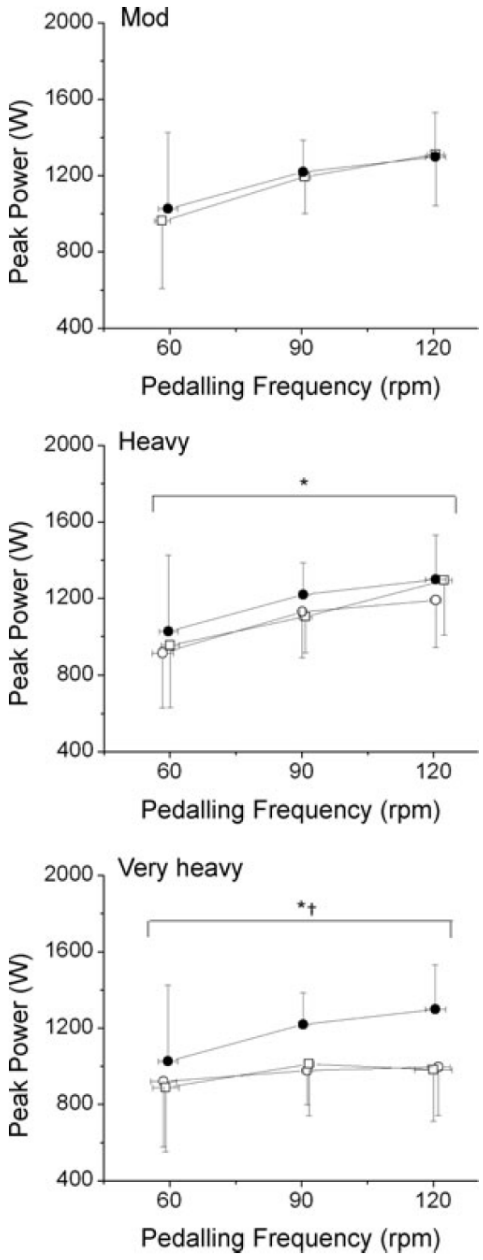

Figure 21. Velocity-specific peak power developed during a maximal isokinetic effort, plotted as a function of pedaling frequency. Velocity-specific power production was unchanged from baseline (●) to 8 minutes (□) of moderate (Mod) intensity cycling. During heavy and very-heavy intensity exercise there was a significant reduction in velocity-specific power production at 3 minutes (○) and 8 minutes (□) of exercise compared to baseline (●). * Significant ($p<0.05$) main effect of exercise duration. † Significant ($p<0.05$) interaction (duration x pedaling frequency) was present. Error bars are S.D. (204)

1.3.6 Dynamics of fatigue in exercise and recovery

As shown above, the onset of fatigue during exercise appears to be very rapid, with reductions in power producing capacity occurring within the first 3 minutes of exercise (200). In addition to this, the recovery from task failure is also rapid. Ferguson *et al.* (208) were the first to measure the recovery of exercise tolerance using the power-duration curve to characterize the recovery of CP and W' after very-heavy intensity exercise to intolerance. They showed that recovery of exercise tolerance in whole-body exercise has a half-time of about 4 minutes. This was shown to be appreciably slower than the recovery of VO_2 but faster than the recovery of blood lactate ($[L^-]$) (Figure 22)

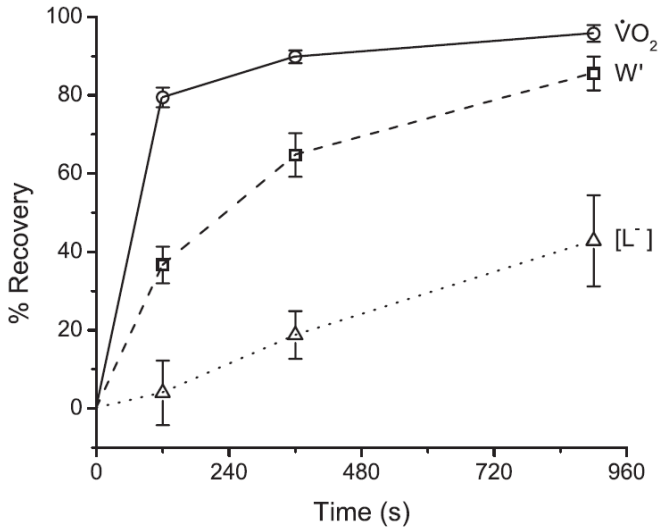

Figure 22. Mean temporal profile of the magnitude of W' recovery (□) relative to recovery of VO_2 (O) and lactate concentration ($[L^-]$, **Δ**); bars represent SD. Note faster recovery kinetics of VO_2 than of W' and slower recovery kinetics of $[L^-]$ than of W' (208).

This has important implications for measurement of fatigue using twitch interpolation or potentiated twitch force measurements after exercise to intolerance (209-215). It is common to quantify muscle fatigue by potentiated twitch force measurements ~10 minutes after a whole-body exercise task. The equipment necessary for the measurements imposes this delay. Fatiguing exercise is performed on a cycle or a treadmill and at termination, the subject is

moved from the ergometer to a chair dynamometer, strapped in, and electrodes placed to stimulate the muscle. The fastest that this can safely be achieved is about 10 minutes. However, this delay means that much of the fatigue present at the limit of tolerance has abated by 10 minutes after the exercise. The residual fatigue measured may be better linked to a type of fatigue termed low-frequency fatigue (199, 216). This is characterized by the slow recovery of force producing capacity, and is associated with impairments in the excitation-contraction coupling. Low-frequency fatigue can last for many hours and, occasionally, days after induction (217). Because of this long duration for recovery low-frequency fatigue is thought to be associated with muscle damage, and impairment of the T tubule and sarcoplasmic reticulum for conduction and/or Ca^{2+} release. While low-frequency fatigue clearly contributes to fatigue, this contribution appears to be small, because the majority of power producing capacity is recovered within ~10 minutes after exercise. Thus, assessments that are made after even a few minutes of recovery may not reflect the true nature of the central and peripheral processes that limited the exercise in the first place.

To better understand these dynamics, Froyd *et al.* (218) studied the time course of fatigue during repetitive concentric extension and flexion exercise of one knee using an isokinetic dynamometer. They assessed neuromuscular function using different forms of electrical stimulation and isometric MVC before the start of a time trial (TT), immediately after each 20% fraction of the TT, and at 1, 2, 4 and 8 minutes after TT termination. They demonstrated that (Figure 23):

- Peripheral fatigue developed rapidly in the first 0-40% of the time trial
- MVC loss mirrored peripheral fatigue early in exercise, but continued to progress during 40-100% of the time trial
- Neuromuscular function began to recover within the first 2 minutes of exercise termination
- Despite this early recovery, neither MVC, nor the peak torque response to a range of methods of electrical stimulation, returned to the baseline value within 8 minutes of resting recovery.

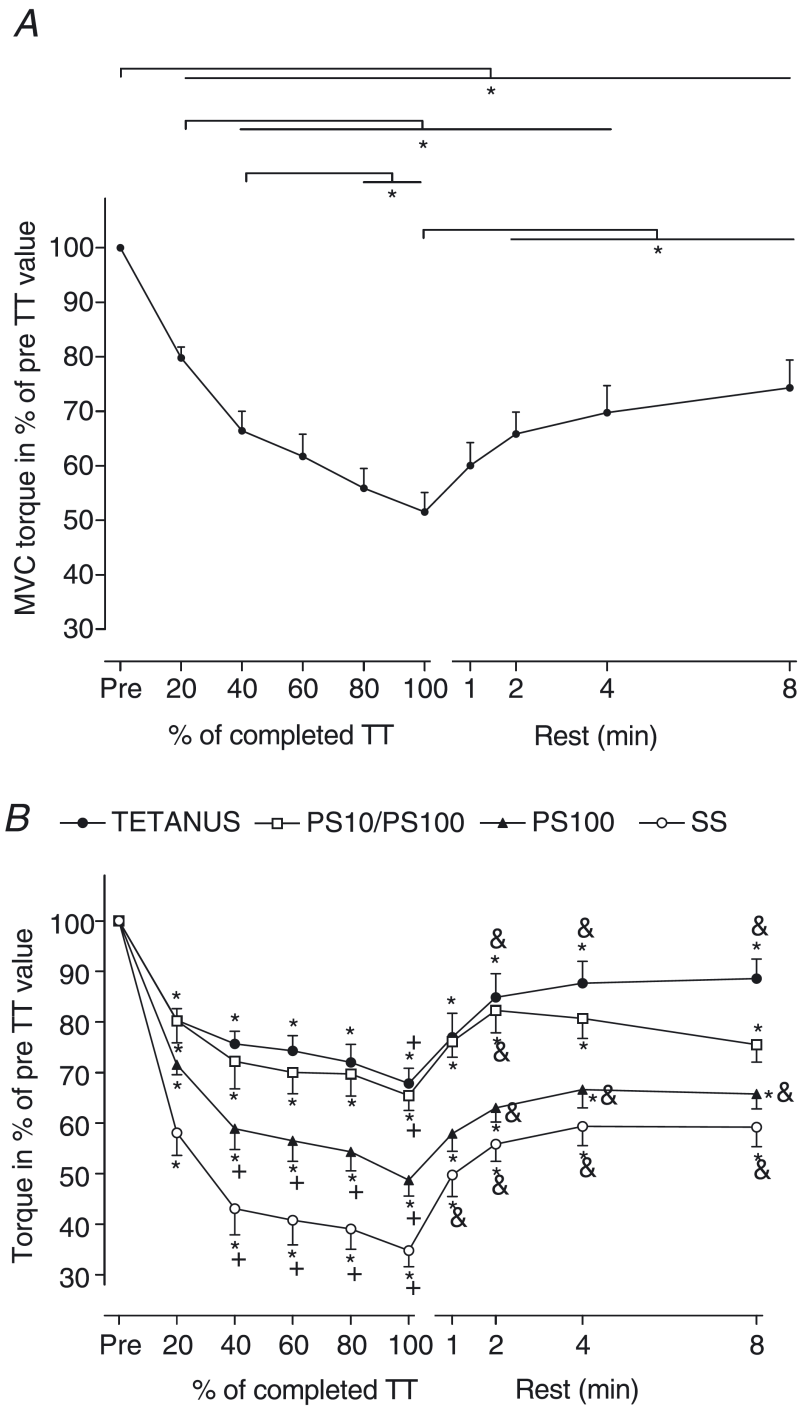

Figure 23. Percentage difference from pre-time trial (TT) values for maximal voluntary contraction (MVC) torque (A), and PT response to ES for single stimulus (SS), paired stimuli at 100 Hz (PS100), paired stimuli at 10 Hz (PS10)/PS100 ratio and tetanic stimulation at 100 Hz (TETANUS; B) during the TT and after 1, 2, 4 and 8 min rest. Values are expressed as means ± SEM, n = 10, *P < 0.05. Statistical differences for B: from the pre-TT value for the same variable, *P < 0.05; from 20% of the TT value for the same variable, $^{+}$P < 0.05; from 100% (=0 min) for the same variable at rest after TT, & P < 0.05. Both during the TT and rest period, TETANUS, PS100, SS and PS10/PS100 were significantly different (P < 0.05) from each other, except between TETANUS and PS10/PS100 during the TT, based on the area under the curve statistics (218).

These recovery dynamics using potentiated twitch force measurement had not previously been reported for self-paced prolonged exercise (219, 220), probably because in previous studies a large degree of recovery had already occurred before the first post-exercise force measurements were performed (221).

These data highlight a range of important gaps in our knowledge about fatigue during exercise including that:

- Any measurement of fatigue should be able to be imposed (essentially) instantaneously at any point during exercise and recovery, in order to avoid recovery in processes contributing to fatigue
- Measurements would ideally assess the loss power during dynamic contractions, and therefore need to be made at a controlled velocity
- Information of central and peripheral contributions to fatigue in whole-body exercise is lacking

The demands require a combination of the approaches discussed, such as combined assessment of RMS EMG, isokinetic power, and/or muscle stimulation instantaneously during whole body exercise: a technically challenging requirement.

2. RATIONALE

Central and peripheral contributions to fatigue are commonly identified using surface stimulation of the muscle, the peripheral nerve (PNS; magnetic or electrical), or the motor cortex (transcranial magnetic stimulation; TMS), with or without combined maximal voluntary contraction (MVC; i.e. twitch interpolation). Due to the localized nature of these stimulation techniques, they provide valuable information on corticospinal (motor cortex to spinal nerves), neuromuscular (lower motor nerve to neuromuscular junction), and skeletal muscle fatigue, and knowledge of these can be used to predict patient outcomes (218, 222-230).

However, the nature of these externally-imposed stimulation techniques to elucidate central and peripheral fatigue necessitates that only isolated muscle (typically single-joint isometric) contractions are evoked. Additionally, the proportion of force that can be generated through surface stimulation, PNS, or TMS alone is a small fraction of MVC force (~10-40%), even under potentiated twitch or when paired stimuli are used (231). Thus, it is the nature of these maneuvers that they have little in common with complex, coordinated, dynamic contractions that provide power during daily activities.

An idealized approach for studying fatigue, therefore, is to measure reduction in power generation during an exercise task that emulates the physiologic conditions under which limitation is manifest (232), and to be able to attribute this reduction to central and peripheral mechanisms of fatigue.

However, measuring fatigue during a whole-body dynamic exercise task (e.g. that achieves maximal rates of ventilation, oxygen uptake, or cardiac output) is extremely challenging, not least because the velocity-dependence of muscle power output (24, 205) confounds fatigue assessment made with traditional ergometry at the limit of tolerance (233-235). The alternative, whole-body exercise followed by external stimulation, often introduces a crucial delay, resulting in a loss of information of the fatiguing processes due to the rapid

recovery dynamics of both neuromuscular and corticospinal fatigue (206, 218, 225, 236). In other words, measuring task failure is straightforward in whole-body exercise, but isolating the contributions of central and peripheral fatigue is not possible because of the limitations in the ergometry needed to emulate whole-body activities (e.g. treadmill, cycle). On the other hand, investigating central and peripheral fatigue with MVC, PNS and TMS is physiologically revealing, but performed swiftly enough at the point of task failure in whole-body exercise in order to provide the desired measurements of central and peripheral fatigue processes.

Consequently, a solution to allow fatigue assessment during whole-body exercise is needed. This assessment would need to be able to measure fatigue during: 1) velocity-specific contractions in a whole-body exercise task; 2) be able to be applied instantaneously during or following exercise; and 3) quantify the muscle activation and muscle fatigue contributions to the total fatigue process. It was reasoned that a fatigue assessment during cycle ergometry, using a rapid switch from cadence-independent (hyperbolic) to maximal-effort isokinetic cycling with crank power measurement (204), coupled with EMG measurements of leg muscles activation, would meet these requirements. Isokinetic ergometry allows the velocity-specific decline in power production to be measured instantaneously during exercise, avoiding the confounding variations of contraction velocity present during standard ergometry (24, 206). Muscle EMG allows changes in MUAPTs during a maximal voluntary effort to be measured. Thus, this thesis proposed that if the relationship between isokinetic power (P_{ISO}) and surface EMG activity in the fresh (unfatigued) condition during cadence-independent cycling could be reasonably predicted (e.g. by a linear relationship), with suitable known confidence, and reproducible within a given subject, then a departure from this fatigue-free EMG-P_{ISO} relationship during or after fatiguing exercise would allow for muscle ‘activation fatigue’ (AF; a reduced muscle activity), and ‘muscle fatigue’ (MF; reduced power at a given muscle activity) to be isolated. In other words, were power during fatigue to be less than expected at the measured muscle activity, then this deficit could be ascribed to MF, with any remaining power reduction being consequent to AF.

Using this rationale this thesis sought to establish a new method for measurement of central and peripheral components of fatigue during whole body exercise eliciting VO_{2max}. Until now, measurement of central and peripheral components of fatigue has been limited to isolated muscle tasks or to time points after exercise where the physiological conditions that brought about the limiting symptoms for exercise have subsided. Thus, development of a method to overcome these limitations, would allow the first demonstration of central and peripheral components of fatigue during a task that elicited maximal strain of the combined neuromuscular and cardiopulmonary systems. As such it was proposed that this method would provide valuable new information about which of the potential integrated physiological systems contributes the most to limiting whole-body exercise.

3. OBJECTIVE

The objective of this thesis was to develop and validate a method for quantifying muscle fatigue (MF), activation fatigue (AF), and their sum (performance fatigue, PF) during whole-body exercise to intolerance in humans.

