CRITICAL ANALYSIS OF TECHNIQUES FOR NORMALISING ELECTROMYOGRAPHIC DATA

From laboratory to clinical research

BY

YUMNA ALBERTUS

DEPARTMENT OF HUMAN BIOLOGY

Submitted in fulfillment of the
requirements for the degree of
DOCTOR OF PHILOSOPHY in Human Biology

University of Cape Town

Supervisors: Assoc Prof. M Lambert
Assoc Prof. W Derman
Dr R Tucker
Prof T Noakes

Cape Town, Western Cape
March, 2008

DECLARATION

PhD THESIS TITLE:

CRITICAL ANALYSIS OF TECHNIQUES FOR NORMALISING ELECTROMYOGRAPHIC DATA
From laboratory to clinical research

I, Yumna Albertus, hereby declare that the work on which this dissertation is based is my original work (except where acknowledgments indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

I empower the university to reproduce for the purpose of research either the whole or any portion of the contents in any matter whatsoever.

SIGNATURE: _____

DATE: _____

ACKNOWLEDGEMENTS

I would like to firstly dedicate this thesis to my loving parents, **Faiq and Bashira Albertus**. They have been my support and strength throughout my life. They have allowed me to choose my career without ever questioning my choices. By fully supporting and encouraging me every step of the way, they have instilled my self-belief. I am truly grateful to them for all the sacrifices they have made to allow my three sisters and me to attend the University of Cape Town and pursue our passion for learning. I just hope I will be as good of a parent as they have been to me.
"*Daddy and Mommy, I love you so very much for giving me the freedom to discover myself, and in this I have found my passion and my love for science. You will never know how much I love you both and appreciate the love and support you have given me.*"

To my husband, **Naasief**, thank you for always being there for me and cheering me on. You have been the most supportive husband. I will always remember how you would make me laugh when I felt down and how you would try to make me do my work whenever I didn't feel like it. I love you for taking such good care of me and always trying to make me feel like I can do anything. This thesis has made us grow closer together and made me realise that you will always be there for me whatever comes our way, and for this I am truly grateful.

To my **family**, thank you for all your encouraging words and interest in my work, I love you all so very much.

To my **friends**, Amaal, Nailah, Saberi, Nezaam and Sharhidd, thank you for being my escape and all the good times, but most of all, for encouraging me and supporting me all the way.

To my **ESSM friends**, Tina, Lize, Ross, Robert, Jeroen, Jill, Kerrith, Miranda, Dale, Jonathon and Liesl, I call you my ESSM support group. You have all been so very helpful and willing to listen to all my problems but most of all, you all have been the best team to do research with and I have learnt so much from each and every one of you.

And now to thank the team that made this thesis what it is today, my supervisors. **Assoc Prof Mike Lambert**, I am extremely grateful that you agreed to supervise me when Zig left. I know you got thrown into the deep end, yet you were always there for me whenever I needed guidance. Your contribution to this thesis has been invaluable. Your knowledge and encouragement has made me always try to do my best. I couldn't have asked for anyone better, because to me, you are a mentor and I will always strive to follow in your footsteps.

Assoc Prof Wayne Derman, thank you for all words of wisdom, your humour, kind words of support, encouragement, enthusiasm and your grammar lessons. I am extremely grateful that you have shown me the clinical side of research, which I am now very interested in pursuing and will be concentrating on in my Postdoctoral Fellowship. You have contributed so much to this thesis, your knowledge and guidance with the PVD study has added another dimension to this research.

Dr Ross Tucker, being one of my good friends, and being my supervisor was enjoyable. I am constantly amazed at your knowledge and reasoning ability at your young age. Thank you so much for moulding this thesis and helping me in displaying the results effectively. I will always remember our conversations about sports science and the world of sport!

Prof Noakes, you embody the word mentor, and I am so very grateful and honoured to have been under your wing for the past 5 years. Your knowledge is overwhelming and your contribution to every student has been invaluable. Thank you for being such a pillar of wisdom and support to all your students.

A special thank you to Assoc Prof Zig St Clair Gibson, for contributing to this thesis. Your supervision and contribution in the 3 years have been invaluable. You have always been so encouraging and optimistic about my research. I am very sorry that you were not able to see it in its full fruition.

Thank you Robert for helping me with the running studies, for always trying to help with equipment problems and for always being optimistic! Thank you to Dr Jeroen Swart and Leslene Titus, for monitoring the patients with PVD throughout the trials. I would also like to thank the Vascular Surgeons at Kingsbury Hospital, Dr Matley and radiologist Gretta, for recruiting patients for the PVD study. And a final thank you to all the cyclists, runners and patients that have contributed their bodies to science and this thesis.

TABLE OF CONTENTS

CONFERENCE PROCEEDINGS ASSOCIATED WITH THE THESIS

- Y Albertus, A St Clair Gibson, M Lambert, TD Noakes. 2005. *Muscle recruitment at exhaustion during peak power output cycling: A study of EMG normalisation and muscle recruitment.* 33rd Meeting of Physiology Society of South Africa. University of Cape Town, Sports Science Institute of South Africa.

- Y Albertus, R Tucker, M Lambert, TD Noakes, A St Clair Gibson. 2006. *Variability of muscle activity during peak power output cycling: The influence of normalisation techniques on muscle recruitment*. 11th European College of Sports Science Conference, Lausanne Switzerland. 5th-8th July 2006.

THESIS ABSTRACT

Measurements of muscle activity derived from surface EMG electrodes are variable due to both intrinsic and extrinsic factors. The intrinsic factors are endogenous in nature (features within the body) and include muscle fiber type, muscle fiber diameter and length, the amount of tissue between muscle and electrode, and depth and location of muscle with respect to the placement of electrodes [24]. These biological factors vary between subjects and cannot be controlled. The extrinsic factors are experimental variables which are influenced by the researcher and can be controlled to some extent. Examples of extrinsic factors include the location, area, orientation, shape of electrodes and the distance between electrodes (inter-electrode distance). In order to measure biological variation in the EMG signal, which is important in studies where surface EMG is used to gain understanding of physiological regulation, it is important to minimise the variation caused by these factors. This is in part achieved through the appropriate method of normalisation.