To achieve this, the work of this thesis first aimed to demonstrate that during isokinetic cycling the EMG-P_{ISO} relationship was linear, and that the slope of this linear relationship was depended on velocity. This would allow a 'frame of reference' to be established for each individual, which would describe the power output (measured from pedal torque) response of fresh muscles to a range of voluntary muscle activation rates (measured by surface EMG). It was then sought to establish that during fatigue in whole-body cycling (under standard clinical exercise testing conditions with velocity-independent control of the imposed ergometry), a maximal isokinetic effort could be reliably applied instantaneously, at any point during the exercise task or recovery. It was the objective of this work to determine whether this short duration (< 5 s) maximal effort could reveal changes in PF, AF and MF that would provide new information about central and peripheral fatigue during whole-body cycling exercise up to VO_{2max}. This new technique should be able to be applied to healthy participants and patients with heightened exercise intolerance. Therefore, the new method was tested in subjects across a wide range of age and physical function.

3.1 Aims and Hypothesis

AIM 1: To combine surface electromyography (EMG) and isokinetic ergometry to characterize the EMG-P_{ISO} relationship.
HYPOTHESIS 1: The baseline (unfatigued) cycling EMG-P_{ISO} relationship is 1) linear; 2) velocity dependent; and 3) reproducible and precise.

AIM 2: To determine the cycling EMG-P_{ISO} relationship at the limit of tolerance during incremental exercise.
HYPOTHESIS 2: At the point of task failure during incremental cycling exercise, fatigue will be characterized by contributions from both central (AF) and peripheral (MF) mechanisms, and measurement of these variables will be reproducible.

AIM 3: To determine the rate with which the baseline (unfatigued) cycling EMG-P_{ISO} relationship is re-established after intolerance during incremental exercise.
HYPOTHESIS 3: The recovery dynamics of the cycling EMG-P_{ISO} relationship will be rapid, as previously demonstrated with isolated muscle tasks.

4. INSTANTANEOUS QUANTIFICATION OF SKELETAL MUSCLE ACTIVATION, POWER PRODUCTION, AND FATIGUE DURING CYCLE ERGOMETRY

Ana C. Coelho, Daniel T. Cannon, Robert Cao, Janos Porszasz, Richard Casaburi, Marli M. Knorst, Harry B. Rossiter.

Journal of Applied Physiology 118, 646-654, 2015.

Instantaneous quantification of skeletal muscle activation, power production, and fatigue during cycle ergometry

*AC Coelho[1,2], *DT Cannon[1], R Cao[1], J Porszasz[1], R Casaburi[1], MM Knorst[2], HB Rossiter[1,3]

[1] Rehabilitation Clinical Trials Center, Division of Respiratory & Critical Care Physiology & Medicine, Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, USA

[2] Programa de Pós-Graduação em Ciências Pneumológicas, Universidade Federal do Rio Grande do Sul, Brazil

[3] School of Biomedical Sciences, Faculty of Biological Sciences, University of Leeds, UK

* authors contributed equally

Running title: Quantifying fatigue during cycle ergometry

Keywords: Isokinetic; Performance fatigue; Muscle fatigue; Electromyography; Exercise testing

Corresponding Author
HB Rossiter, PhD
Division of Respiratory & Critical Care Physiology & Medicine
Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center
1124 W Carson St, CDCRC Building
Torrance, CA 90502, USA
424 201 3000 x7270
hrossiter@ucla.edu

Abstract (250 words)

A rapid switch from hyperbolic to isokinetic cycling allows the velocity-specific decline in maximal power to be measured, i.e. fatigue. We reasoned that should the baseline relationship between isokinetic power (P_{iso}) and electromyography (EMG) be reproducible, then contributions to fatigue may be isolated from: 1) the decline in muscle activation (muscle activation fatigue, AF); and 2) the decline in P_{iso} at a given activation (muscle fatigue, MF). We hypothesized that the EMG-P_{iso} relationship is linear, velocity-dependent, and reliable for instantaneous fatigue assessment at intolerance during and following whole-body exercise. Healthy participants (n=13) completed short (5 s) variable-effort isokinetic bouts at 50, 70, 100 rpm to characterize baseline EMG-P_{iso}. Repeated ramp incremental (RI) exercise tests were terminated with maximal isokinetic cycling (5 s) at 70 rpm. Individual baseline EMG-P_{iso} relationships were linear (r^2=0.95±0.04), and velocity dependent (ANCOVA). P_{iso} at intolerance (two legs, 335±88 W) was ~45% less than baseline (630±156 W, $CI_{Difference}$ 211, 380 W, p<0.05). Following intolerance, P_{iso} recovered rapidly (F=44.1; p<0.05; η^2=0.79): Power was reduced (p<0.05) *vs.* baseline only at 0 min ($CI_{Difference}$ 80, 201 W) and 1 min recovery ($CI_{Difference}$ 13, 80 W). AF and MF (one leg) were 97±55 W and 60±50 W. Mean bias ± limits of agreement for reproducibility were: baseline P_{iso} 1±30 W; P_{iso} at 0 min recovery 3±35 W; and EMG at P_{iso} 3±14%. EMG-power is linear, velocity dependent, and reproducible. Deviation from this relationship at the limit of tolerance can quantify the ‘activation’ and ‘muscle’ related components of fatigue during cycling.

Abbreviations

AF, activation fatigue

BL, baseline (non-fatigued) state

EMG, electromyogram

LT, lactate threshold

MF, muscle fatigue

MVC, maximal voluntary contraction

PCr, phosphocreatine

PF, performance fatigue

P_{iso}, isokinetic power

PNS, peripheral nerve stimulation

R0, maximal isokinetic effort measured at the limit of tolerance, ‘0 min recovery’

R1, maximal isokinetic effort measured at 1 min into recovery

R2, maximal isokinetic effort measured at 2 min into recovery

R3, maximal isokinetic effort measured at 3 min into recovery

TMS, transcranial magnetic stimulation

$\dot{V}O_2$, rate of pulmonary O_2 uptake

$\dot{V}O_{2peak}$, peak rate of pulmonary O_2 uptake

Introduction

Exercise tolerance is the ability to produce force or power adequate to accomplish a task. Maintenance of a high quality of life is dependent on adequate exercise tolerance to perform the activities of daily living. In addition to quality of life, exercise tolerance is also strongly predictive of mortality, in both health and disease (5, 25, 26, 37). While whole-body endurance exercise tolerance correlates with the morphological structures and physiological processes that support oxygen flux between the atmosphere and the muscle mitochondrion, the integrated neuromuscular mechanisms determining task failure remain poorly understood. This is largely due to the complexity in measuring, and localizing the causes of fatigue during whole-body exercise.

Fatigue, an exercise-induced reduction in power or force that is reversible with rest, may be generally categorized as: 1) a reduction in efferent activity of the motor cortex, spinal cord, or motor neurons that innervate the skeletal muscles; and/or 2) a disruption to skeletal muscle excitation-contraction coupling from depletion of energy stores or accumulation of metabolites. These are often referred to as ‘central’ and ‘peripheral’ fatigue, respectively. Central and peripheral contributions to fatigue are commonly identified using surface stimulation of the muscle, the peripheral nerve (PNS; magnetic or electrical), or the motor cortex (transcranial magnetic stimulation; TMS), with or without combined maximal voluntary contraction (MVC; i.e. twitch interpolation). Due to the localized nature of these stimulation techniques, they provide valuable information on corticospinal (motor cortex to spinal nerves), neuromuscular (lower motor nerve to neuromuscular junction), and skeletal muscle fatigue, and knowledge of these can be used to predict patient outcomes (7, 9, 14-16, 19, 21, 27, 34, 38).

The nature of external stimulation necessitates isolated, single-joint isometric muscle contraction. Additionally, the proportion of force that can be generated through surface stimulation, PNS, or TMS alone is a small fraction of MVC force (~10-40%), even under potentiated twitch or when paired stimuli are used

(38). Thus, these maneuvers have little in common with complex, coordinated, dynamic contractions that provide power for daily tasks.

An idealized approach for studying fatigue, therefore, is to measure power generation during an exercise task that emulates the physiologic conditions under which limitation is manifest (9). However, measuring fatigue during a whole-body dynamic exercise task (e.g. that achieves maximal rates of ventilation, oxygen uptake, or cardiac output) is extremely challenging, not least because the velocity-dependence of muscle power output (3, 4) confounds fatigue assessment made with traditional ergometry at the limit of tolerance (8, 23, 24). The alternative, whole-body exercise *followed* by external stimulation, often introduces a crucial delay, resulting in a loss of information of the fatiguing processes due to the rapid recovery dynamics of both neuromuscular and corticospinal fatigue (14, 16, 30, 36). Consequently, an ideal assessment of fatigue during whole-body exercise would: 1) be task- and velocity-specific; 2) be able to be applied instantaneously during or following exercise; and 3) quantify the muscle activation and muscle fatigue contributions to the total fatigue process.

We reasoned that a fatigue assessment during cycle ergometry, using a rapid switch from cadence-independent (hyperbolic) to maximal-effort isokinetic cycling with crank power measurement (10), coupled with electromyographic (EMG) measurements of leg muscles activation, would meet these requirements. Isokinetic ergometry allows the velocity-specific decline in power production to be measured instantaneously during exercise, avoiding the confounding variations of contraction velocity present during standard ergometry (3, 30). Muscle EMG allows changes in motor unit action potential trains during a maximal voluntary effort to be measured. Thus, were the relationship between isokinetic power (P_{iso}) and surface EMG activity during cycling to be predictable with known confidence and reproducible in a given subject, then a departure from the baseline (fatigue-free) EMG-P_{iso} relationship during or after fatiguing exercise would allow for muscle ‘activation fatigue’ (AF; a reduced muscle activity), and ‘muscle fatigue’ (MF; reduced power at a given muscle activity) to be isolated. In other words, were power during fatigue to be

less than expected at the measured muscle activity, then this deficit could be ascribed to MF, with any remaining power reduction being consequent to AF.

We hypothesized that the baseline (non-fatigued) EMG-P_{iso} relationship would be linear, velocity dependent, reproducible, and sufficiently precise to provide a reference for comparison with instantaneous EMG-P_{iso} during cycle ergometry. This would allow an individual's muscle activation and muscle fatigue to be measured during, or following, whole-body exercise to the limit of tolerance.

Materials and Methods

Ethical Approval and Participants

The Institutional Review Board, Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center approved this study, and all procedures complied with the latest revision of the *Declaration of Helsinki* and *Belmont Report*. Written informed consent was obtained from healthy, active volunteers (n=13, 42±14 yr [range 29-72 yr], 171±8 cm, 75±14 kg, $\dot{V}O_{2peak}$ 3.2±0.7 $L.min^{-1}$) prior to their participation in the study. Volunteers were screened for cardiovascular disease risk with a resting ECG and a health history questionnaire.

Exercise Protocol

Volunteers visited the laboratory on two occasions for duplicate measures of an identical protocol. On each visit, the participants completed two phases: 1) short (~5 s) bouts of variable effort isokinetic cycling at three pedaling frequencies; and 2) a ramp-incremental exercise test, followed by a short (~5 s) maximal effort isokinetic bout performed immediately at the limit of tolerance and at each minute of recovery.

Measurement of Baseline EMG-P_{iso}. Volunteers cycled on an ergometer (Lode BV) with pedaling rate constrained at 50, 70, or 100 rpm (isokinetic). An example of the protocol at 70 rpm is shown in Figure 1. Participants were asked to give 4 to 5 variable efforts at approximately 25%, 50%, 75% and 100% of maximum effort. Each of the efforts lasted for approximately 3 to 5 s,

and were separated by approximately 0.5 to 5 minutes of unloaded cycling (longer recovery following the maximal efforts). The process was repeated 2-3 times at each pedaling frequency (50, 70, and 100 rpm). The mean EMG (Figure 1A) and isokinetic crank power (Figure 1B) at each pedaling frequency (Figure 1C) were determined from three consecutive isokinetic crank revolutions, and used to model the baseline (fatigue-free) velocity-dependent EMG-P_{iso} relationship (Figure 1D) (see EMG-P_{iso} Relationship and Fatigue Characterization for details of the analysis).

Measurement of EMG-Piso Following Ramp Exercise. Following a rest period of ~20-30 min, participants returned to the ergometer to complete a ramp incremental test. This test consisted of ~2 min at rest, ~4 min of 0 W cycling, a ramp phase of 15-30 $W.min^{-1}$ until the limit of tolerance, followed by recovery at 0 W. During the ramp phase, the ergometer power was cadence-independent (hyperbolic). The limit of tolerance was defined as being unable to maintain a frequency above 55 rpm, despite strong encouragement. At the limit of tolerance, the ergometer was switched instantaneously to the isokinetic mode constrained to 70 rpm. This instantaneously reduced the flywheel breaking to zero (because cadence was below 70 rpm at the limit of tolerance), and volunteers were strongly encouraged to give a maximal effort lasting ~5 s; thus the flywheel was rapidly (within ~1-2 s) accelerated to, and held at, 70 rpm while crank power was measured for 4 to 5 revolutions. This maneuver is similar to the baseline maximal effort isokinetic bout during *Measurement of Baseline EMG-P_{iso}*, with which the participants were well familiarized. A maximal ~5 s isokinetic bout at 70 rpm was repeated each minute during recovery until P_{iso} was similar to the baseline.

Eleven of 13 participants completed duplicate laboratory visits separated by at least 48 hours. This duplicate visit included both phases for *Measurement of Baseline EMG-P_{iso}* and *Measurement of EMG-P_{iso} Following Ramp Exercise.*

Ergometry

All exercise tests were undertaken on a computer-controlled electromagnetically-braked cycle ergometer (Excalibur Sport PFM, Lode BV, Groningen, NL). In addition to the standard application and measurement of power at the electromagnetically-braked flywheel, the ergometer was instrumented with force transducers in the bottom bracket spindle. Left and right torque (Nm) was measured independently (peak force 2000 N, < 0.5 N resolution and measurement uncertainty of < 3%). Instantaneous angular velocity of the crank ($Rad.s^{-1}$) was measured with a resolution of 2° using three independent sensors sampling in series (measurement uncertainty of < 1%). Power (W) was calculated every 2° from torque and angular velocity measurements. There was no systematic difference in the power production between the left and right cranks, and therefore power from the right crank only was used (10), and averaged over 3 crank revolutions to provide a paired datum with EMG from the same leg (described below).

Electromyography

Surface EMG was measured in five muscles of the right leg: *vastus lateralis*, *rectus femoris*, *vastus medialis*, *biceps femoris*, and *gastrocnemius lateralis*. Placement sites were shaved, abraded with gauze, and cleaned with 70%vol. isopropyl alcohol. Wireless transmitting Ag bipolar parallel-bar surface electrodes were placed over the muscle belly using Surface Electromyography for the Non-Invasive Assessment of Muscles (SENIAM) recommendations (Trigno Wireless System, Delsys Inc., Boston, MA). Electrodes were placed over the *vastus lateralis* $2/3^{rds}$ of the distance from the anterior superior iliac spine to the lateral side of the patella, over the *rectus femoris* halfway between the anterior superior iliac spine and the superior border of the patella, over the *vastus medialis* $8/10^{ths}$ of the distance from the anterior superior iliac spine to the joint space in front of the anterior border of the medial ligament, over the *biceps femoris* halfway between the ischial tuberosity and lateral epicondyle of the tibia, and over the *gastrocnemius lateralis* $1/3^{rd}$ the distance between the head of the fibula and the calcaneus. The longitudinal axis of the electrode was aligned parallel to the long axis of the muscle.

EMG signals were differentially amplified and sampled at 2 kHz with 16-bit resolution. Each sensor had a signal bandwidth of 20-450 Hz and common mode rejection ratio of >80 dB. During post-processing, signals were filtered with a second-order Butterworth band-pass filter (3dB, 10-500 Hz) and smoothed via root mean square (RMS) with a 100 ms moving window with no overlap. The peak activity (μV; from the 100 ms RMS) from each crank revolution was used as an estimate of muscle activity. The earliest three consecutive isokinetic crank revolutions that were appropriately constrained at the desired angular velocity were identified in the output from the cycle ergometer, and the peak RMS EMG from these were ensemble averaged for each muscle; these were typically the 2^{nd}, 3^{rd}, and 4^{th} crank revolutions after switching to isokinetic cycling. The RMS EMG values from the 5 muscles of the right leg were averaged to provide an EMG datum to pair with P_{iso} produced at the crank from the same leg. The muscle selection reflected the weighted power contributions from knee extension/flexion and plantarflexion (12).

EMG-P_{iso} Relationship and Fatigue Characterization

The characterization of the EMG-P_{iso} relationship is specific to the electrode placement (skin preparation, conduction, etc.), and therefore the baseline 'physiologic normalization' (e.g. Figure 1D) was repeated at each laboratory visit, and the RMS EMG values normalized to the visit maximum. The baseline relationship between power production and EMG activity (*Measurement of Baseline EMG-P_{iso}*) was characterized using linear regression at each angular velocity.

Measurements made at the limit of tolerance and in recovery from ramp incremental exercise were used to calculate three indices of fatigue. Performance fatigue (PF) is the reduction in P_{iso} (W) from the baseline maximum. The proportion of PF resulting from activation fatigue (AF, expressed in W) is calculated from the power equivalent of the reduction in RMS EMG activity, using the baseline linear regression between EMG and P_{iso} at 70rpm. Muscle fatigue (MF) was calculated from the balance (MF = PF – AF;

with lower bounds constrained at 0 W), i.e. the deviation in W from the baseline EMG-P_{iso} relationship at the measured EMG value (for a graphical representation, see Figure 4A).

Pulmonary Gas Exchange

Respired gases were measured breath-by-breath with a commercial metabolic measurement system (VMax Spectra, Sensormedics, Yorba Linda, CA, USA). The system was calibrated immediately prior to each testing session. A 3 L syringe (Hans Rudolph Inc., Shawnee, KS, USA) was used to calibrate the flow sensor (hot-wire anemometer) from ~0.2 to 8 $L.s^{-1}$, mimicking flow rates expected at rest and during exercise. The CO_2 and O_2 analyzers were calibrated using gases of known concentrations (O_2 26.0% and 16.0%; CO_2 0.0% and 4.0%). ECG (Cardiosoft, GE Healthcare, Little Chalfont, UK) and finger pulse oximetry (Radical-7, Masimo Corp, Irvine, CA) was monitored throughout exercise.

Statistical Analyses

Means were compared, where appropriate, with t-tests, ANOVA, or repeated measures ANOVA. ANCOVA was used to assess the velocity-dependence of each individual's baseline EMG-P_{iso} relationship. The standard error of the estimate (SEE) of the baseline EMG-P_{iso} regression was calculated as an index of sensitivity of muscle fatigue measurement. Bland-Altman plots were generated for an index of reproducibility (6). Statistical significance was determined at $p<0.05$. Statistics were computed using IBM SPSS v20. Data are presented as mean±SD.

Results

Measurement of Baseline EMG-P_{iso}. As expected, participants' peak single-leg isokinetic power (measured at the right crank arm, mean over 3 revolutions) during baseline assessment was strongly cadence-dependent: 232±56 W at 50 rpm; 307±75 W at 70 rpm; and 373±114 W at 100 rpm (n=13; F[1.1,13.3]=49.9;

$p<0.05$; $\eta^2=0.81$; all Bonferroni *post hoc* comparisons $p<0.05$; Figure 2). Importantly, individual EMG-P_{iso} relationships were strongly linear over the ranges measured (between ~25-100% effort, $r^2=0.95\pm0.04$). Individual EMG-P_{iso} slopes differed across the 3 pedaling cadences ($p<0.01$; ANCOVA) (Figure 2) in all but one participant ($p=0.07$; ANCOVA). The precision of the EMG-P_{iso} regression at 70 rpm was determined using SEE. These values were used to estimate the 'sensitivity' to detect a difference from baseline of a single EMG-P_{iso} measurement during fatigue. The SEE was not different among cadences ($F[2,24]=0.2$; $p>0.05$; $\eta^2=0.02$), and averaged 4.2±2.0%, 4.5±2.2%, and 4.2±2.6% of peak P_{iso} at baseline, at 50, 70, and 100 rpm respectively.

Measurement of EMG-P_{iso} Following Ramp Exercise. A representative example of crank power and cadence during the ramp incremental test and instantaneous isokinetic fatigue measurement is shown in Figure 3. During the ramp incremental test peak power measured at the flywheel was 261±58 W and $\dot{V}O_{2peak}$ was 3.2±0.7 L.min^{-1}. As a group, P_{iso} at the limit of tolerance (termed 'R0', for recovery at 0 minutes; 335±88 W, two-times one leg) was greater than flywheel power (261±58 W, $CI_{Difference}$ 28, 120 W, $p<0.05$), but only 55±14% of baseline P_{iso} (630±156 W, $CI_{Difference}$ 211, 380 W, $p<0.05$).

A representative example of the baseline EMG-P_{iso} relationship, and P_{iso} measured during fatigue is shown in Figure 4. The difference in P_{iso} between baseline and R0 determined PF (158±80 W, one leg): On the EMG-P_{iso} plot (Figure 4A), PF is the total displacement in the power (y) dimension. There was a significant inverse relationship between $\dot{V}O_{2peak}$ and PF normalized to baseline peak P_{iso} ($r^2=0.49$; $p<0.05$), i.e. a greater oxidative capacity associated with lesser relative PF at the limit of ramp incremental exercise (Figure 4C).

The baseline EMG-P_{iso} relationship was then used to characterize activation and muscle fatigue in each participant at the limit of tolerance (R0) and the first 3 minutes during recovery (R1, R2, R3). A representative example is shown in Figure 4B, and group data in Figure 5. Performance fatigue is rank-ordered in

Figure 5A, with activation and muscle fatigue in the hashed and closed bars, respectively. Activation and muscle fatigue were 97±555 W and 60±50 W, respectively. Fatigue assessment during recovery from intolerance revealed that P_{iso} varied across time (n=13; F[1.7, 19.9]=44.1; p<0.05; η^2=0.79) and was reduced (p<0.05, Bonferroni) *vs*. baseline measures at R0 ($CI_{Difference}$ 80, 201 W) and R1 ($CI_{Difference}$ 13, 80 W) (Figure 5B).

Reproducibility of the primary measurements required for fatigue assessment for 11 participants was determined from the mean bias and limits of agreement (LoA) using a Bland-Altman plot. Mean bias ± LoA were: $\dot{V}O_{2peak}$ 0.1±0.5 $L.min^{1}$ (Figure 6A); baseline P_{iso} 1±30 W (Figure 6B); P_{iso} at R0 3±35 W (Figure 6C); and EMG at P_{iso} 3±14% (Figure 6D).

Discussion

Our main findings are that the relationship between leg muscle activity and isokinetic power during cycle ergometry is linear and velocity-dependent (Figure 2), and was reproducible across visits. Using standard incremental exercise followed immediately by measurement of maximal voluntary isokinetic power and leg muscle surface EMG, we were able to make sensitive assessments of the proportion of PF that resulted from a reduction in maximal voluntary muscle activity (activation fatigue), and that from the reduction in muscle power production for the achieved muscle activity (muscle fatigue) (Figure 4, and Figure 5). Additionally, we showed that the dynamics of power recovery are rapid (30), returning to baseline in approximately 2-3 min (half-time ~30-60 s; Figure 5B). The reproducibility of the primary measurements was similar to the test-retest reproducibility of $\dot{V}O_{2peak}$ measurement, i.e. ~10% (1) (Figure 6), suggesting that this new fatigue assessment is useful for localizing the variables that determine exercise intolerance during whole-body exercise.

The EMG-P_{iso} Relationship During Isokinetic Cycle Ergometry

As a reference for the power production expected from a given muscle activity, we measured the relationship between surface EMG and P_{iso} in the rested, or baseline, state. We reasoned that should this relationship be confidently predictable and reproducible, then we would be able to generate a 'physiologic normalization' for an individual that could be used as a baseline comparator for measurements made in a fatigued state. The EMG-P_{iso} relationship was strikingly linear and highly reproducible over the range of ~25-100% maximal effort (Figure 2), providing a robust and sensitive framework for determining fatigue components in whole-body exercise.

The EMG-P_{iso} was significantly dependent on contraction velocity (steepening between 50 and 100 rpm). This reinforces that any fatigue comparisons need to be made at an isokinetic velocity (8). This velocity-dependence of the EMG-P_{iso} relationship is expected from the parabolic nature of the muscle power-velocity curve. Motor activity is maximal at any point on the power-velocity curve; in this study each individual's absolute RMS EMG was not different across maximal efforts at each contraction velocity. Thus, maximal motor activity at 50 rpm gives rise to a lower power than at 100 rpm. The fact that the difference between EMG-P_{iso} slopes was greater between 50 rpm and 70 rpm than between 70 rpm and 100 rpm reflects the power-velocity curvature, and that at 100 rpm individuals are approaching the velocity at which isokinetic power is maximal (3).

Power Reserve at the Limit of Tolerance

Previous attempts to identify a reserve in power production at the limit of tolerance have led to the controversial conclusion that exercise is constrained not by inability to produce power, but by perception of effort, and that a large reserve in power production capacity is present (24). Unfortunately, this conclusion was heavily influenced by an ergometry protocol that did not control for contraction velocity (8, 23). Conversely, we showed a large (55%) reduction in peak P_{iso} from 630 W at baseline to 335 W at the limit of tolerance, which was associated with a relatively small (18±11% of P_{iso}) reserve in power at the

limit of tolerance (CI_{95} 28, 120 W) in agreement with our previous work using isokinetic ergometry (13). Our data confirm that the limit of tolerance is reached with a severe depression in the locomotor muscle power generating capacity, and this power is not substantially more than that produced at the end of ramp incremental exercise [*cf.* (24)]. The difference, 74±75 W, may result from the ~1-2 s of recovery as the participant accelerates the ergometer flywheel from ~50 rpm at the limit of tolerance in the ramp to the reference isokinetic velocity of 70 rpm. However, we believe that this difference more likely reflects (a very limited) capacity for short-term power production. In fact, large swings in instantaneous power, measured at the crank arm, were clearly visible during the ramp incremental phase (Figure 3). In other words, the very smooth linear increase in power at the flywheel is not indicative of the rapid fluctuation in power measured at the crank arm. The flywheel power represents the mean power required for the task, while the fluctuations in crank power can exceed the flywheel power by more than 30%. In some participants, these swings amplify during the final minutes and seconds of ramp exercise as the motor system becomes increasingly challenged by the demands of task, demonstrating some capacity for fleeting increases in power production as intolerance encroaches.

How local metabolic factors, afferent feedback, and motor system excitability combine to modulate muscle power and impose limits on exercise is unknown. However, our method provides information on the proportion of performance deficit that is attributable to muscle fatigue: a reduction in power at the achieved maximal voluntary motor activation. The balance of the performance deficit, therefore, is due to a reduction in muscle activation. Reduced activation may result from feedback generated by the muscle fatigue, or fatigue-related metabolites, or other physiological processes and perceptions contributing to symptom limitation, depending on the exercise condition or state of health (18). In this study of healthy volunteers there was a strong association between $\dot{V}O_{2peak}$ and relative PF, suggesting that individuals with greater aerobic capacity are better protected from fatigue, and are able to reach a greater proportion of their baseline peak P_{iso} before reaching intolerance. While the average contribution of MF to the performance deficit at the limit of tolerance

was approximately 35%, there was variability among individuals in the MF fraction, e.g. participants 5, 7, 9, and 13 (Figure 5A). None of the variables measured in this study could clearly explain these exceptions. Thus, the next steps are to employ the method to: 1) associate the magnitude of these fatigue components to physiologic, morphologic, or sensory differences among individuals; and 2) identify how interventions modify the components of fatigue, especially in patients with chronic cardiopulmonary and muscle diseases that predispose to poor exercise tolerance.

Recovery Dynamics and Alternatives for Measuring Fatigue

Central to our approach was finding an alternative assessment for fatigue that could be applied essentially instantaneously during or following whole-body exercise. Alternative methods such as PNS or TMS necessitate isometric contraction, and are limited to following immediately from similar single-joint exercise, such as knee-extension (7, 14), and therefore rarely elicit maximal cardiorespiratory limits. Alternatively, the time delay of transferring from a treadmill or cycle ergometer in order to make the fatigue measurements, limits the ability to assess the mechanisms of fatigue instantaneously at the point of limitation (2). Further, the effect of partially potentiated twitch may confound the otherwise strong (r^2>0.8) relationship with MVC-measured force (27), while fully potentiated twitch appears to be more robust and reproducible (22, 27).

Our data show that recovery of voluntary power production following cycling intolerance at $\dot{V}O_{2peak}$ is restored rapidly back to near-baseline levels within 2-3 min (30, 36). This is in agreement with previous isokinetic measurements (30), the dynamics of supraspinal and neuromuscular fatigue recovery after single-leg knee-extensor exercise and magnetic/electrical simulation (14, 16), and conforms closely to the recovery dynamics of intramuscular fatigue-related metabolites accumulated during exercise (28, 29). In our data, peak P_{iso} at 2 min of recovery (R2) was restored back to baseline P_{iso} (*p*=n.s.), reinforcing the necessity for fatigue measurements to be made at, or following rapidly, the time of interest (14, 16).

Limitations

Our aim was to develop a method that could evaluate the activation and muscle fatigue contributions to exercise intolerance during whole-body exercise that elicits maximal cardiorespiratory strain. Although methods allowing external neuromuscular stimulation during whole-body voluntary exercise are available (31-33, 35), to quantify muscle power and simplify the application of fatigue assessment we chose to rely on voluntary activation. Thus, our measurements at the limit of tolerance, and in recovery, rely on the participant to give a maximal voluntary effort, and are not independently verified by the imposition of controlled or standardized external stimuli. Naturally, the same argument could be made for most exercise tests. However, in our case, there is an opportunity to interpret a reduction in surface EMG and cycling power to some 'activation fatigue' when it may result from a sub-maximal effort. This is of particular concern when no skeletal muscle fatigue can be identified using the method, and represents an opportunity for a 'false positive' to identify activation fatigue. We do not yet have a solution to this limitation. However, we are confident in the reproducibility of both baseline and R0 isokinetic power measurements (LoA ± 10 W (~1% of baseline P_{iso}) and 20 W (~6% of R0 P_{iso}), respectively); the likelihood is low that a repeated sub-maximal efforts could be delivered independently at $\dot{V}O_{2peak}$ with such precision. It is likely that familiarization to maximal isokinetic efforts afforded by the baseline assessment is important in minimizing these limits of agreement, and these brief efforts can be reliably repeated as needed. Nonetheless, this method remains a functional assessment dependent upon a maximal effort by a well-motivated participant.

The EMG-P_{iso} relationship must be assessed at each placement of the EMG electrodes. Even with indelible markings on the skin, changes in skin temperature, hydration, skin preparation, and conductance, rule out comparisons between days without normalization in the EMG axis. We found that there was generally little difference in absolute EMG values within an individual between visits; however, this was not the case for every test. Thus, a 'physiologic normalization' needs to be performed on each visit by each participant, adding time and effort to the exercise test. Nevertheless, using this

approach, we found good agreement between EMG at R0 after normalization (LoA ±14%). It is worth noting that the fatiguing exercise bouts in this study were relatively short (~10 minutes). We do not know whether longer bouts of exercise that result in large core and muscle temperature change might result in a distortion of the EMG-P_{iso} relationship.

Finally, the effects of amplitude cancellation and changes in signal conduction velocity with fatigue might partly explain the variability in EMG (Figure 6D). Here we use EMG amplitude to reflect muscle activity. However, amplitude cancellation increases act to reduce the apparent EMG amplitude for a given muscle activation, the magnitude of which is influenced by fatigue. Conduction velocity may also change with fatigue, increasing or decreasing EMG amplitude for a given muscle activation. In addition, these effects interact with each other variably, depending on %MVC. These effects confound interpretation of EMG amplitude either in terms of muscle activity or as an index of neural drive (11, 20). During cycling, EMG activity in the knee extensors during a single maximal pedal stroke is ~25% of knee extension MVC (17). Even in normalized EMG at relatively low power, some degree of amplitude cancellation is likely present. Nevertheless, at ~25% MVC, EMG amplitude is relatively unaffected by conduction velocity compared to high %MVC maneuvers (11), making predictions about muscle activity from EMG somewhat less problematic. While it is tempting to conclude that EMG amplitude is an indicator of the central motor drive, and this has often been the approach used in the past, it is currently not certain how the motor input is related to the EMG signal.

Conclusion

Establishing an individual's isokinetic EMG-power relationship for fatigue assessment is a reliable addition to a standard cycle ergometer exercise test. This relationship is linear, velocity dependent, and reproducible. The method is sufficiently sensitive to quantify the muscle activation and muscle fatigue components of performance fatigue during whole-body exercise. Deviation from the baseline isokinetic EMG-power relationship during or following

exercise can provide insight on the nature of exercise intolerance in health and disease.

Acknowledgements

This study was supported by the Pulmonary Education & Research Foundation (PERF). ACC was supported by a scholarship from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Brazil (3468/13-5). RC occupies the Grancell/Burns Chair in the Rehabilitative Sciences. We thank the volunteers for their time and dedication.

Figure Legends

Figure 1. Representative participant data showing the characterization of the isokinetic EMG-power relationship. Measurements were taken during four variable effort isokinetic bouts. **Panel A:** Raw EMG measured in a representative muscle (*vastus lateralis*). **Panel B:** Power measured at the right crank arm every 2° of rotation. **Panel C:** Pedaling frequency (velocity) during the variable effort isokinetic bouts. Data show the ergometer tightly constraining the angular velocity, despite large changes in pedal forces. **Panel D**: Isokinetic EMG-Power relationship (EMG-P_{iso}). EMG is normalized to maximum activity while power is plotted in W. Dotted lines represent 95% prediction bands for linear regression.