The isometric maximal voluntary contraction (MVC) has been used as a standard method of normalisation for both static and dynamic exercises. However, researchers have recently improved the methods of normalisation by developing alternative techniques for the measurement of EMG during dynamic activities. By using the same type of movement for normalisation as during the trial, experimental errors can be reduced. The appropriate method of normalisation is defined as a method that is capable of showing repeatability, reliability (low intra-subject variation) and sensitivity to changes in EMG amplitude that is due to biological change and not the contribution of experimental factors.

The aim of this thesis was to critically analyse alternative methods of EMG normalisation during dynamic exercise. The data should provide possible guidelines to researchers who are planning studies involving measurement of EMG activity during cycling, running and in clinical populations. Furthermore, the thesis aimed to illustrate that decisions regarding the most appropriate method of normalisation should be based on the study design, research question (absolute muscle activity or changes in muscle pattern) and the muscles being investigated.

Study One

The aim of this study was to evaluate possible methods of normalisation for EMG measured during cycling. The ideal method of normalisation is required to show repeatability, intra-subject reliability and sensitivity to changes in exercise. Four methods for normalising EMG during cycling were evaluated and their repeatability, reliability and sensitivity to change in workload were compared. The methods of normalisation investigated in this study were; 1) MVC method, 2) Active Period MVC method (AP MVC), 3) Sprint method and 4) 70 % Peak Power Output Method.

Thirteen well trained cyclists performed the same experimental protocol on three separate occasions separated by 5 - 7 days of normal training. EMG activity was measured on 6 muscles on the right leg namely; Vastus medialis (VM); Vastus lateralis (VL), Rectus femoris (RF), Biceps femoris (BF) and Medial and Lateral gastrocnemius (MG and LG respectively). Subjects firstly performed maximum voluntary contractions (MVC) using the Biodex Dynamometer 3, followed by a 10 s maximal sprint on an electronically braked cycle ergometer starting at 200W. Following this they performed an incremental test to exhaustion in order to obtain the Peak Power Output (PPO), where work rates began at 3.33 $W.kg^{-1}$ body mass. After a resting period they performed a submaximal cycle at 70 % of PPO for 5 minutes at 90 rpm. The study showed the 70 % PPO method to be the most repeatable method as it demonstrated 'good' repeatability for all muscles, ICC values ranged between R= 0.82-0.92. The 70 % PPO method also demonstrated the least intra-subject variability for 5 out of 6 muscles. The MVC method showed the highest percentage of intra-subject variation for most muscles except VM. The AP MVC and Sprint methods were found to be the most sensitive in detecting changes in muscle activity with increasing workloads. However, when the data were presented as power output/EMG ratio (the method with a slope close to zero suggests that the method is more sensitive in tracking changes in power output), the 70 % PPO method was the most sensitive method for tracking changes in power output for all muscles except VM.

In conclusion, based on the results of the present study, these data suggest that normalising EMG to dynamic methods is the most appropriate method of normalisation for examining muscle activity during cycling over different days and for

once-off measurements. The method of normalisation most appropriate for EMG measurement during cycling was the 70 % PPO method. The 70 % PPO method is recommended for measurement of trials to exhaustion and in repeated trial study designs. The Sprint method is recommended for the measurement of maximal muscle activity.

Study Two

This study aimed to find the most appropriate method of normalisation for muscle activity during running. These methods should be in accordance to the requirements of an ideal method of normalisation. The study investigated three methods for normalising EMG during running, the methods were; 1) MVC method, 2) Sprint method and 3) 70 % Peak Running Speed Method

Twelve well trained runners performed the same experimental protocol on three separate occasions separated by 5 - 7 days of normal training. EMG activity was measured on 6 muscles on the right leg namely; Vastus medialis (VM); Vastus lateralis (VL), Rectus femoris (RF), Biceps femoris (BF) and Medial and Lateral gastrocnemius (MG and LG respectively). Subjects firstly performed maximum voluntary contractions (MVC) using the Biodex Dynamometer 3, followed by two 20 m maximal sprints on an indoor running track. Following this, they performed the Peak Running Speed test (PRS) until exhaustion, where running speed started at 10 $km.h^{-1}$ and increased by 0.5 $km.h^{-1}$ every 30 s. After a resting period they performed a submaximal run at 70 % of PRS for 5 minutes. The MVC and Sprint methods were the most repeatable for normalising EMG activity during running. The MVC method showed 'good' ICC values for VM, BF and MG ("good" repeatability where ICC R > 0.80). The Sprint method showed 'good' repeatability for RF, VL and LG. The 70 % PRS method demonstrated the least intra-subject variability between trials. Furthermore, the 70 % PRS method had the greatest ability to detect changes in EMG with increasing running speed for most muscles except RF. Presenting the data as running speed/EMG ratio, the 70 % PRS method also had the greatest ability to track changes in running speed. However, the Sprint and MVC methods also showed low slope values for the running speed/EMG relationship and thus could also be considered as good methods for tracking EMG to exercise intensity.

In conclusion, based on the results of the present study, the methods of normalisation most appropriate for the measurement of EMG during running were the Sprint and 70 % PRS methods. These two methods of normalisation are recommended for trials to exhaustion and in repeated trial study designs, while the Sprint and MVC methods are recommended when measuring maximal muscle activity during a trial.

Study Three

This study aimed to apply the previously determined appropriate methods of normalisation for cycling trials and in particular to measure maximal muscle activity during incremental cycling to exhaustion. The study utilised the MVC and Sprint methods of normalisation, as these methods are associated with a maximal effort during a static isometric contraction and dynamic cycling sprint respectively.

A similar research design was used as in the first study. Considering the advantages and disadvantages of each method, the Sprint method of normalisation was considered to be the most reliable and repeatable method, as well as being representative of the muscle movement used in cycling. The Sprint method was therefore chosen as the appropriate method of normalisation to evaluate muscle activity at exhaustion during cycling. The main finding of the study was that muscle activity at exhaustion, in all the major muscles during cycling, was submaximal as compared to EMG activity during a maximal cycling sprint. All six muscles measured in the study achieved peak activation of between 44 % - 65 % of that achieved during a 10 second all-out sprint. The intra-subject variability showed that only a few muscles had CV values which were less than 12 % at exhaustion. This is an indication that muscle activity at exhaustion varies from day-to-day.