Figure 2. The velocity-dependence of the isokinetic EMG-power relationship. **Panel A:** Representative participant values for EMG-power relationship at 50, 70, and 100 rpm. **Panel B:** Group mean values for the isokinetic EMG-power relationship at 50 (●), 70 (○), and 100 (▲) rpm. Error bars are SD. ***** denotes significant (p<0.05) difference for slope across pedaling frequencies via ANCOVA.

Figure 3. Representative participant data during a ramp incremental test terminated with a maximal effort, 5 s, isokinetic bout. The dotted lines indicate that data are continuous, where the x-axis is expanded to better visualize pedal-by-pedal power measurements. Grey shading along the x-axis represents hyperbolic ergometry, or cadence independent power. Black shading along the x-axis represents isokinetic ergometry at 70 rpm. **Panel A:** Average crank arm power measured during ramp and isokinetic exercise. **Panel B:** Pedaling frequency (velocity) measured throughout the experiment. During the isokinetic phase, cadence is constrained at the target velocity.

Figure 4. EMG-power relationship from a representative participant and group data for the relationship between performance fatigue and $\dot{V}O_{2peak}$. Data are variable-effort isokinetic bouts (●), maximal isokinetic effort at the limit of tolerance (○). All measurements were taken at 70 rpm. **Panel A:**

Representative participant showing approximately equal components of muscle and activation fatigue to total performance fatigue. PF arrow represents the total reduction in power generation. MF arrow represents the proportion of PF that can be attributed to muscle fatigue i.e. a lower power than expected for the measured EMG. The balance, AF, represents the proportion of PF from a reduced muscle activity. BL: baseline, R0: 0 min recovery (immediately following the limit of tolerance), PF: performance fatigue, MF: muscle fatigue, AF: activation fatigue. **Panel B:** Same data as in Panel A, but open symbols with crosses/lines represent maximal isokinetic efforts during recovery. R1, R2, R3: recovery at 1, 2, 3 min post-limit of tolerance. **Panel C:** Relationship between PF (normalized to P_{iso} at baseline) and $\dot{V}O_{2peak}$. r^2=0.49, p<0.05

Figure 5. Contributions of muscle and activation fatigue to performance fatigue for all participants at the limit of tolerance, and the recovery dynamics of maximal isokinetic power. **Panel A:** Each bar represents a participant's performance fatigue (PF) (individuals are arranged in order of declining performance fatigue), and solid filled portion shows muscle fatigue (MF). **Panel B:** Maximal isokinetic power in the right crank arm measured at 70 rpm during baseline (BL), at the limit of tolerance with no recovery (R0), and at 1, 2, and 3 min of recovery (R1, R2, R3). *Significantly different from BL value.

Figure 6. Bland-Altman plots for reproducibility of the primary variables. In each case, the differences between two measurements, obtained on separate days, are plotted as a function of their mean (n=11). Dashed lines show limits of agreement (±1.96 SD). **A:** $\dot{V}O_{2peak}$, **B:** Baseline peak isokinetic power (P_{iso}), **C:** Isokinetic power (P_{iso}) at the limit of tolerance (R0), **D:** EMG during P_{iso} at the limit of tolerance (R0).

Figures

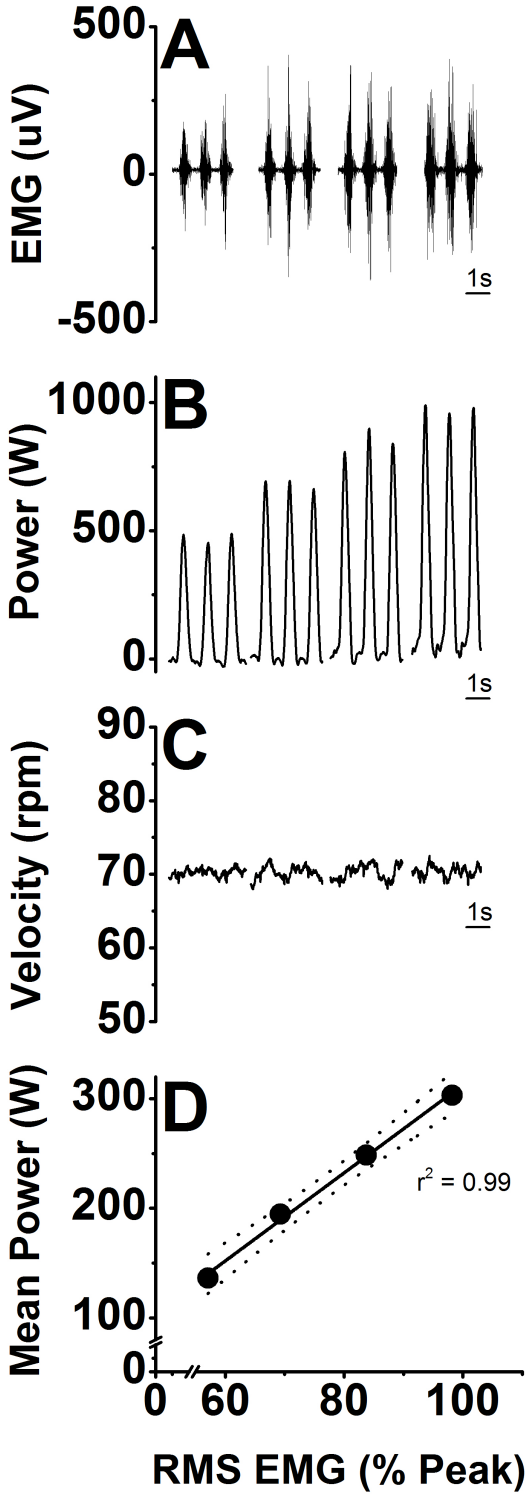

Figure 1.

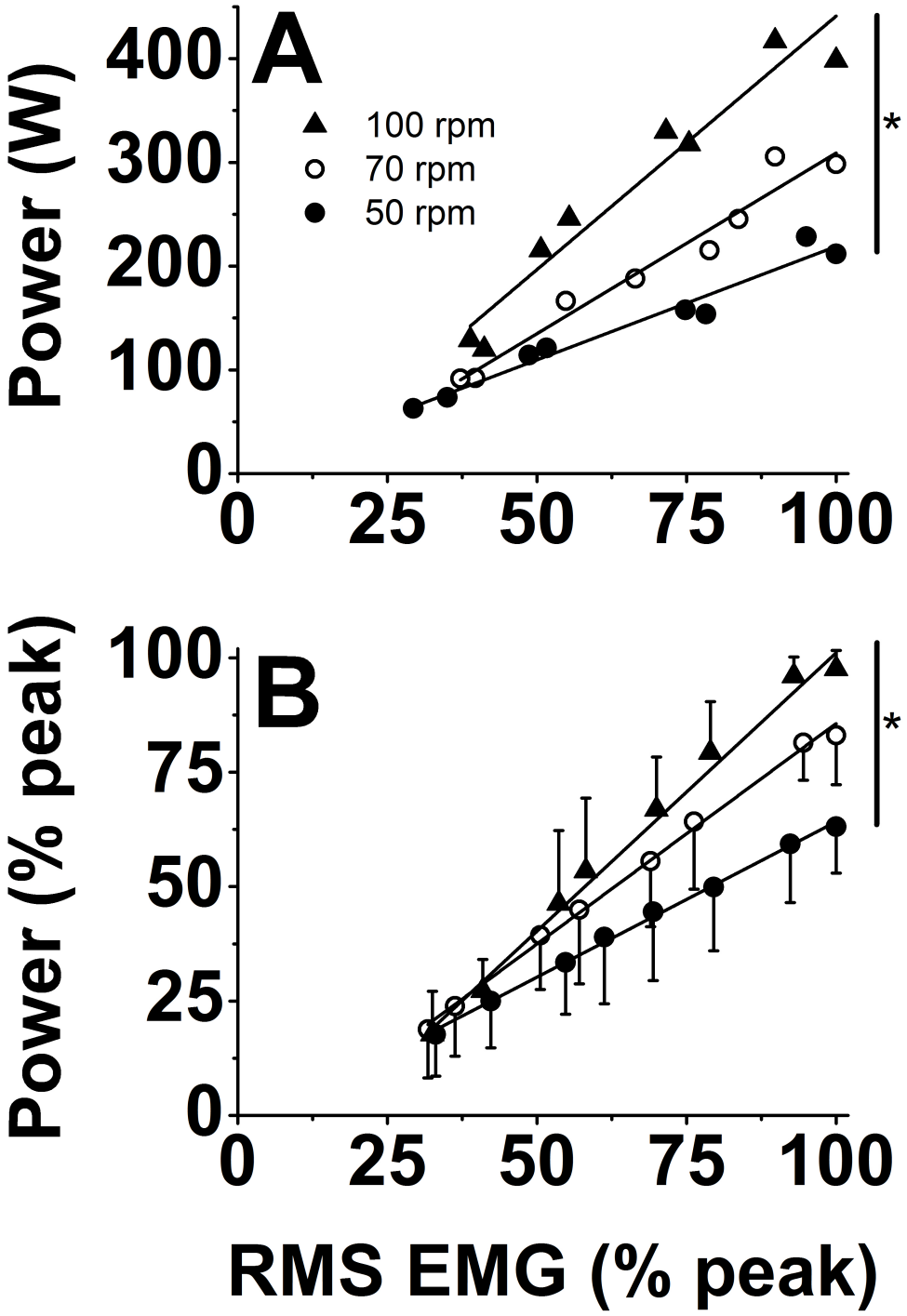
A
100 rpm
70 rpm
50 rpm
Power (W)
400
300
200
100
0
0
25
50
75
100
*
B
Power (% peak)
100
75
50
25
0
0
25
50
75
100
*
RMS EMG (% peak)

Figure 2.

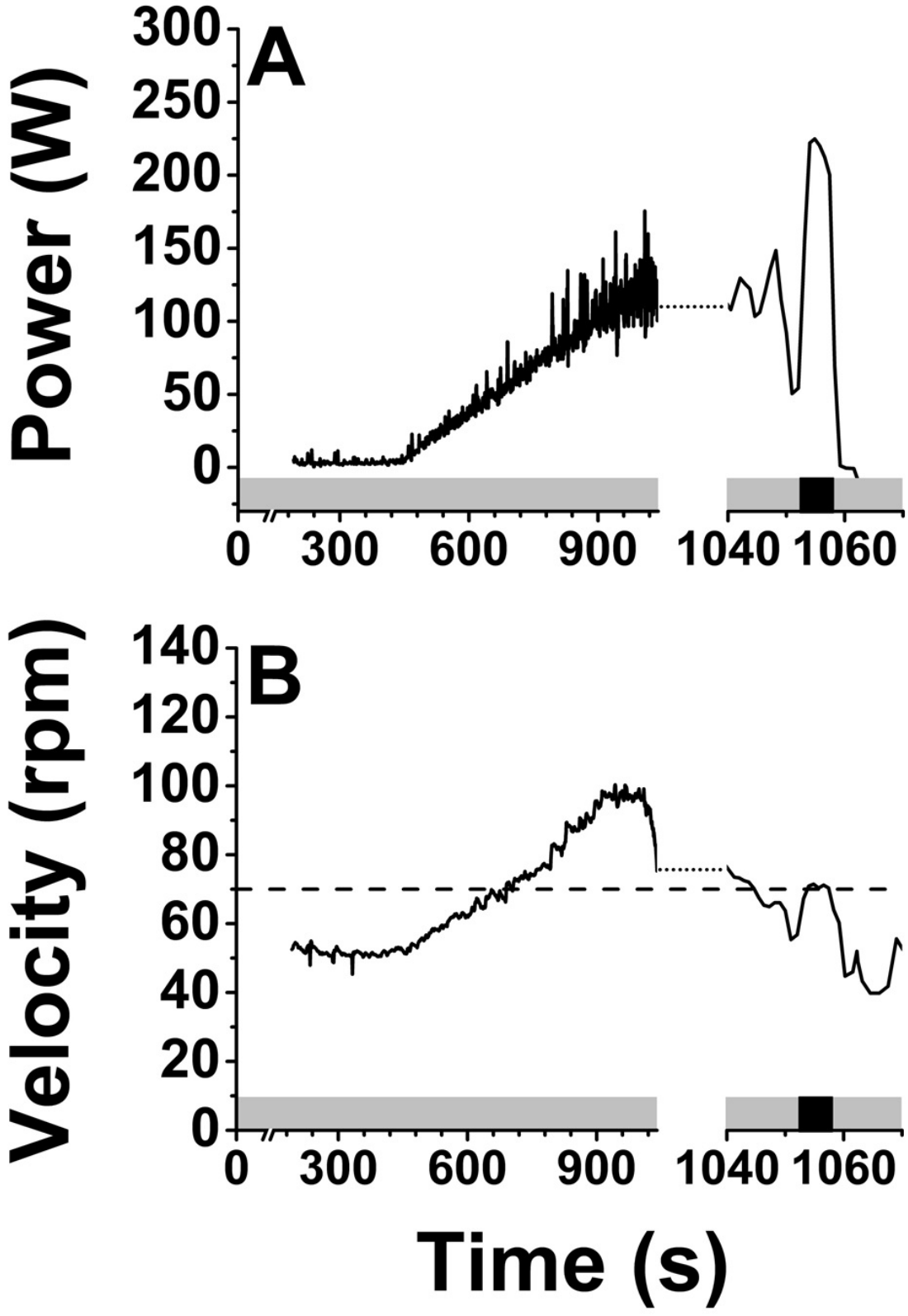
A
Power (W)
300
250
200
150
100
50
0
0
300
600
900
1040
1060
B
Velocity (rpm)
140
120
100
80
60
40
20
0
0
300
600
900
1040
1060
Time (s)

Figure 3.

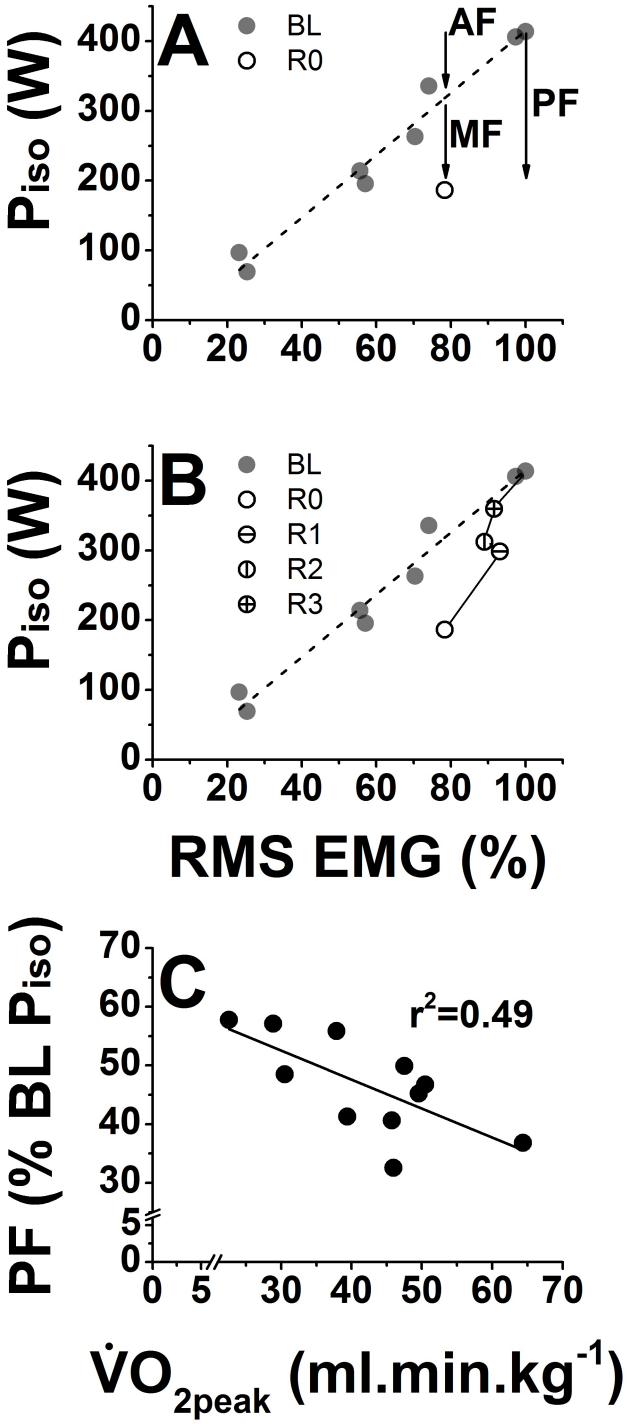
A
BL
R0
AF
MF
PF
Piso (W)
400
300
200
100
0
0
20
40
60
80
100
B
BL
R0
R1
R2
R3
Piso (W)
RMS EMG (%)
C
r2=0.49
PF (% BL Piso)
70
60
50
40
30
5
0
0
5
30
40
50
60
70
V̇O2peak (ml.min.kg-1)

Figure 4.

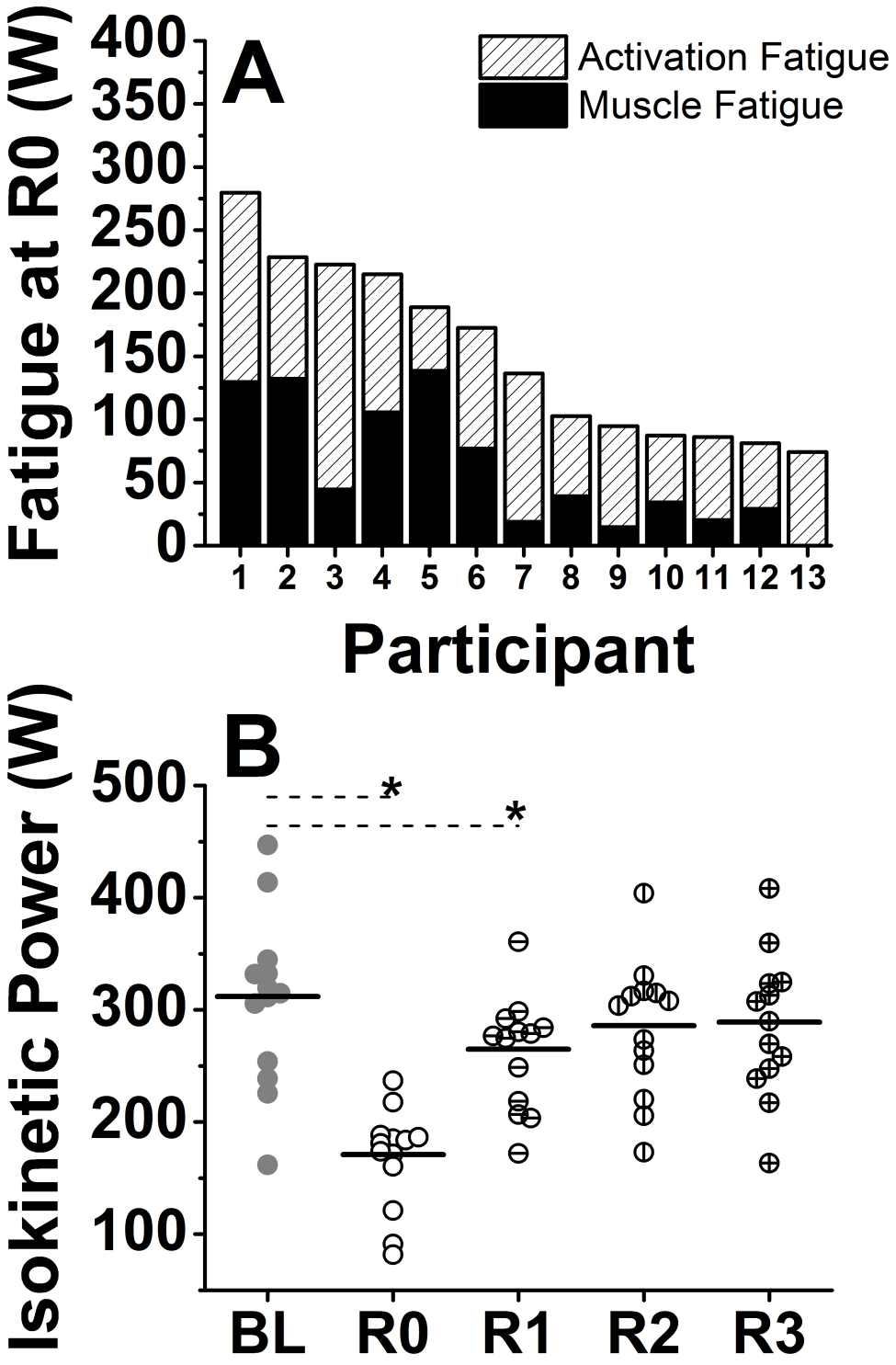
A
Activation Fatigue
Muscle Fatigue
Fatigue at R0 (W)
400
350
300
250
200
150
100
50
0
1 2 3 4 5 6 7 8 9 10 11 12 13
Participant
B
Isokinetic Power (W)
500
400
300
200
100
*
*
BL R0 R1 R2 R3

Figure 5.

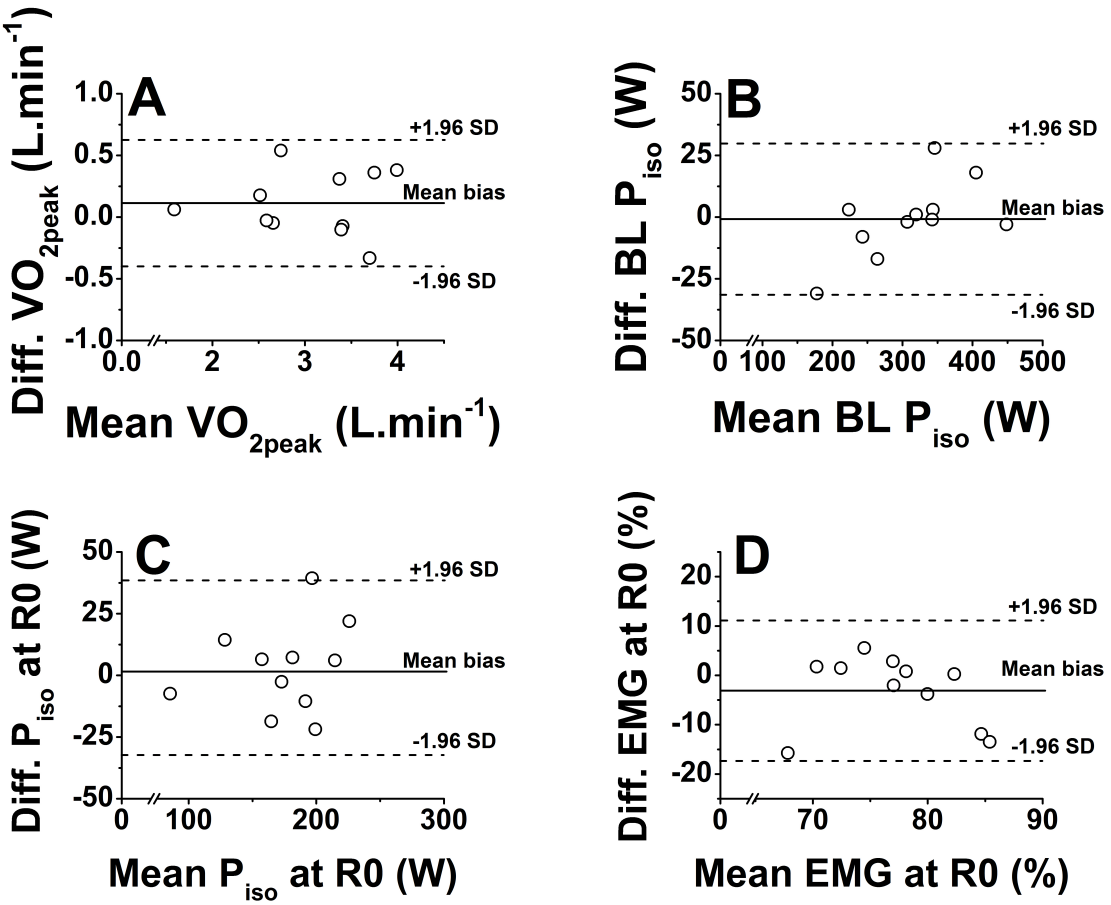
A
+1.96 SD
Mean bias
-1.96 SD
Diff. VO2peak (L.min-1)
Mean VO2peak (L.min-1)
B
+1.96 SD
Mean bias
-1.96 SD
Diff. BL Piso (W)
Mean BL Piso (W)
C
+1.96 SD
Mean bias
-1.96 SD
Diff. Piso at R0 (W)
Mean Piso at R0 (W)
D
+1.96 SD
Mean bias
-1.96 SD
Diff. EMG at R0 (%)
Mean EMG at R0 (%)

Figure 6.

References

1. ATS/ACCP Statement on cardiopulmonary exercise testing. *Am J Respir Crit Care Med* 167: 211-277, 2003.

2. Amann M, and Dempsey JA. Locomotor muscle fatigue modifies central motor drive in healthy humans and imposes a limitation to exercise performance. *J Physiol* 586: 161-173, 2008.

3. Beelen A, and Sargeant AJ. Effect of fatigue on maximal power output at different contraction velocities in humans. *J Appl Physiol* 71: 2332-2337, 1991.

4. Beelen A, and Sargeant AJ. Effect of prior exercise at different pedalling frequencies on maximal power in humans. *Eur J Appl Physiol Occup Physiol* 66: 102-107, 1993.

5. Blair SN, Kohl HW, 3rd, Barlow CE, Paffenbarger RS, Jr., Gibbons LW, and Macera CA. Changes in physical fitness and all-cause mortality. A prospective study of healthy and unhealthy men. *JAMA* 273: 1093-1098, 1995.

6. Bland JM, and Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1: 307-310, 1986.

7. Burnley M. Estimation of critical torque using intermittent isometric maximal voluntary contractions of the quadriceps in humans. *J Appl Physiol (1985)* 106: 975-983, 2009.

8. Burnley M. The limit to exercise tolerance in humans: validity compromised by failing to account for the power-velocity relationship. *Eur J Appl Physiol* 109: 1225-1226, 2010.

9. Cairns SP, Knicker AJ, Thompson MW, and Sjogaard G. Evaluation of models used to study neuromuscular fatigue. *Exerc Sport Sci Rev* 33: 9-16, 2005.

10. Cannon DT, White AC, Andriano MF, Kolkhorst FW, and Rossiter HB. Skeletal muscle fatigue precedes the slow component of oxygen uptake kinetics during exercise in humans. *J Physiol* 589: 727-739, 2011.

11. Dideriksen JL, Enoka RM, and Farina D. Neuromuscular adjustments that constrain submaximal EMG amplitude at task failure of sustained isometric contractions. *J Appl Physiol (1985)* 111: 485-494, 2011.

12. Ericson MO, Bratt A, Nisell R, Arborelius UP, and Ekholm J. Power output and work in different muscle groups during ergometer cycling. *Eur J Appl Physiol Occup Physiol* 55: 229-235, 1986.

13. Ferguson C, Wylde LA, Cannon DT, Benson AP, and Rossiter HB. No reserve in maximal voluntary cycling power at the limit of tolerance using isokinetic ergometry. *Med Sci Sports Exerc* 45: S598, 2013.

14. Froyd C, Millet GY, and Noakes TD. The development of peripheral fatigue and short-term recovery during self-paced high-intensity exercise. *J Physiol* 591: 1339-1346, 2013.

15. Goodall S, Charlton K, Howatson G, and Thomas K. Neuromuscular Fatigability during Repeated-Sprint Exercise in Male Athletes. *Med Sci Sports Exerc* 2014.

16. Gruet M, Temesi J, Rupp T, Levy P, Verges S, and Millet GY. Dynamics of corticospinal changes during and after high-intensity quadriceps exercise. *Exp Physiol* 99: 1053-1064, 2014.

17. Hunter AM, St Clair Gibson A, Lambert M, and Noakes TD. Electromyographic (EMG) normalization method for cycle fatigue protocols. *Med Sci Sports Exerc* 34: 857-861, 2002.

18. Jones NL, and Killian KJ. Exercise limitation in health and disease. *N Engl J Med* 343: 632-641, 2000.

19. Jubeau M, Rupp T, Perrey S, Temesi J, Wuyam B, Levy P, Verges S, and Millet GY. Changes in voluntary activation assessed by transcranial magnetic stimulation during prolonged cycling exercise. *PLoS One* 9: e89157, 2014.

20. Keenan KG, Farina D, Maluf KS, Merletti R, and Enoka RM. Influence of amplitude cancellation on the simulated surface electromyogram. *J Appl Physiol (1985)* 98: 120-131, 2005.

21. Klass M, Roelands B, Levenez M, Fontenelle V, Pattyn N, Meeusen R, and Duchateau J. Effects of noradrenaline and dopamine on supraspinal fatigue in well-trained men. *Med Sci Sports Exerc* 44: 2299-2308, 2012.

22. Kufel TJ, Pineda LA, and Mador MJ. Comparison of potentiated and unpotentiated twitches as an index of muscle fatigue. *Muscle Nerve* 25: 438-444, 2002.

23. MacIntosh BR, and Fletcher JR. The parabolic power-velocity relationship does apply to fatigued states. *Eur J Appl Physiol* 111: 319-320, 2011.

24. Marcora SM, and Staiano W. The limit to exercise tolerance in humans: mind over muscle? *Eur J Appl Physiol* 109: 763-770, 2010.

25. Myers J, Prakash M, Froelicher V, Do D, Partington S, and Atwood JE. Exercise capacity and mortality among men referred for exercise testing. *N Engl J Med* 346: 793-801, 2002.

26. Paffenbarger RS, Jr., Hyde RT, Wing AL, Lee IM, Jung DL, and Kampert JB. The association of changes in physical-activity level and other lifestyle characteristics with mortality among men. *N Engl J Med* 328: 538-545, 1993.

27. Polkey MI, Kyroussis D, Hamnegard CH, Mills GH, Green M, and Moxham J. Quadriceps strength and fatigue assessed by magnetic stimulation of the femoral nerve in man. *Muscle Nerve* 19: 549-555, 1996.

28. Rossiter HB, Ward SA, Howe FA, Kowalchuk JM, Griffiths JR, and Whipp BJ. Dynamics of intramuscular 31P-MRS P(i) peak splitting and the slow components of PCr and O2 uptake during exercise. *J Appl Physiol* 93: 2059-2069, 2002.

29. Rossiter HB, Ward SA, Kowalchuk JM, Howe FA, Griffiths JR, and Whipp BJ. Dynamic asymmetry of phosphocreatine concentration and O(2) uptake between the on- and off-transients of moderate- and high-intensity exercise in humans. *J Physiol* 541: 991-1002, 2002.

30. Sargeant AJ, and Dolan P. Effect of prior exercise on maximal short-term power output in humans. *J Appl Physiol* 63: 1475-1480, 1987.

31. Sidhu SK, Cresswell AG, and Carroll TJ. Motor cortex excitability does not increase during sustained cycling exercise to volitional exhaustion. *J Appl Physiol (1985)* 113: 401-409, 2012.

32. Sidhu SK, Hoffman BW, Cresswell AG, and Carroll TJ. Corticospinal contributions to lower limb muscle activity during cycling in humans. *J Neurophysiol* 107: 306-314, 2012.

33. Sidhu SK, Lauber B, Cresswell AG, and Carroll TJ. Sustained cycling exercise increases intracortical inhibition. *Med Sci Sports Exerc* 45: 654-662, 2013.

34. Swallow EB, Reyes D, Hopkinson NS, Man WD, Porcher R, Cetti EJ, Moore AJ, Moxham J, and Polkey MI. Quadriceps strength predicts mortality in patients with moderate to severe chronic obstructive pulmonary disease. *Thorax* 62: 115-120, 2007.

35. Szecsi J, Straube A, and Fornusek C. Comparison of the pedalling performance induced by magnetic and electrical stimulation cycle ergometry in able-bodied subjects. *Med Eng Phys* 36: 484-489, 2014.

36. Szubski C, Burtscher M, and Loscher WN. Neuromuscular fatigue during sustained contractions performed in short-term hypoxia. *Med Sci Sports Exerc* 39: 948-954, 2007.

37. Vanhees L, Fagard R, Thijs L, and Amery A. Prognostic value of training-induced change in peak exercise capacity in patients with myocardial infarcts and patients with coronary bypass surgery. *Am J Cardiol* 76: 1014-1019, 1995.

38. Verges S, Maffiuletti NA, Kerherve H, Decorte N, Wuyam B, and Millet GY. Comparison of electrical and magnetic stimulations to assess quadriceps muscle function. *J Appl Physiol (1985)* 106: 701-710, 2009.

39. Vogiatzis I, and Zakynthinos S. The physiological basis of rehabilitation in chronic heart and lung disease. *J Appl Physiol (1985)* 115: 16-21, 2013.