In conclusion, by using the most appropriate method of EMG normalisation, this study found that muscle activity was submaximal at exhaustion during PPO cycling. The findings support the central regulation of exercise and confirm that only a certain percentage of the limb muscle mass is active during maximal exercise. These findings are not compatible with the peripheral model of fatigue, as this model assumes that the total muscle mass is active at exhaustion.

Study Four

This study attempted to answer the same question that was addressed in the previous study but used running as the mode of exercise. The study utilised the MVC and Sprint methods as methods of normalisation as these methods measure muscle activity during static isometric contraction and dynamic running sprints respectively.

The same protocols were used as in the Study Two. After tabulating the advantages and disadvantages of each method of normalisation, the Sprint method was considered to be the most appropriate method. The Sprint method demonstrated greater intra-subject reliability and sensitivity to increasing running speed. By applying the Sprint method for normalisation, the main finding of this study showed that muscle activity at exhaustion was submaximal during running in all the measured muscles. This finding supports the results of Study Three. All six muscles measured in the study achieved peak activation of between 42 % - 66 % of that achieved during a 20 m maximal sprint. The intra-subject reliability showed only a few muscles had CV values which were less than 12 % at exhaustion. This indicates that muscle activity is never totally fixed at exhaustion.

In conclusion, the findings from this study concur with Study Three, that muscle activity is submaximal at exhaustion during 'maximal' exercise. These findings support the central regulation of exercise and confirm that only a certain percentage of the limb muscle mass is active during maximal exercises.

Study Five

Measuring EMG activity in patients with Peripheral Vascular Disease (PVD) requires an appropriate method of normalisation to assess muscle activity and exercise intolerance in clinical populations. The MVC and Mean Dynamic methods of normalisation are the most recommended methods in the literature. The aims of the study were to apply an appropriate method of normalisation to measure and evaluate muscle activity in patients with PVD. This study describes the detailed findings in a case series of patients with PVD and functional incapacity, who undergo an angioplasty intervention.

Two patients (AA and AB) were recruited with diagnosed PVD. After partaking in the familiarisation trial, the patients arrived at the laboratory prior to their angioplasty procedure. They were prepared for the placement of the electrodes on four muscles (VM, VL, MG and LG) on both the right and left leg. They then performed maximal voluntary contractions on the Biodex Dynamometer 3. Following this, the patients were instructed to walk on the treadmill until exhaustion using the Graded Treadmill exercise test (GTET) protocol, which involved walking at a constant speed of 3.2 $km.h^{-1}$ starting at a 2 % gradient which was increased by 2 % every 2 minutes until exhaustion. Heart rate and brachial blood pressure were monitored every two minutes during exercise. Patients were asked to report their perceived pain using a perception of pain scale and rate their perceived exertion by indicating a value on the Borg scale. During exercise, oxygen uptake (VO_2) was measured using the Oxycon Alpha Analyzer (Oxycon, Viasis, Hoechberg, Germany). Prior to the initiation of exercise, a resting venous blood sample (two millilitres) was directly drawn from a subcutaneous forearm vein in patient AB. An additional two millilitre blood sample was collected at three, four and five minutes post exercise. The blood was then analysed to determine peak blood lactate concentrations. Three days after the Pre-Angioplasty trial, the patients underwent angioplasty of the popliteal artery (patient AA) and of the femoral artery (patient AB). Once the blood flow was restored, the same radiographer measured the blood flow velocity using a duplex Doppler, to measure change in blood velocity. Following a three day recovery period from the angioplasty, subjects returned to the laboratory where they repeated the exercise trial (Post-Angioplasty trial).

EMG data analysis using both methods of normalisation (i.e. MVC and Mean Dynamic methods) resulted in contrasting findings. When normalising muscle activity using the MVC method, both patients had a decrease in muscle activity in the diseased leg after angioplasty, for most muscles. In contrast when normalised using the Mean Dynamic method, muscle activity in the diseased leg, increased after angioplasty in both patients. The Mean Dynamic method was chosen as the more appropriate method, as the MVC method could have produced abnormally low EMG activity during the MVC (due to possible muscle inhibition). In addition, the MVC method of normalisation is considered less sensitive than the Mean Dynamic method in tracking changes in muscle activity with increasing exercise intensity. By using the Mean Dynamic method of normalisation, the study was able to

demonstrate a reduction in muscle activity during the state of peripheral vascular disease. Muscle activity in the diseased leg then increased to greater activity than the non-symptomatic (healthy) leg after angioplasty. The physiological variables of both patients improved after angioplasty. For example peak blood lactate concentration (which has been assumed to be the reason for claudication pain) was less than that of healthy controls and patients with other chronic diseases.

The alteration in muscle activity after angioplasty is a novel finding. It can be assumed that these changes in the muscle activity could be seen as a readjustment of the central drive to the lower limbs and a possible regulatory mechanism to protect the body from being harmed, especially in this diseased state.

The findings of this thesis have established that different methods of normalisation should be used for different studies. It is important that researchers use a method of normalisation that is suitable to their study design and research question. Choosing the most appropriate method of normalisation reduces experimental error and increases the chance of detecting biological variation and hence improves the interpretation of the data.

LIST OF ABBREVIATIONS

Ankle Brachial Index	ABI
American College of Sports Medicine	ACSM
Analysis of Variance	ANOVA
Active Period Maximum Voluntary Contraction	AP MVC
Biceps femoris	BF
Body Mass Index	BMI
Coronary Artery Bypass Grafting	CABG
Central Nervous System	CNS
Coefficient of Variation	CV
Electrocardiogram	ECG
Electromyography	EMG
Graded Treadmill Exercise Test	GTET
Heart Rate	HR
Intra-Class Correlation	ICC
Intergrated Electromyography	iEMG
Lateral gastrocnemius	LG
Maximal Voluntary Contraction	MVC
Median Frequency	MDF
Medial gastrocnemius	MG
Physical Activity Readiness Questionnaire	PAR-Q
Peak Power Output	PPO
Peak Running Speed	PRS
Percutaneous Transluminal Angioplasty	PTA
Peak Treadmill Running Speed	PTRS
Peripheral Vascular Disease	PVD
Rectus femoris	RF
Root Mean Square	RMS

Measurements

Beats per minute	bpm
Centimeters per second	$cm.s^{-1}$
Degrees Celsius	°C
EMG per unit force	μv.Nm