5. CONCLUSIONS

The objective of this thesis was to develop and validate a method for quantifying MF, AF, and their sum (PF) during whole-body exercise to intolerance in humans. The new method developed was successful in this objective, and showed a similar level of reproducibility to standard cardiopulmonary measurements such as VO_{2max}. It was shown that this method could reliably determine components of fatigue at the instant of intolerance in incremental cycle ergometry in healthy participants ranging in age from 29 to 72 years, and ranging in aerobic capacity between moderate (23.5 ml/min/kg) and endurance trained (62.4 ml/min/kg). Therefore, the new method for determining components of fatigue has promise to shed light on the mechanisms limiting exercise tolerance in patients where it is limited. Comparisons among the various physiological responses to exercise (gas exchange, ventilation, circulation, muscle oxygenation) and magnitude of AF and MF during and following exercise may help to elucidate the primary variables contributing to fatigue and therefore limiting whole body exercise in humans.

Specifically, this thesis tested three hypotheses. Firstly, that the baseline (unfatigued) cycling EMG-P_{ISO} relationship would be sufficiently predictable to act as a frame of reference for determining AF and MF during exercise. As we hypothesized the baseline EMG-P_{ISO} relationship was well modelled by a linear function, which was reproducible day-to-day. The variability of the individual EMG-P_{ISO} measurements between ~25% and 100% effort, around the linear model, was sufficiently tight that the baseline linear relationship allowed for a precise quantification of AF and MF at the limit of tolerance and in recovery from a maximal aerobic exercise task. It was also demonstrated that the EMG-P_{ISO} relationship was velocity dependent, as expected from the parabolic nature power-velocity curve.

The second hypothesis tested was that measurement of the single value EMG-P_{ISO} at the point of intolerance would be reproducible, and allowed the contributions of AF and MF to performance fatigue to be apportioned. It was

found that performance fatigue at the limit of incremental exercise resulted in an approximate 45% decline in power generating capacity compared to baseline. Both AF and MF contributed to this fatigue. While AF contributed ~60% on average to PF, this was somewhat variable among participants, and future work may focus on why some participants are able to minimize AF contributions to fatigue while others cannot. Thus, the second hypothesis of this thesis was accepted.

The final hypothesis was that recovery of fatigue would be rapid, as previously demonstrated in isolated muscle tasks (218). In fact, it was shown the performance fatigue was recovered to ~95% of baseline within 2 minutes of intolerance in incremental exercise. This recovery time course matches well to the recovery of intramuscular PCr (237) and Pi (207) following two-leg very heavy intensity knee-extension exercise, the latter being strongly implicated in the mechanism in MF. Interestingly, the time course of PF recovery measured in cycling exercise in this thesis was even faster than previously demonstrated in isolated muscle tasks. For example, Froyd et al (218) showed that voluntary MVC only recovered to 75% in 8 minutes single leg knee extension time-trial exercise, although tetanic stimulation of the muscle itself showed that force production was recovered to ~90% within 4 minutes. The differences between these previous findings, and the data from this thesis, likely lie in the different modalities used for the exercise. Isolated muscle tasks are likely to produce far greater intramuscular strain and therefore greater muscle fatigue. Whole-body tasks, on the other hand, may be more susceptible to a large contribution from central fatigue, where exercise-related signals from the locomotor muscles, respiratory muscles, cardiac muscles, or sense of dyspnea could each feedback and contribute to inhibiting muscle activity at the spinal or supraspinal levels. The dynamics of recovery of central could be faster than those of muscle fatigue. However, as yet, there is not data to test this hypothesis in whole-body exercise.

6. SUGGESTIONS FOR FUTURE RESEARCH

The work in this thesis has potential utility for future research.

Firstly, the new method developed here showed that this model was tolerated and reproducible in older subjects (up to 72 years old). This means that it can be reliably applied to investigate fatigue in aging and chronic diseases states where fatigue and exercise tolerance are primary symptoms. For example, it would be very interesting to know whether MF and AF change with age. It is well known that non-fatigable type I fiber expression is increased in the elderly (238). It has been proposed that for this reason elderly muscles (studied in isolation) are less fatigable than those of younger subjects (238). Also, elderly subjects report increased 'fatigue' (or tiredness) during daily activities. This new method is well placed to determine whether AF and/or MF are altered in aging during a whole-body bipedal exercise task. In addition using this method it is also possible to measure other physiological variables that may be associated with this fatigue, such as VO_2, V_E, HR or CO, or muscle and cerebral oxygenation.

Secondly, this thesis demonstrated that while both AF and MF contributed to PF, contribution of AF was greater on average and somewhat variable among participants. Thus, future research may focus on why some participants are able to minimize AF contributions to fatigue while others cannot. With this in mind, a better understanding of the mechanisms contributing to central fatigue, in particular, during whole body exercise (central fatigue being far less studied than peripheral fatigue) may reveal new ways to extend the time of exercise in professional athletes and also in patients with chronic disease.

Thirdly, one interesting question related to this thesis is: How might central and peripheral fatigue differ between subjects with heart or lung disease? It could be hypothesized that patients with heart disease will have high levels of MF, because of poor peripheral perfusion, whereas patients with lung diseases (at least those without severe desaturation) will have greater levels of AF induced by the sensations related to dyspnea. Applying this new method in patients with

heart and lung disease may help to reveal the locus (central or peripheral) of fatigue in these patient groups, and therefore be used to guide therapeutic interventions to increase exercise tolerance and physical activity.

Another question that could be addressed by this technique is whether reducing ventilatory demands of exercise in COPD, e.g. by increasing inspired oxygen fraction, would increase exercise tolerance by reducing AF or MF? It is possible that high F_iO_2 could increase O_2 delivery to the muscles therefore reduce MF. Alternatively, high F_iO_2 may reduce ventilatory demands and reduce dyspnea and AF. Were AF to be linked to dyspnea (or some measure of respiratory muscle work or fatigue) it would have profound implications for the mechanisms by which exercise is limited in these patients. Currently it is assumed that dyspnea may lead to exercise intolerance i.e. at a 'single point' when dyspnea reaches intolerance levels the exercise task would be slowed or ceased. However, it is unknown whether dyspnea contributes to fatigue – the dynamic process that ends in task failure. A demonstration that neural feedback from the pulmonary system can progressively reduce motor outflow (i.e. increase AF) would be a new mechanism by which exercise is regulated by the CNS. Which of these mechanisms provide the major operational limitation in COPD (AF or MF) could help to determine which patients would benefit more from O_2 therapy compared to rehabilitation of the peripheral muscles.

Thus, it is hoped that the new method developed in this thesis will contribute to developing a better understanding of the mechanisms limiting exercise tolerance in health and disease, and therefore help improve the lives of patients where exercise intolerance is a major symptom.

7. REFERENCES

1. Oja P, Bull FC, Fogelholm M, Martin BW. Physical activity recommendations for health: what should Europe do? BMC Public Health. 2010;10:10.
2. Booth FW, Lees SJ. Fundamental questions about genes, inactivity, and chronic diseases. 2007;28(2):146-57.
3. Garber CE, Blissmer B, Deschenes MR, Franklin BA, Lamonte MJ, Lee IM, et al. American College of Sports Medicine position stand. Quantity and quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently healthy adults: guidance for prescribing exercise. Med Sci Sports Exerc. 2011;43(7):1334-59.
4. Koeneman MA, Verheijden MW, Chinapaw MJM, Hopman-Rock M. Determinants of physical activity and exercise in healthy older adults: A Systematic Review. International Journal of Behavioral Nutrition and Physical Activity. 2011(8):142.
5. Jones NL, Killian KJ. Exercise Limitation in Health and Disease. New England Journal of Medicine. 2000;343(9):632-41.
6. Huxley A, Niedergerke R. Structural changes in muscle during contraction; interference microscopy of living muscle fibres
. Nature. 1954;173(4412):971.
7. Huxley H, Hanson J. Changes in the cross-striations of muscle during contraction and stretch and their structural interpretation. Nature 1954;173 (4412):973.
8. Saladin K. Anatomy and Physiology: The Unity of Form and Function. New York: McGraw Hill; 2012.
9. Jones D, Round J. Skeletal muscle in health and disease: Manchester University Press; 1992.
10. Powers SK, Howley ET. Skeletal Muscle: Structure and Function. 6th ed. New York: McGraw-Hill; 2007.
11. Bigland-Ritchie B, Jones DA, Woods JJ. Excitation frequency and muscle fatigue: Electrical responses during human voluntary and stimulated contractions. Experimental Neurology. 1979;64(2):414–27.

12. De Luca CJ, Erim Z. Common drive of motor units in regulation of muscle force. Trends in Neurosciences. 1994;17(7):299-305.
13. Whipp B, Sargeant A. Physiological Determinants of Exercise Tolerance in Humans. London: The Physiological Society; 1999.
14. McArdle W, Katch F, Katch V. Exercise Physiology: Nutrition, Energy, and Human Performance. 7th ed. Philadelphia: Lippincott Williams & Wilkins; 2009.
15. Saladin K. Anatomy and Physiology. 3rd ed. New York: Watnick; 2010.
16. Martini FH, Timmons MJ, Tallitsch RB. Human Anatomy: Benjamin Cummings; 2008.
17. Larsson L, Moss R. Maximum velocity of shortening in relation to myosin isoform composition in single fibres from human skeletal muscle. J Physiol 1993(472):595-614.
18. Sant'Ana Pereira J, Wessels A, Nijtmara L, Moorman A, Sargeant A. New mwthod for the accurate chraracterisation of single human skeletal muscle fibres demonstrates a relation between mATPase and MyHC expression in pure and hybrid fibres types. J Musc Res Cell Motility 1995(16):21-34.
19. Sant'Ana Pereira J, Sargeant A, de Haan A, Rademarker A, van Mechelen W. Myosin heavy chain isoform expression and high energy phosphate content of human muscle fibres at rest and post-exercise. J Physiol 1996. 1996;492(2):583-8.
20. Stienen G, Kiers J, Bottinelli R, Reggiani C. Myofibrillar ATPase activity in skinned human skeletal muscle fibres: fibre type and temperature dependence. J Physiol. 1996;493(2):299-308.
21. Henneman E, Mendell L. Functional organization of motoneurons pool and its inputs. In: VB B, editor. Handbook of Physiology I. II. Bethesda: American Physiological Society; 1981. p. 423-507.
22. Maughan R, Gleeson M. The Biochemical Basis of Sports Performance. 2nd ed. New York: Oxford University Press; 2010.
23. Woledge R, Curtin N, Homsher E. Energetic Aspects of Nuscle Contraction. London: Academic Press; 1985.
24. Beelen A, Sargeant AJ. Effect of fatigue on maximal power output at different contraction velocities in humans. 1991;71(6):2332-7.

25. Gregor RJ, Broker JP, Ryan MM. The biomechanics of cycling. Exerc Sport Sci Rev. 1991;19:127–69.
26. De Luca CJ. Use of the surface EMG signal for performance evaluation of back muscles. Muscle Nerve 1993;16:210–6.
27. De Luca CJ. Electromyography. Encyclopedia of Medical Devices and Instrumentation. Webster JG, editor: John Wiley Publisher; 2006.
28. Basmajian JV, De Luca CJ. Muscles Alive. 5th ed. Baltimore: Williams & Wilkins; 1985.
29. Mirca GA. The quantification of EMG normalization error. Ergonomics. 1991;34:343–52.
30. Knutson LM, Clarke WR, Sodberg GL. A study of various normalization procedures for within day electromyographic data. J Electromyogr Kinesiol. 1994;4:47–59.
31. Hill A. Adenosine triphosphate and muscular contraction. Nature. 1949;163:320.
32. Blomstrand E, Ekblom B, Newsholme E. Maximum activities of key glycolytic and oxidative enzymes in human muscle from differently trained individuals. J Physiol. 1986(381):111-8.
33. Newsholme E, Start C. Regulation in metabolism. London: John Wiley; 1973.
34. Chasiotis D, Sahlin K, Hultman E. Regulation of glycogenolysis in human muscle at rest and during exercise. J Appl Physiol. 1982;53:708-15.
35. Katz A, Sahlin K. Regulation of lactic acid production during exercise. J Appl Physiol. 1988(65):509-18.
36. Hultman E. Pyruvate dehydrogenase as a regulator of substrate utilization in skeletal muscle. Maughan R, Shirreffs S, editors. Champaign: Human Kinetics; 1996.
37. Dyck D, Putman C, Heigenhauser G, Hultman E, Spriet L. Regulation of fat-carbohydrate interaction in skeletal muscle during intense aerobic cycling. Am J Physiol 1993(265):E852-E9.
38. Sugden M, Holness M. Interactive regulation of the pyruvate dehydrogenase complex and carnitine palmitoyltransferase system. FASEB J. 1994;8:54-61.

39. Kowalchuk J, Heigenhauser G, Lindinger M, Sutton J, Jones N. Factors influencing hydrogen ion concentration in muscle after intense exercise. J Appl Physiol 1988(65):2080-9.
40. Harris R, Sahlin K, Hultman E. Phosphagen and lactate contents of m. quadriceps femoris of man after exercise. J Appl Physiol 1977(43):852-7.
41. Sahlin K, Harris R, Hultman E. Creatine kinase equilibrium and lactate content compared with muscle pH in tissue samples obtained after isometric exercise. Biochem J. 1975(152):173-80.
42. Bergstrom J, Hermansen L, Hultman E, Saltin B. Diet, muscle glycogen and physical performance. Acta Physiol Scand. 1967(71):140-50.
43. Connett R, Gayeski T, Honig C. Lactate accumulation in fully aerobic, working, dog gracilis muscle. Am J Physiol 1984. 1984;246:H120-H8.
44. Connett R, Honig C, Gayeski T, Brooks G. Defining hypoxia: a systems view of VO2, glycolysis, energetics, and intracellular PO2. J Appl Physiol 1990(68):833-42.
45. Graham T, Saltin B. Estimation of mitochondrial redox state in human skeletal muscle during exercise. J Appl Physiol. 1989(66):561-6.
46. Rowell L. Human cardiovascular control. New York: Oxford University Press; 1993.
47. West J. Respiratory Physiology: The Essentials. 9th ed. Baltimore: Lippincott Williams & Wilkins; 2012.
48. Wasserman K, Hansen JE, Sue DY, Stringer WW, Sietsema KE, Sun X-G, et al. Principles of Exercise Testing and Interpretation 5th ed. Philadelphia: Lippincott Williams & Wilkins; 2012.
49. Fick A. The output of the heart. Physikalisch-Medicinische Gesellschaft. 1870(2):XVI.
50. Stringer W, Hansen J, Wasserman K. Cardiac output estimated noninvasively from oxygen uptake during exercise. Journal of Applied Physiology. 1997(82):908-12.
51. Sullivan M, Knight J, Higginbotham M, Cobb F. Relation between central and peripheral hemodynamics during exercise in patients with chronic heart failure. Circulation. 1989(80):769–81.
52. Weber K, Janicki J. Cardiopulmonary exercise testing for evaluation of chronic heart failure. Am J Cardiol. 1985(55):22A–31A.

53. Guyton A, Jones C, Coleman T. Cardiac output in muscular exercise. Philadelphia: W.B. Saunders; 1973.
54. De Troyer A. Respiratory muscle function. Gibson J, Geddes D, Costabel U, editors. London: Saunders; 2003.
55. Henke K, Sharratt M, Pegelow D, Dempsey J. Regulation of end-expiratory lung volume during exercise. Journal of Applied Physiology. 1988(64):135–46.
56. Dempsey J, Johnson B. Demand vs. capacity in the healthy pulmonary system. Schweizerische Zeitschrift fur Sportmedizin. 1992(40):55–64.
57. Jensen D, Ofir D, O'Donnell D. Effects of pregnancy, obesity and aging on the intensity of perceived breathlessness during exercise in healthy humans. Respiratory Physiology and Neurobiology. 2009(167):87–100.
58. ATS/ACCP, Statement on cardiopulmonary exercise testing. American Journal of Respiratory and Critical Care Medicine. American Journal of Respiratory and Critical Care Medicine. 2003;167(2):211–77.
59. Sun X, Hansen J, Oudiz R, Wasserman K. Exercise pathophysiology in patients with primary pulmonary hypertension. Circulation. 2001(104):429-35.
60. Oudiz R, Midde R, Hovenesyan A, et al. Usefulness of right-to-left shunting and poor exercise gas exchange for predicting prognosis in patients with pulmonary arterial hypertension. American Journal of Cardiology. 2010(105):1186-91.
61. Ponikowski P, Francis D, Piepoli M, et al. Enhanced ventilatory response to exercise in patients with chronic heart failure and preserved exercise tolerance: marker of abnormal cardiorespiratory reflex control and predictor of poor prognosis. Circulation. 2001(103):967–72.
62. Rahn H, Fenn W. A Graphical Analysis of the Respiratory Exchange: The O2-CO2 Diagram. Washington: American Physiological Society; 1955.
63. Dempsey J, Wagner P. Exercise-induced arterial hypoxemia. Journal of Applied Physiology. 1999(87):1997-2006.
64. Nici L. Mechanisms and measures of exercise intolerance in chronic obstructive pulmonary disease. Clinics in Chest Medicine. 2000(21):693-704.
65. Johnson B, Weisman I, Zeballos R, Beck K. Emerging concepts in the evaluation of ventilatory limitation during exercise: the exercise tidal flow-volume loop. Chest. 1999(116):488-503.