Force	Nm
Hertz	Hz
Hour	h
Kilogram	kg
Kilometer	km
Kilometer per hour	$km.h^{-1}$
Meters per second	$m.s^{-1}$
Micro-volt per second	$\mu v.s^{-1}$
Micro-volt second per second	$(\mu v.s)s^{-1}$
Milligrams per day	$mg.day^{-1}$
Millimeter	mm
Milliliter per kilogram per minute	$ml.kg^{-1}.min^{-1}$
Millimole per liter	$mmol.l^{-1}$
Minute	min
Revolutions per minute	rpm
Oxygen uptake	VO_2
Watts	W
Watts per kilogram	$W.kg^{-1}$

CHAPTER 1

ELECTROMYOGRAPHY: DEVELOPMENT AND FACTORS INFLUENCING MEASUREMENT IN LABORATORIES

REVIEW OF THE LITERATURE

Electromyography is defined as the study of muscle function by examination of the electrical signals that emanate from skeletal muscle [13].

1.1 HISTORICAL DEVELOPMENT OF ELECTROMYOGRAPHY

The detection of electrical signals within muscles originates from research performed as early as 1658. Swammerdam introduced the mechanics of muscular contractions by performing experiments on muscle and nerve irritation (cited in [28]). He discovered that stroking the innervating nerve of a frog's Medial gastrocnemius muscle generated a contraction. Thereafter, Galvani published research on muscular irritation in 1792, where he discovered frog muscle could be excited by an electrical current [100]. However, due to limited instrumentation, his work was not fully accepted until almost 40 years later. Electric current was only shown to be linked to muscular contraction in 1838. This was demonstrated by Matteucci using a galvanometer (machine which is able to detect and measure small electric current) [38; 100].

However, the most important development in electromyography (EMG) was accomplished by Duchenne de Boulogne in 1850. de Boulogne studied the dynamics and functions of intact skeletal muscles and electrical stimulations. He developed techniques for registration and stimulation of both muscle and nerve, and applied electrodes for recording the path electric current in contracting muscle fibers(cited in [30]). Piper was, however considered to be the first researcher to study the EMG signal [133]. In 1907 he developed the metal surface electrode and in 1912

recorded human voluntary muscle activity by utilising the Einthoven's string galvanometer [154]. Thereafter, Proebster in 1928, was able to describe spontaneous irregular action potentials in the denervated muscle of a human subject [154]. He was thus seen as a key contributor to the field of clinical electromyography.

In 1929, Adrian and Bronk, developed the concentric needle electrode and which enabled them to conduct studies on motor unit action potential [5]. These researchers amplified muscle action potential activities and used this amplified signal to produce an output from a loudspeaker. The information carried by the resulting sound aided in interpreting the EMG signal [5]. By using this technique, the researchers determined that there was no spontaneous electrical activity in completely relaxed normal muscle. This approach, for the first time enabled the measurement and observation of electrical activity associated with individual or small groups of muscle fibers [13].

The first visual format of EMG was introduced by Gasser and Erlanger in 1922. They used a cathode ray oscilloscope in place of the galvanometer, to show electrical signals from muscles [13]. The cathode ray oscilloscope helped neurophysiologists to study the undistorted shape of muscle activity [154]. Subsequently, the development of sensitive electronic equipment and the invention of new techniques to study muscle, contributed significantly to the analysis of EMG [154]. However, kinesiological study (which is the analysis of function and coordination of muscles in different movements and posture in a variety of human conditions) only advanced after the development of electrically stable silver-chloride electrode and the non-obtrusive wire electrode in 1960 [13]. With the introduction of the above mentioned advancements, the accuracy of the EMG signal interpretation was improved and its application has branched from traditional diagnosis of muscle disorders to exercise physiology, movement analysis, rehabilitation, biofeedback and myoelectric control of prostheses.

EMG has contributed significantly to research in the aetiology of fatigue, and has become a fundamental research tool in exercise physiology and clinical studies. Earlier investigations of fatigue have measured EMG data during sustained muscular contractions [42; 76; 92; 102; 116; 188]. The sustained isometric contraction allowed fatigue to be localised to the muscle or group of synergistic muscles

performing the contraction [13]. From as early as 1954, researchers considered localised fatigue to be influenced peripherally (within muscle tissue) and centrally (in brain and spinal cord). Important theories were derived to explain the increase in amplitude and frequency shift of EMG signal observed during sustained, constant force isometric contractions. Due to these studies and the development of new technology, research has extended into studying fatigue during dynamic movement. This has been achieved by applying the interpretation of fatigue developed from static isometric contraction, to understand data obtained during dynamic activity. However, EMG becomes complicated during dynamic activity due to changes in muscle morphology and other biological and extrinsic factors which influences the EMG signal (which is discussed in further detail from Section 1.2.3.1). The thesis thus focuses on the measurement of muscle activity during dynamic activity and attempts to clarify the difficulty in measuring EMG in dynamic exercise performed in athletic and clinical populations.

1.2 ELECTROMYOGRAPHY

1.2.1 Muscle Physiology

Skeletal muscle fibers are innervated in groups called motor units which are activated by the central nervous system (CNS) via α- motorneurons. α-Motorneurons are neural structures whose cell bodies are located in the anterior horn of the spinal cord and through the large diameter axon and terminal branches, innervates a group of muscle fibers [13]. The motor units are continuously activated by the CNS for as long as the muscle is required to generate force. To produce greater forces, the CNS increases excitation to the motor units, which results in a greater number of motor units being activated and an increase in the firing rates of all active motor units [38]. The electrical signal that originates from the activation of muscle fibers, and which is within the detectable range of an electrode, is called a motor unit action potential [38; 141]. The motor unit action potential propagates from the innervation zone in both directions along each muscle fiber.

1.2.2 EMG signal

The EMG signal is comprised of a time and frequency domain [47]. Amplitude analysis (area under the curve) is derived from the time domain and requires rectification of the raw EMG signal, either linearly, by forming the absolute values of the raw signal, or quadratically, by calculating the squares of the raw signal. This rectified data must then undergo a 'moving' or 'weighted' averaging over a suitable time window. With linear rectification, the outcome is referred to as average rectified value. In quadratic rectification, the root is taken of every average reading and is referred to as the root mean square (RMS) and is the preferred recommendation for smoothing [77]. The size of the amplitude reflects both the number of active motor units and their firing rates. During a sustained contraction, the resultant increase in amplitude has been suggested to be due to both the recruitment of new motor units and the synchronisation of motor unit firing rates [37].

The frequency domain of the EMG signal can be expressed by the frequency spectrum of the EMG signal. The most common method used for estimating the spectrum (frequency content) of the EMG signal is the Fast Fourier Transformation algorithm which can be described as a decomposition of the EMG signal to its underlying sinus contents [199]. For example, if the most dominant sine wave is recognised at 100 Hz, the EMG has the most power at this frequency. If this power distribution analysis is done continuously over a certain Hertz range, a frequency distribution graph or total power spectrum is created. The total power spectrum can be calculated again by the following frequency parameters, namely the median and mean frequency. The median frequency divides the area of the spectrum into two equal halves and the mean frequency is the mathematical mean value of the spectrum curve [132].

Frequency analysis is used to estimate contractile fatigue, fiber type compositions and identify pathologies [13]. The mean or median frequency of the spectrum is influenced by muscle fatigue. During fatigue states there is an increase in the amplitude of the low frequency band and a relative decrease in the amplitude of higher frequency band. This results in a frequency shift to the left. The frequency shift has been attributed to changes in conduction velocity, changes in intra-muscular pH, altered recruitment and synchronisation of the motor units and the fiber type [13; 160]. The frequency aspect of the EMG signal is beyond the scope of

this thesis and thus the following chapters will focus on the amplitude characteristic of the EMG signal.

Two main types of electrodes are used to measure or detect EMG signals; needle/fine-wire electrodes (which are inserted into the muscle) and surface electrodes (placed on the skin surface overlying the muscle).

1.2.3 Needle/Fine-wire EMG

Needle or fine-wire EMG recording involves the insertion of an electrode into the muscle tissue. The needle electrode contains either one (monopolar) or two (bipolar) insulated wires in the cannula. The tip of the wire is exposed and acts as a detection surface [13]. Motor unit action potentials are detected in a small number near the needle tip [13; 133]. Fine - wire electrodes are extremely fine and cause relatively little pain during insertion. They are manufactured from mainly highly oxidised, stiff wire with insulation [13]. The action potentials of the muscle fibers closest to the active surface of the needle or wire are recorded with higher amplitudes than the more distant fibers [13]. Needle EMG is useful in detecting changes in motor unit size and is able to detect abnormal muscle function. Therefore this method of EMG recording is useful in studying clinical conditions including the effects of loss of nerve supply to the muscle, regeneration of nerves and the skeletal muscle myopathies. In addition, needle EMG techniques allow the study of motor unit recruitment and firing patterns, which provides information about CNS motor control [181]. By using needle EMG, researchers are able to measure the effects of fatigue on muscle by measuring conduction velocity, mean and median frequency.

The disadvantages of utilising needle EMG are; 1) it is an invasive measurement and is therefore difficult to use when measuring EMG on more that one occasion (due to the re-insertion of the needle in the same place and possible damage caused to the muscle fibers); 2) the use of needle EMG may cause minor damage to the muscle fibers from where the signal is detected, consequently influencing the shape of the motor unit action potential and 3) once the electrode is inserted the subject is required to remain steady as slight movement could result in changes in motor unit action potential.

1.2.4 Surface EMG

Surface electromyography (EMG) is obtained from the summation of electrical activity of the active motor units, detected by the electrodes placed on the skin overlying the muscle. Bipolar electrodes, which consist of two electrodes placed next to each other, are commonly used in modern day research. The EMG signal is thus an electric potential difference between the two electrodes. The information received from EMG is considered a global measure of motor unit activity since the surface electrodes are unable to detect specific activity levels of a single motor unit. Therefore the surface EMG signal is viewed as a more complex signal to that of the more invasive methods (needle/fine-wire).

Surface EMG signal is dependant on the membrane properties of the muscle fiber, the timing of motor unit action potentials and motor unit firing rates. It is therefore considered to reflect both central (central nervous system and spinal cord) and peripheral properties (for example, muscle fiber, metabolites, ion concentrations) of the neuromuscular system [60].

During trials involving static or isometric exercise, techniques including fine-wire, intramuscular EMG and transcranial magnetic stimulation have been used as a direct measure of the motor unit activation. However, during dynamic exercise, these techniques are not yet practical, and so the use of surface EMG remains the only feasible alternative method of measuring muscle activity. Surface EMG measurement of muscle activity is technically easier than the above methods and allows for the simultaneous and frequent measurement of multiple muscle groups. Therefore surface EMG is seen as the more appropriate measurement and should thus be improved upon, by simplifying the signal. Until researchers are able to produce a less complex signal, the interpretation of data derived from surface EMG should be made with caution.

Measurements of muscle activity derived from surface EMG electrodes are however variable due to both intrinsic and extrinsic factors. The intrinsic factors are biological in nature (features within the body) and include characteristics of the muscle fiber such as, muscle fiber type, muscle fiber diameter and length, the amount of tissue between muscle and electrode, and depth and location of muscle with respect to the

placement of electrodes [24]. These biological factors vary between subjects and cannot be controlled.

The extrinsic factors are experimental variables which are influenced by the researcher and can be controlled to some extent. Examples of extrinsic factors include the location, area, orientation and shape of electrodes and the distance between electrodes (inter-electrode distance) [24]. Recording factors including processing of the EMG signal, amplification filters, various noise contributions (ambient and transducer) and motion artefacts are also classified as extrinsic factors but are beyond the scope of the thesis.

It is important to note that due to the influence of the above mentioned intrinsic and extrinsic factors on the EMG signal, surface EMG should interpreted with caution. Indeed interpretation of surface EMG in dynamic tasks is complicated further by additional factors including the signal non-stationarity, shift of electrodes relative to muscle fiber during contraction and the change in conductivity properties of tissues separating the electrodes and muscle fibers [58]. These factors will be described in more detail in subsequent sections.

The biological and experimental factors, which are common to both static and dynamic contractions, have been investigated extensively and are summarised in Figure 1.1.