66. Gandevia B, Hugh-Jones P. Terminology for measurements of ventilatory capacity; a report to the thoracic society. Thorax. 1957(12):290-3.
67. Sliwinski P, Jan S, Gauthier A, Macklem P. nfluence of global inspiratory muscle fatigue on breathing during exercise. J Appl Physiol. 1996(80):1270-8.
68. Johnson B, Reddan W, Pegelow D, Seow K, Dempsey J. Flow limitation and regulation of functional residual capacity during exercise in a physically active aging population. American Review of Respiratory Disease. 1991(143):960-7.
69. Johnson B, Reddan W, Seow K, Dempsey J. Mechanical constraints on exercise hyperpnea in a fit aging population. American Review of Respiratory Disease. 1991(143):968-77.
70. Pepe P, Marini J. Occult positive end-expiratory pressure in mechanically ventilated patients with airflow obstruction: the auto-PEEP effect. American Review of Respiratory Disease. 1982(126):166–70.
71. Calverley P, Koulouris N. Flow limitation and dynamic hyperinflation: key concepts in modern respiratory physiology. European Respiratory Journal. 2005(25):186–99.
72. Johnson B, Scanlon P, Beck K. Regulation of ventilatory capacity during exercise in asthmatics. Journal of Applied Physiology. 1995(79):892-901.
73. O'Donnell D, Laveneziana P. Physiology and consequences of lung hyperinflation in COPD. European Respiratory Review. 2006(15):61–7.
74. Dawson S, Elliott E. Wave speed limitation on expiratory flow: A unifying concept. Journal of Applied Physiology Respiratory Environmental and Exercise Physiology. 1977(43):498–515.
75. Hyatt R. Expiratory flow limitation. Journal of Applied Physiology Respiratory Environmental and Exercise Physiology. 1983(55): 1-7.
76. Marin J, Hussain S, Gibbons W, Polverino M, Levy R, Cosio M. Relationship of resting lung mechanics and exercise pattern of breathing in patients with chronic obstructive lung disease. Chest,. 1993(104):705-11.
77. O'Donnell D, Hamilton A, Webb K. Sensory mechanical relationships during high-intensity, constant work-rate exercise in COPD. Journal of Applied Physiology. 2006(101):1025–35.
78. Laveneziana P, Webb K, Ora J, Wadell K, O'Donnell D. Evolution of dyspnea during exercise in chronic obstructive pulmonary disease: impact of

critical volume constraints. American Journal of Respiratory and Critical Care Medicine. 2011(184):1367–73.

79. Wagner P. Determinants of maximal oxygen transport and utilization. Annual Review of Physiology. 1996(58):21–50.

80. Powers S, Lawler J, Dempsey J, S D, Landry G. Effects of incomplete pulmonary gas exchange on VO2max. Journal of Applied Physiology. 1989;66(6):2491-5.

81. Saltin B, Blomqvist G, Mitchell J, Johnson Jr R, Wildenthal K, Chapman C. Response to exercise after bed rest and after training. Circulation. 1968;38(5):VII1-78.

82. Ekblom B, Astrand P, Saltin B, Stenberg J, Wallström B. Effect of training on circulatory response to exercise. Journal of applied physiology. 1968;24(4):518-28.

83. Holloszy J, Coyle E. Adaptations of skeletal muscle to endurance exercise and their metabolic consequences. Journal of Applied Physiology Respiratory Environmental and Exercise Physiology. 1984;56(4):831–8.

84. Green D, Naylor L, George K, Dempsey J, Stickland M, Katayama K. Cardiovascular and pulmonary adaptations to endurance training. New York: Elsevier; 2008.

85. Di Bello V, Santoro G, Talarico L. Left ventricular function during exercise in athletes and in sedentary men. Medicine and Science in Sports and Exercise. 1996;28(2):190–6.

86. Levine B, Lane L, Buckey J, Friedman D, Blomqvist C. Left ventricular pressure-volume and Frank-Starling relations in endurance athletes: Implications for orthostatic tolerance and exercise performance. Circulation. 1991;84(3):1016–23.

87. Levy W, Cerqueira M, Abrass I, Schwartz R, Stratton J. Endurance exercise training augments diastolic filling at rest and during exercise in healthy young and older men. Circulation. 1993;88 (1):116–26.

88. Nixon J, Wright A, Porter T, Roy V, Arrowood J. Effects of exercise on left ventricular diastolic performance in trained athletes. American Journal of Cardiology. 1991;68(9):945–9.

89. Stickland M, Wels R, Petersen S. Does fitness level modulate the cardiovascular hemodynamic response to exercise? Journal of Applied Physiology. 2006;100(6):1895–901.
90. Howley E, Bassett Jr D, Welch H. Criteria for maximal oxygen uptake: review and commentary. Medicine and Science in Sports and Exercise. 1995(27):1292-301.
91. Franklin B. ACSM's Guidelines of Exercise Testing and Prescription. New York: Lippincott Williams & Wilkins; 2000.
92. Gibbons R, Balady G, Beasley J, et al. ACC/AHA guidelines for exercise testing: a report of the American College of Cardiology/American Heart Association task force on practice guidelines (Committee on Exercise Testing. Journal of the American College of Cardiology. 1997;30(1):260-311.
93. Whipp BJ. Domains of aerobic function and their limiting parameters. In: Steinacker JM, Ward SA, editors. The physiology and pathophysiology os exercise tolerance. New York: Plenum Press; 1996. p. 83-9.
94. Scherr J, Wolfarth B, Christle JW, Pressler A, Wagenpfeil S, Halle M. Associations between Borg's rating of perceived exertion and physiological measures of exercise intensity. Eur J Appl Physiol. 2013;113(1):147-55.
95. Özyener F, Rossiter HB, Ward SA, Whipp BJ. Influence of exercise intensity on the on- and off-transient kinetics of pulmonary oxygen uptake in humans. J Physiol. 2001;533:891-902.
96. Roston WL, Whipp BJ, Davis JA, Effros RM, Wasserman K. Oxygen uptake kinetics and lactate concentration during exercise in man. Amer Rev Resp Dis. 1987;135:1080-4.
97. Casaburi R, Storer TW, Ben-Dov I, Wasserman K. Effect of endurance training on possible determinants of VO2 during heavy exercise. 1987;62(1):199-207.
98. Poole D, Ward S, Gardner G, Whipp B. Metabolic and respiratory profile of the upper limit for prolonged exercise in man. Ergonomics. 1988(31):1265–79.
99. Jones A, Wilkerson D, DiMenna F, Fulford J, Poole D. Muscle metabolic responses to exercise above and below the "critical power" assessed using 31P-MRS. Am J Physiol Regul Integr Comp Physiol. 2008(294):R585–R93.

100. Özyener F, Rossiter H, Ward S, Whipp B. Influence of exercise intensity on the on- and off-transient kinetics of pulmonary oxygen uptake in humans. J Physiol. 2001(533):891–902.
101. Chance B, Leigh Jr JS, Clark BJ, Maris J, Kent J, Nioka S, et al. Control of oxidative metabolism and oxygen delivery in human skeletal muscle: a steady state analysis of the work/energy cost transfer function. Proc Natl Acad Sci. 1985;82:8385-8.
102. Funk CI, Clark Jr A, Connett R. A simple model of aerobic metabolism: applications to work transitions in muscle. Amer J Physiol. 1990;258:C995-C1005.
103. Kushmerick MJ, Meyer RA, Brown TR. Regulation of oxygen consumption in fast - and - slow twitch muscle. Amer J Physiol. 1992;263:C598-C606.
104. Barstow TJ, Lamarra N, Whipp BJ. Modulation of muscle abd pulmonary O2 uptakes by circulatory dynamics during exercise. J Appl Physiol. 1990;68:979-89.
105. Whipp BJ, Ward SA. Physiological determinants of pulmonary gas exchange kinetics during exercise. Med Sci Sports Exerc. 1990;22:62-71.
106. Linnarsson D. Dynamics of pulmonary gas exchange and heart rate changes at start and end of exersice Acta Physiol Scand. 1974;415:1-68.
107. Murgatroyd SR, Ferguson C, Ward SA, Whipp BJ, Rossiter HB. Pulmonary O2 uptake kinetics as a determinant of high-intensity exercise tolerance in humans. 2011;110(6):1598-606.
108. Whipp BJ, Ward SA, Lamarra N, Davis JA, Wasserman K. Parameters of ventilatory and gas exchange dynamics during exercise. J Appl Physiology. 1982;52:1506-13.
109. Lamarra N, Whipp BJ, Blumenberg M, Wasserman K. Model-order estimation of cardiorepiratory dynamics during moderate exercise. . In: Whipp BJ, Wiberg DM, editors. Modelling and control of breathing New York: Elsevier; 1983. p. 338-45.
110. Whipp BJ, Mahler M. Dynamics of pulmonary gas exchange during exercise. In: West JB, editor. Pulmonary Gas Exchange. II. New York: Academic Press; 1980. p. 33-96.

111. Cerretelli P, Di Prampero PE. Gas Exchange in exercise. In: Fahri LE, Tenney SM, editors. Handbook of Physiology 3 The Respiratory System. IV. Bethesda: American Physiology Society; 1987. p. 297-339.
112. Griffiths TL, Henson LC, Whipp BJ. Influence of peripheral chemoreceptors on the dynamics of the exercise hyperpnea in man. J Physiol. 1986;380:387-403.
113. Barstow TJ, Lamarra N, Whipp BJ. Modulation of muscle and pulmonay O2 uptakes by circulatory dinamics during exercise. J Appl Physiol. 1990;68:979-89.
114. Paterson DH, Whipp BJ. Asymmetries of oxygen uptake transients at the on-and off-set of heavy exercise in humans. J Appl Physiol. 1991;443:575-86.
115. Helson LC, Poole DC, Whipp BJ. Fitness as a determinant of oxygen uptake response to constant-load exercise. Europ J Appl Physiol. 1989;59:21-8.
116. Whipp BJ. Dynamics of pulmonar gas exchange. Circulation. 1987;76:VI-18-VI-28.
117. Astrand PO, Rodhal K. Textbook of work physiology. New York: McGraw-Hill; 1970.
118. Wilkie DR. Equations describing power input by humans as a function of duration of exercise. In: Cerretelli P, Whipp BJ, editors. Exercise bioenergetics and gas exchange. Amsterdam: Elsevier; 1980. p. 75-80.
119. Poole D, Ward S, Whipp B. The effects of training on the metabolic and respiratory profile of high-intensity cycle ergometer exercise. Eur J Appl Physiol. 1990(59):421–9.
120. Whipp BJ. The slow component of O2 uptake kinetics during heavy exercise Med Sci Sports Exerc. 1994;26:1319-26.
121. Morton R. The critical power and related whole-body bioenergetic models. Eur J Appl Physiol. 2006(96):339–54.
122. Copp S, Hirai D, Musch T, Poole D. Critical speed in the rat: implications for hindlimb muscle blood flow distribution and fibre recruitment. J Physiol. 2010(588):5077–87.
123. Barker T, Poole DC, Noble ML, Barstow TJ. Human critical power–oxygen uptake relationship at different pedalling frequencies. Experimental Physiology. 2006;91(3):621-32.

124. Monod H, Scherrer J. The work capacity of a synergic muscle group. Ergonomics. 1965(8):329–38.
125. Gaesser G, Poole D. The slow component of oxygen uptake kinetics in humans. Exerc Sport Sci Rev. 1996;24(35):35–71.
126. Bulbulian R, Jeong J, Murphy M. Comparison of anaerobic components of the Wingate and critical power tests in males and females. Med Sci Sports Exerc. 1996(28):1336–41.
127. Hill D, Smith J. A comparison of methods of estimating anaerobic work capacity. Ergonomics 1993(36):1495–500.
128. Miura A, Endo M, Sato H, Barstow T, Fukura Y. Relationship between the curvature constant parameter of the power-duration curve and muscle cross-sectional area of the thigh for cycle ergometry in humans. Eur J Appl Physiol. 2002;87(238):238–44.
129. Nebelsick G, Housh T, Johnson G, Bauge S. Comparison between methods of measuring anaerobic work capacity. Ergonomics. 1988(31):1413–9.
130. Allen D, Lamb G, Westerblad H. Skeletal muscle fatigue: cellular mechanisms. Physiol Rev. 2008(88):287–332.
131. Coats E, Rossiter H, Day J, Miura A, Fukuba Y, Whipp B. Intensity-dependent tolerance to exercise after attaining VO2max in humans. J Appl Physiol. 2003(95):483–90.
132. Burnley M, Jones A. Oxygen uptake kinetics as a determinant of sports performance. Eur J Sports Sci. 2007(7):63–79.
133. Fukuba Y, Miura A, Endo M, Kan A, Yanagawa K, Whipp B. The Curvature Constant Parameter of the Power-Duration Curve for Varied-Power Exercise. Med Sci Sports Exerc. 2003;35(8):1413–8.
134. Carter H, Pringle J, Boobis L, Jones A, Doust J. Muscle glycogen depletion alters oxygen uptake kinetics during heavy exercise. Med Sci Sports Exerc. 2004(36):965–72.
135. Murgatroyd SR, Wylde LA. The power–duration relationship of high-intensity exercise: from mathematical parameters to physiological mechanisms. The Journal of Physiology. 2011;589(10):2443-5.
136. Ferguson C, Whipp B, Cathcart A, Rossiter H, Turner A, Ward S. Effects of prior very-heavy intensity exercise on indices of aerobic function and high-intensity exercise tolerance. J Appl Physiol. 2007;103:812–22.

137. Barstow T, Jones A, Nguyen P, Casaburi R. Influence of muscle fiber type and pedal frequency on oxygen uptake kinetics of heavy exercise. J Appl Physiol. 1996(81):1642–50.
138. Endo M, Kobayakawa M, Kinugasa R, Kuno S, Akima H, Rossiter H, et al. Thigh muscle activation distribution and pulmonary VO2 kinetics during moderate, heavy, and very heavy intensity cycling exercise in humans. Am J Physiol Regul Integr Comp Physiol. 2007(293):R812–R20.
139. Poole D, Barstow T, Gaesser G, Willis W, Whipp B. VO2 slow component: physiological and functional significance. Med Sci Sports Exerc. 1994(26):1354–8.
140. Vanhatalo A, Poole D, Dimenna F, Bailey S, Jones A. Muscle fiber recruitment and the slow component of O2 uptake: constant work rate vs. all-out sprint exercise. Am J Physiol Regul Integr Comp Physiol. 2011(300):R700–R7.
141. Grassi B, Rossiter HB, Zoladz JA. Skeletal Muscle Fatigue and Decreased Efficiency: Two Sides of the Same Coin? Exercise and Sport Sciences Reviews. 2015;43(2):75-83.
142. Cannon DT, Bimson WE, Hampson SA, Bowen TS, Murgatroyd SR, Marwood S, et al. Skeletal muscle ATP turnover by 31P magnetic resonance spectroscopy during moderate and heavy bilateral knee extension. The Journal of Physiology. 2014;592(23):5287-300.
143. Vanhatalo A, Fulford J, DiMenna FJ, Jones AM. Influence of hyperoxia on muscle metabolic responses and the power–duration relationship during severe-intensity exercise in humans: a 31P magnetic resonance spectroscopy study. Experimental Physiology. 2010;95(4):528-40.
144. Valli G, Cogo A, Passino C, Bonardi D, Morici G, Fasano V, et al. Exercise intolerance at high altitude (5050 m): critical power and W'. Respir Physiol Neurobiol. 2011;177(3):333-41.
145. Neder J, Jones P, Nery L, Whipp B. Determinants of the Exercise Endurance Capacity in Patients with Chronic Obstructive Pulmonary Disease. The Power–Duration Relationship. Am J Respir Crit Care Med. 2000;162:497–504.
146. van der Vaart H, Murgatroyd SR, Rossiter HB, Chen C, Casaburi R, Porszasz J. Selecting Constant Work Rates for Endurance Testing in COPD:

The Role of the Power-Duration Relationship. COPD: Journal of Chronic Obstructive Pulmonary Disease. 2014;11(3):267-76.
147. Rossiter HB. Exercise: Kinetic Considerations for Gas Exchange. Compr Physiol. 2011;1:203-44.
148. Hill DW, Poole DC, Smith JC. The relationship between power and the time to achieve VO2max. Med Sci Sports Exerc. 2002;34:709-14.
149. Edwards R. Human muscle function and fatigue. Ciba Found Symp. 1981(82):1–18.
150. Booth F, Thomason D. Molecular and cellular adaptation of muscle in response to exercise: perspectives of various models. Physiol Rev. 1991;71:541–85.
151. Bigland-Ritchie B, Woods J. Changes in muscle contractile properties and neural control during human muscular fatigue. Muscle Nerve. 1984;7:691–9.
152. NHLBI. Workshop on Respiratory Muscle Fatigue. Am Rev Respir Dis. 1990;142:474–80.
153. Hickie I, Hooker A, Hadzi-Pavlovic D, Bennett B, Wilson A, Lloyd A. Fatigue in selected primary care settings: sociodemographic and psychiatric correlates. Med J Aust. 1996;164:585–8.
154. Gandevia SC. Spinal and Supraspinal Factors in Human Muscle Fatigue. Physiological Reviews. 2001;81(4):1725-89.
155. Garner SH, Ha L, Ma J. Prolongation of twitch potentiating mechanism throughout muscle fatigue and recovery. Eur J Neurol. 1989;103:277–81.
156. Powers RK, Binder MD. Effects of low-frequency stimulation on the tension-frequency relations of fast-twitch motor units in the cat. J Neurophysiol. 1991;66:905–18.
157. Rankin LL, Enoka RM, Volz KA, Stuart DG. Coexistence of twitch potentiation and tetanic force decline in rat hindlimb muscle. J Appl Physiol. 1988(65):2687–95.
158. Vandervoort AA, Quinlan J, Mccomas AJ. witch potentiation after voluntary contraction. Eur J Neurol. 1983;81:141–52.
159. Reid C. The mechanism of voluntary muscular fatigue. J Physiol. 1928;29:17–42.
160. Merton PA. Voluntary strength and fatigue. J Physiol. 1954;123:553–64.

161. McKenna MJ, Hargreaves M. Resolving fatigue mechanisms determining exercise performance: integrative physiology at its finest! J Appl Physiol 2008;104(1):286–7.
162. Amann M, Calbet JA. Convective oxygen transport and fatigue. J Appl Physiol 2008;104(3):861–70.
163. Noakes TD, St Clair Gibson A. Logical limitations to the “catastrophe” models of fatigue during exercise in humans. Br J Sports Med. 2004;38(5):648–9.
164. Secher NH, Seifert T, Van Lieshout JJ. Cerebral blood flow and metabolism during exercise: implications for fatigue. J Appl Physiol. 2008;104(1):306–14.
165. Walsh ML. Whole body fatigue and critical power: a physiological interpretation. Sports Med 2000;29(3):153–66.
166. Barry BK, Enoka RM. The neurobiology of muscle fatigue: 15 years later. Integr Comp Biol. 2007;47 (4):465–73.
167. Enoka RM, Duchateau J. Muscle fatigue: what, why and how it influences muscle function. J Physiol. 2008;586(1):11–23.
168. Yoon T, Schlinder Delap B, Griffith EE, Hunter SK. Mechanisms of fatigue differ after low- and high-force fatiguing contractions in men and women. Muscle Nerve. 2007;36(4):515–24.
169. Hortobagyi T, Mizelle C, Beam S, DeVita P. Old adults perform activities of daily living near their maximal capabilities. J Gerontol A Biol Sci Med Sci. 2003;58(5):M453–M60.
170. Fitts RH. The cross-bridge cycle and skeletal muscle fatigue. J Appl Physiol. 2008;104(2):551–8.
171. McKenna MJ, Bangsbo J, Renaud JM. Muscle K+, Na+, and Cl disturbances and Na+-K+ pump inactivation: implications for fatigue. J Appl Physiol. 2008;104(1):288–95.
172. Brehm JW, Self EA. The intensity of motivation. Annu Rev Psychol. 1989;40:109–31.
173. Marcora SM. Do we really need a central governor to explain brain regulation of exercise performance? Eur J Appl Physiol. 2008;104(5):929–31.
174. Marcora SM, Bosio A, de Morree HM. Locomotor muscle fatigue increases cardiorespiratory responses and reduces performance during intense

cycling exercise independently from metabolic stress. Am J Physiol Regul Integr Comp Physiol. 2008;294(3):R874–R83.

175. Marcora SM, Staiano W, Manning V. Mental fatigue impairs physical performance in humans. J Appl Physiol. 2009;106(3):857–64.

176. Marcora S. Perception of effort during exercise is independent of afferent feedback from skeletal muscles, heart, and lungs. J Appl Physiol 2009;106(6):2060–2.

177. Martin BJ. Effect of sleep deprivation on tolerance of prolonged exercise. Eur J Appl Physiol Occup Physiol 1981;47 (4):345–54.

178. Jacobs I, Bell DG. Effects of acute modafinil ingestion on exercise time to exhaustion. Med Sci Sports Exerc. 2004;36(6):1078–82.

179. Wilmore JH. Influence of motivation on physical work capacity and performance. J Appl Physiol 1968;24(4):459–63.

180. Loscher WN, Cresswell AG, Thorstensson A. Central fatigue during a long-lasting submaximal contraction of the triceps surae. Exp Brain Res. 1996;108:305–14.

181. Loscher WN, Cresswell AG, Thorstensson A. Excitatory drive to the alpha-motoneuron pool during a fatiguing submaximal contraction in man. J Physiol. 1996;491:271–80.

182. Mckenzie DK, Allen GM, Butler JE, Gandevia SC. Task failure with lack of diaphragm fatigue during inspiratory resistive loading in human subjects. J Appl Physiol. 1997;82:2011–9.

183. Murphy RM, Stephenson DG, Lamb GD. Effect of creatine on contractile force and sensitivity in mechanically skinned single fibers from rat skeletal muscle. Am J Physiol Cell Physiol. 2004;287:C1589–C95.

184. Allen DG, Westerblad H. Role of phosphate and calcium stores in muscle fatigue. The Journal of Physiology. 2001;536:657-65.

185. Westerblad H, Allen DG, Lännergren J. Muscle fatigue: lactic acid or inorganic phosphate the major cause? News Physiol Sci. 2002;17:17–21.

186. Woods J, Furbush F, Bigland-Ritchie B. Evidence for a fatigue induced reflex inhibition of motoneuron firing rates. J Neurophysiol. 1987(58):125-37.

187. Kniffki K, Mense S, Schmidt R. Responses of group IV afferent units from skeletal muscle to stretch, contraction and chemical stimulation. Exp Brain Res. 1978(31):511-22.

188. Sinoway L, Hill J, Pickar J, Kaufman M. Effects of contraction and lactic acid on the discharge of group III muscle afferents in cats. J Neurophysiol. 1993(69):1053-9.
189. Rotto D, Kaufman M. Effect of metabolic products of muscular contraction on discharge of group III and IV afferents. J Appl Physiol. 1988(64):2306-13.
190. Gandevia S. Neural control in human muscle fatigue: changes in muscle afferents, motoneurones and motor cortical drive. Acta Physiol Scand. 1998(163):305.
191. Gandevia S, Killian K, McKenzie D, Crawford M, Allen G, Gorman R, et al. Respiratory sensations, cardiovascular control, kinaesthesia and transcranial stimulation during paralysis in humans. The Journal of Physiology. 1993(470):85-107.
192. Fitts R. Cellular mechanisms of muscle fatigue. Physiol Rev. 1994(74):49-94.
193. Fitts R. Substrate supply and energy metabolism during brief high intensity exercise: importance in limiting performance. Lamb D, Gisolfi C, editors. Dubuque, Iowa: Brown & Benchmark; 1992.
194. Cairns SP. Lactic acid and exercise performance: culprit or friend? Sports Med. 2006;36(4):279-91.
195. Sutton JR. Limitations to maximal oxygen uptake. Sports Med. 1992;13:127–33.
196. Barcroft H, Millen JL. The blood flow through muscle during sustained contraction. J Physiol. 1939;97:17–31.
197. Wright JR, McCloskey DI, Fitzpatrick RC. Effects of muscle perfusion pressure on fatigue and systemic arterial pressure in human subjects. J Appl Physiol. 1999;86:845–51.
198. Romer LM, Haverkamp HC, Lovering AT, Pegelow DF, Dempsey JA. Effect of exercise-induced arterial hypoxemia on quadriceps muscle fatigue in healthy humans. Am J Physiol Regul Integr Comp Physiol. 2006;290:R365–R75.
199. Amann M, Romer LM, Pegelow DF, Jacques AJ, Hess CJ, Dempsey JA. Effects of arterial oxygen content on peripheral locomotor muscle fatigue. J Appl Physiol. 2006(101):119–27.

200. Hogan MC, Richardson RS, Haseler LJ. Human muscle performance and PCr hydrolysis with varied inspired oxygen fractions: a 31P-MRS study. J Appl Physiol 1999;86:1367–73.
201. Place N, Bruton JD, Westerblad H. Mechanisms of fatigue induced by isometric contractions in exercising humans and in mouse isolated single muscle fibres. Clin Exp Pharmacol Physiol. 2009;36(3):334-9.
202. Jones D, Round J. Skeletal muscle in health and disease. Manchester: Manchester University Press; 1990.
203. Rossman MJ, Garten RS, Venturelli M, Amann M, Richardson RS. The role of active muscle mass in determining the magnitude of peripheral fatigue during dynamic exercise. 2014;306(12):R934-R40.
204. Cannon DT, White AC, Andriano MF, Kolkhorst FW, Rossiter HB. Skeletal muscle fatigue precedes the slow component of oxygen uptake kinetics during exercise in humans. J Physiol. 2011;589(Pt 3):727-39.
205. Beelen A, Sargeant AJ. Effect of prior exercise at different pedalling frequencies on maximal power in humans. Eur J Appl Physiol Occup Physiol. 1993;66:102-7.
206. Sargeant AJ, Dolan P. Effect of prior exercise on maximal short-term power output in humans. J Appl Physiol. 1987;63:1475-80.
207. Rossiter HB, Ward SA, Howe FA, Kowalchuk JM, Griffiths JR, Whipp BJ. Dynamics of intramuscular 31P-MRS P(i) peak splitting and the slow components of PCr and O2 uptake during exercise. J Appl Physiol (1985). 2002;93(6):2059-69.
208. Ferguson C, Rossiter HB, Whipp BJ, Cathcart AJ, Murgatroyd SR, Ward SA. Effect of recovery duration from prior exhaustive exercise on the parameters of the power-duration relationship. J Appl Physiol. 2010;108: 866–74.
209. Saey D, Debigaré R, LeBlanc P, Mador MJ, Côté CH, Jobin J, et al. Contractile Leg Fatigue after Cycle Exercise. American Journal of Respiratory and Critical Care Medicine. 2003;168(4):425-30.
210. Millet GY, Millet GP, Lattier G, Maffiuletti NA, Candau R. Alteration of neuromuscular function after a prolonged road cycling race. Int J Sports Med. 2003;24(3):190-4.

211. Kremenic IJ, Glace BW, Ben-Avi SS, NIicholas SJ, Mchugh MP. Central Fatigue after Cycling Evaluated Using Peripheral Magnetic Stimulation. Medicine & Science in Sports & Exercise. 2009;41(7):1461-6.
212. Sidhu SK, Bentley DJ, Carroll TJ. Locomotor exercise induces long-lasting impairments in the capacity of the human motor cortex to voluntarily activate knee extensor muscles. 2009;106(2):556-65.
213. Amann M, Regan MS, Kobitary M, Eldridge MW, Boutellier U, Pegelow DF, et al. Impact of pulmonary system limitations on locomotor muscle fatigue in patients with COPD. 2010;299(1):R314-R24.
214. Goodall S, González-Alonso J, Ali L, Ross EZ, Romer LM. Supraspinal fatigue after normoxic and hypoxic exercise in humans. The Journal of Physiology. 2012;590(11):2767-82.
215. Amann M, Proctor LT, Sebranek JJ, Pegelow DF, Dempsey JA. Opioid-mediated muscle afferents inhibit central motor drive and limit peripheral muscle fatigue development in humans. The Journal of Physiology. 2009;587(1):271-83.
216. Katayama K, Amann M, Pegelow DF, Jacques AJ, Dempsey JA. Effect of arterial oxygenation on quadriceps fatigability during isolated muscle exercise. Am J Physiol Regul Integr Comp Physiol. 2007;292:R1279–R86.
217. Edwards RH, Hill DK, Jones DA, Merton PA. Fatigue of long duration in human skeletal muscle after exercise. J Physiol. 1977;272:769–78.
218. Froyd C, Millet GY, Noakes TD. The development of peripheral fatigue and short term recovery during self-paced high-intensity exercise. J Physiol. 2013;591:1339-46.
219. Lattier G, Millet GY, Martin A, Martin V. Fatigue and recovery after high-intensity exercise. Part II: recovery interventions. Int J Sports Med. 2004;25:509–15.
220. Racinais S, Girard O, Micallef JP, Perrey S. Failed excitability of spinal motoneurons induced by prolonged running exercise. J Neurophysiol. 2007;97:596–603.
221. MacIntosh BR, Shahi MR. A peripheral governor regulates muscle contraction. Appl Physiol Nutr Metab. 2011;36:1–11.

222. Burnley M. Estimation of critical torque using intermittent isometric maximal voluntary contractions of the quadriceps in humans. J Appl Physiol. 2009(106):975-83.
223. Cairns SP, Knicker AJ, Thompson MW, Sjogaard G. Evaluation of models used to study neuromuscular fatigue. Exerc Sport Sci Rev. 2005(33):9-16.
224. Goodall S, Charlton K, Howatson G, Thoms K. Neuromuscular Fatigability during Repeated-Sprint Exercise in Male Athletes. Medicine & Science in Sports & Exercise. 2015;47(3):528-36.
225. Gruet M, Temesi J, Rupp T, Levy P, Verges S, Millet GY. Dynamics of corticospinal changes during and after high-intensity quadriceps exercise. Exp Physiol. 2014;99:1053-64.
226. Jubeau M, Rupp T, Perrey S, Temes IJ, Wuyam B, Levy P, et al. Changes in voluntary activation assessed by transcranial magnetic stimulation during prolonged cycling exercise. PLoS One. 2014;9:e89157.
227. Klass M, Roelands B, Levenez M, Fontenelle V, Pattyn N, Meeusen R, et al. Effects of noradrenaline and dopamine on supraspinal fatigue in well-trained men. Med Sci Sports Exerc 2012(44):2299-308.
228. Polkey MI, Kyroussis D, Hamnegard CH, Mills GH, Green M, Moxham J. Quadriceps strength and fatigue assessed by magnetic stimulation of the femoral nerve in man. Muscle Nerve 1996;19:549-55.
229. Swallow EB, Reyes D, Hopkinson NS, Man WD, Porcher R, Cetti EJ, et al. Quadriceps strength predicts mortality in patients with moderate to severe chronic obstructive pulmonary disease. Thorax. 2007;62:115-20.
230. Verges S, Maffiuletti NA, Kerherve H, Decorte N, Wuyam B, Millet GY. Comparison of electrical and magnetic stimulations to assess quadriceps muscle function. JAppl Physiol. 2009;1985(106):701-10.
231. Verges S, Maffiuletti NA, Kerherve H, Decorte N, Wuyam B, Millet GY. Comparison of electrical and magnetic stimulations to assess quadriceps muscle function. JAppl Physiol 2009;1985(106):701-10.
232. Cairns SP, Knicker AJ, Thompson MW, Sjogaard G. Evaluation of models used to study neuromuscular fatigue. Exerc Sport Sci Rev. 2005;33:9-16.

233. Burnley M. The limit to exercise tolerance in humans: validity compromised by failing to account for the power-velocity relationship. Eur J Appl Physiol. 2010;109:1225-6.
234. MacIntosh BR, Fletcher JR. The parabolic power-velocity relationship does apply to fatigued states. Eur J Appl Physiol. 2011;111:319-20.
235. Marcora SM, Staiano W. The limit to exercise tolerance in humans: mind over muscle? Eur J Appl Physiol. 2010;109:763-70.
236. Szubski C, Burtscher M, Loscher WN. Neuromuscular fatigue during sustained contractions performed in short-term hypoxia. Med Sci Sports Exerc. 2007;39:948-54.
237. Rossiter HB, Ward SA, Kowalchuk JM, Howe FA, Griffiths JR, Whipp BJ. Dynamic asymmetry of phosphocreatine concentration and O2 uptake between the on- and off-transients of moderate- and high-intensity exercise in humans. The Journal of Physiology. 2002;541(3):991-1002.
238. Christie A, Snook EM, Kent-Braun JA. Systematic Review and Meta-Analysis of Skeletal Muscle Fatigue in Old Age. Medicine & Science in Sports & Exercise. 2011;43(4):568-77.