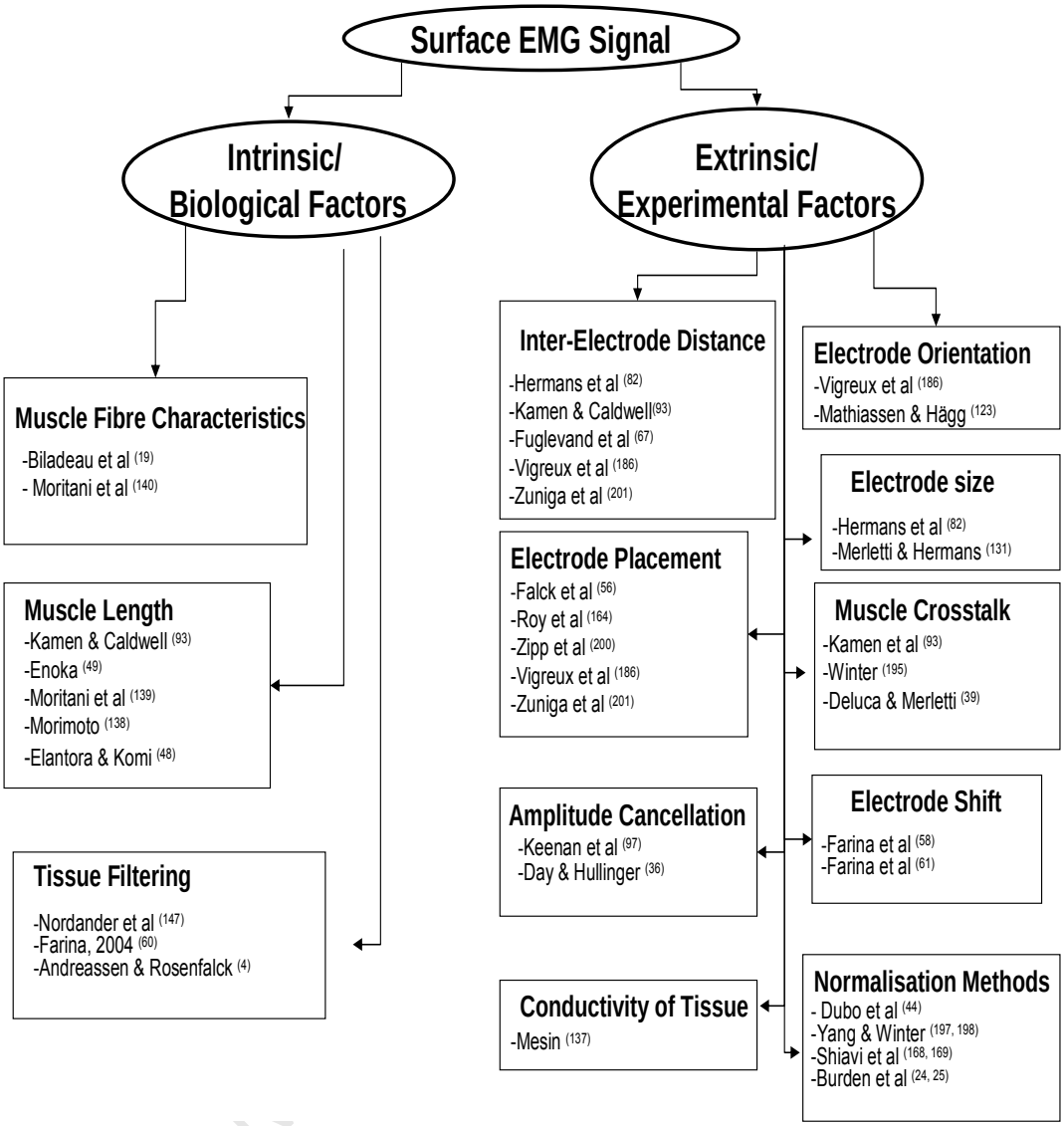


Figure 1.1: Summary of important intrinsic biological and extrinsic experimental factors that are common to both static and dynamic contractions.

1.2.4.1 Intrinsic/Biological factors

As mentioned previously, intrinsic or biological factors include muscle fiber type, muscle fiber diameter and length, the amount of tissue between muscle and electrode, and depth and location of muscle with respect to placement of electrodes (24).

1.2.4.1.1 Muscle fiber characteristics

Action potentials have greater amplitude in Type II muscle fibers than in Type I muscle fibers, due to higher conduction velocities. Furthermore, action potentials derived from Type II muscle fibers have an intrinsically higher frequency content than those action potentials transmitted from Type I muscle fibers. Therefore EMG measurement from a muscle with higher proportion of Type II muscle fibers should have higher frequency than EMG from a muscle with a higher proportion of Type I muscle fibers [19; 140]. This has implications for comparisons between individuals, since the proportion of Type I and II muscle fibers are different between individuals and therefore the EMG signal will be influenced.

1.2.4.1.2 Muscle length

Muscle length changes with movement. As the muscle length increases, the muscle fiber diameter decreases resulting in an alteration of action potentials, and a decrease in EMG amplitude [81; 93; 138]. Furthermore, the conduction velocity decreases due to smaller fiber diameter [138].

Studies have also shown that the rate of change of muscle length alters the EMG amplitude [81]. For example, EMG amplitudes are greater during dynamic muscle activity (involving lengthening and shortening actions, and referred to as eccentric and concentric respectively) than in static (isometric, no change in muscle length) actions. In isometric actions, muscle fibers experience difficulty in producing force in this shortened state. In addition, eccentric muscle actions generate less EMG amplitude than concentric actions at the same workload [48; 193].This can be explained by the less favourable length-tension relationship of the actin-myosin crossbridges [139]. As the muscle fiber lengthens, as occurs during eccentric actions, the actin and myosin filaments overlap declines until the two can no longer overlap, and no force is generated [23]. Furthermore, during eccentric actions the lengthening of muscle fibers requires lower levels of voluntary activation by the nervous system to achieve a given muscle force. Thus resulting in a decrease in EMG amplitude [49; 153]. Therefore the changes in the muscle length-tension relationship accounts for the greater EMG amplitude measured during dynamic contraction [21]. For this

reason the interpretation of activity can be complicated during dynamic exercise where the muscle lengthens and shortens during the bout.

1.2.4.1.3 Tissue filtering (i.e. tissue between electrode and muscle, depth of muscle)

The tissue between the electrode and muscle fibers consists of skin, fat and connective tissue. These tissues cause attenuation in the signal strength, resulting in decreased signal amplitude and frequency as the depth of the tissue increases. This is known as the low pass filtering effect [62; 93]. The largest motor units are detectable at depths of approximately 35 mm [67]. Nordander et al [147] found EMG amplitude to decrease with increasing muscle-electrode distance, furthermore they demonstrated that not only the distance, but also the electrical properties of subcutaneous tissue, are of importance for the attenuation of the EMG signal. The electrical properties are affected by fatigue, temperature and ionic concentrations including sodium and potassium [93]. Ionic fluctuations alter the excitability of the muscle fiber membrane [7], thereby influencing the amplitude and frequency domains of the EMG [54]. Furthermore, metabolic

1.2.4.2 Extrinsic/Experimental factors

1.2.4.2.1 Electrode orientation

Two factors are important with regards to the surface electrode position. Firstly, the electrode orientation, defined as the direction of the sensor in relation to the orientation of the muscle fibers, is of importance. Secondly, the positioning of the electrode, defined as the location of the centre of the sensor on the muscle, is perhaps equally as important.

Electrodes should be orientated and placed parallel to the muscle fibers as less than 50 % of the amplitude is captured when the electrodes are placed perpendicular, rather than parallel, to the orientation of the muscle fibers **[186]**. However, De Luca [40] recommends that the electrode placement should be perpendicular to the length of the muscle fibers, since there is less variability in the

EMG signal using this orientation. Hermans et al. [82], examined 144 peer reviewed papers on the applied surface EMG sensor properties and sensor placement procedures, and found that there was a general concensus about orienting the surface electrode parallel to the muscle fibers. The orientation of the surface electrodes impacts on experiments where EMG data are measured on different days, as it is important that the researcher re-aligns the electrodes to similar orientation and position to the muscle belly as used in the previous testing sessions.

1.2.4.2.2 Inter-electrode distance

Inter-electrode distance is defined as the centre-to-centre distance between the conductive areas of two electrodes [82]. There is high variability in EMG amplitude for inter-electrode distances [82]. For most muscles a certain distance range between electrodes were found to provide optimum EMG signals eg. Biceps femoris: 20-50 mm, Rectus femoris: 1-50 mm, gastrocnemius: 10-50 mm [82]. Thus, the preferred inter-electrode distance used by researchers is approximately 20 mm [82]. A further consideration is that inter-electrode distance should be determined such that the two electrodes do not approach the area of motor neuron innervation or tendon zones [29; 82] as this may cause large variation in EMG amplitude.

1.2.4.2.3 Electrode placement

Electrode placement is perhaps the most significant variable determining the fluctuation in EMG amplitude and frequency over different testing sessions. In addition to the above mentioned factors, the placement of electrodes over the motor neuron innervation zone could also result in a cancellation of the signals from both surface electrodes given that motor unit action potentials propagate bi-directionally from the innervation zone, resulting in highly variable recordings from EMG [93]. The EMG signal is less stable and reproducible if placed on or near the innervation zone, since a slight shift of the electrode sensor could move it to a place near or over the innervation zone [82]. The most reliable EMG signals are obtained from the muscle belly area between the innervation zone and the most distal tendon [29]. During complex movements in sport and physical activity, the muscle belly shortens

in the proximal direction during concentric contractions and the electrode on the skin may shift to a position over the distal tendon.

The location of the innervation zone of the muscle can be determined by using 100 ms segments of raw surface EMG signals from adjacent channels (electrode array). The data are then plotted to observe the location where the reversal of the signal direction occurs. The location of the innervation zone under the electrode array is then estimated by evaluating which electrodes show the reversal in signal direction. Rainoldi et al. [161] studied the locations of the innervation zones using linear electrode array. The study examined 13 muscles and found that 8 out of the 13 muscles studied, provided information on the ability to provide electrode locations based on innervation zone location and bony landmarks. They suggest that for Medial gastrocnemius, Soleus, Gluteus medius, Peroneus longus and Rectus femoris muscles, it is necessary to determine each individual's innervation zone location before the placement of the electrodes. However, the study does not make mention of the effect of innervation zone shifts on EMG amplitude.

The study thus recommends that researchers use the electrode array in identifying innervation zones before electrode placement. Although it is possible to use the electrode array method to determine electrode placement, electrode arrays are costly, and the availability in physiology laboratories is thus limited. It is thus proposed that if EMG needs to be measured during repetitive contractions, and the electrode array is unavailable, that the electrodes are placed over the visual midpoint or muscle belly of the contracted muscle by using anatomical landmarks and standardised measurements in the placement of the electrodes [27; 31].

1.2.4.2.4 Electrode size

Hermans et al.[82] reported that the majority of the research has used circular electrodes with a diameter ranging between 8 mm to 10 mm. Small electrodes are preferred since larger electrodes introduce excessive low-pass filtering effects which attenuates the EMG signal. Therefore, when two electrodes are placed next to each other, the inter-electrode distance should be reduced to the range of 20 mm

as previously discussed. The recommended size of electrodes should therefore not exceed 10 mm [131].

1.2.4.2.5 Muscle crosstalk

Muscle crosstalk refers to the phenomenon where a signal recorded over a particular muscle is in fact generated by a nearby muscle [60]. Thus the measured EMG signal is not discernable from the signals originating from deep underlying or adjacent muscles. The effect of crosstalk can be minimised through careful use of appropriate procedures [195]. Placing pairs of electrodes at least 20 mm apart minimises crosstalk of the signal. Winter et al. [195] suggested a technique to identify and measure the presence of EMG crosstalk. This technique involves the subject performing a resistance test to isolate specific muscle groups. EMG activity is then examined in the non-active muscles. If the non-active muscle displays a resultant EMG signal, then one can assume the presence of crosstalk.

1.2.4.2.6 Electrode shift during muscle contraction

During muscle contraction and subsequent movement, the electrode on the skin overlying the muscle may shift with respect to the muscle fibers originally being recorded [61]. This results in the electrode having a different position throughout the range of movement relative to the muscle fibers. This causes an unwanted signal component which is difficult to predict or remove [58]. Farina et al.[195] investigated the effect of leg movement on the shift of the innervation zone relative to the electrodes. The study demonstrated during knee and ankle movement that the innervation zone shifted approximately 10 mm for Vastus lateralis and Vastus medialis, while for Rectus femoris the innervation zone shifted 5 mm, Biceps femoris 30 mm and Medial gastrocnemius 10 mm. Lateral gastrocnemius however, did not show any shift in innervation zone over the range of movement.

It is possible that the use of electrode arrays (to maximise recording area) and spatial filtering [63] might minimise this technical problem; yet, these techniques are expensive and time consuming. Thus the potential problem of electrode shift over muscles during dynamic exercise is still not adequately answered.

1.2.4.2.7 Conductivity of tissues

The movement of a joint results in changes of muscle fiber diameter, length and orientation thus altering conductivity of muscle tissue. At each point along a muscle, relative fiber direction changes with movement [137], thus conductivity of tissues surrounding the fibers are changed resulting in an alteration in the measured action potentials at skin surface. In addition, the increasing sweat rate during dynamic activity alters electrode-skin resistance. The electrode contact stability is therefore reduced by sweating excessively. Thus the changing conductivity of tissues during dynamic activity is an important contributor to EMG variation.

1.2.4.2.8 Amplitude cancellation

Amplitude cancellation occurs when the positive and negative phases of the motor unit action potentials cancel one another. This occurrence reduces the amplitude of the EMG signal therefore underestimating the true measurement of motor unit activity [36; 97]. This limits the interpretation of recruitment patterns and muscle activity. The magnitude of the signal lost due to amplitude cancellation can be calculated by comparing the signal amplitude obtained by summing motor unit action potentials before and after rectification (through filtering and RMS) of each potential [36; 60].

A possible technique to minimise amplitude cancellation is to normalise EMG activity to maximal levels of muscle excitation which can be obtained from maximal voluntary contractions. Day & Hulliger [36] demonstrated this technique by using a computational model which simulated surface EMG. They demonstrated a minimal amplitude cancellation of less than 5 % when normalising to maximal values. The amplitude cancellation increased up to 17 % when normalised to intermediate levels of muscle excitation.

1.2.4.2.9 Normalisation of the EMG signal

Most factors which introduce experimental error (discussed above) are common to both static and dynamic conditions. These factors are often negligible in static

conditions but can significantly complicate the interpretation of the EMG signal during movement [58].

By reducing variability caused by these experimental factors during dynamic movement, one is able to measure more accurately the true biological variation in the EMG signal. A method that could possibly assist in reducing the variation in EMG signal during dynamic exercise trials is the use of an appropriate method of normalisation. Normalising the EMG recording to a reference value obtained during standardised and reproducible conditions, allows for the comparison of muscle activity between different muscles over time and between individuals. The method of obtaining the reference value used in a normalisation equation has, however varied across studies [103].

The most common method of normalisation is based on isometric maximum voluntary contractions (MVC). This method has proved popular amongst researchers [9; 44; 53; 120; 158; 162] but has certain limitations. For example, it is assumed that subjects provide maximum effort during testing and that the maximal contraction achieved represents 100 % of muscle activity [24]. Although researchers can encourage the subject to generate a MVC, the force generated ultimately depends on the level of motivation of the subject.

This method also assumes a linear EMG/force relationship, since both force and EMG is modulated by a combination of motor unit recruitment [17]. The amplitude of the EMG signal is also affected by the size of the individual muscle fiber potentials. However, the amplitude of the EMG signals cannot be assumed to be homogeneous for different fiber types and under different experimental conditions. Bigland-Ritchie [17] found that all muscles producing non-linear EMG/force responses were of mixed fiber composition, whereas linear responses were found during contraction of muscles with uniform fiber composition.

Despite these limitations few studies have addressed the appropriateness of the MVC method or proposed alternative methods of normalisation during exercise trials. To address this challenge, the first requirement is to determine an appropriate method of normalisation that fulfils certain criteria namely;

a) Repeatability

An appropriate method of normalisation would need to be highly repeatable. Repeatability should consider two different and complementary aspects; 1) reliability, which addresses between-day variations of measured variables; and 2) constancy, which addresses within-day variations of measured variables. Repeatability would ensure precision during the measurement and lowest variation in repeated trials with altered electrode positioning [25; 159].

Determination of the Intra-class correlation (ICC) is the most commonly used statistical method to identify repeatability of the EMG signal [21; 113; 124; 142; 159]. A high ICC (closest to 1) is associated with a small within-subject variance relative to the between-subjects variance [84]. This statistical method describes how closely the values of one trial track the values of another trial from individual to individual. Furthermore, the ICC statistic represents how well the rank order of subjects in one trial is replicated in the second and additional trials. The closer the correlation gets to 1, the better the replication [84].

b) Reliability

Reliability refers to the extent to which measurements are consistent, dependable, and free from error [157]. Reliability also refers to the stability and consistency of measures with respect to time so that changes between the measurements can be attributed to the intervention [99]. Improved reliability means improved precision of single measurements and improved tracking of changes in measurements during experimental trials with longitudinal design [84]. A reliable measurement should also show reproducibility. According to this definition other researchers should obtain similar measures with testing using the same method [103].

One of the main contributors to reliability is the within-subject variation which affects the precision of estimates of change in the variable of an experimental trial [84]. Thus the smaller the within-subject variation, the easier it is to measure the change in the variable being investigated [84]. The

coefficient of variation (CV) is the statistical method often used to evaluate intra-subject variability.

c) Sensitivity

Sensitivity is defined as the ability to detect true biological variations. In the context of the thesis, the ability of the method to assist in the measurement of actual change in muscle activity rather than the measurement of the combination of the biological and experimental factors that affect the signal, is a requirement of a good method of normalisation. It is reasonable to expect muscle force output and EMG amplitude to have a linear relationship, as they both depend on the number of motor units recruited [60]. For this reason, EMG amplitude has been shown to increase with increasing power output/workload [18; 51; 52; 119]. However, EMG and force relationship are not always linear, particularly in high force activity and fatigue states [93]. Thus an ideal method of normalisation should to some extent, be able to identify changes in muscle activity due to the change in workload or exercise intensity, if fatigue is not present. Furthermore, a method of normalisation should not only be sensitive to a changing workload, but should also be sensitive to changes in a clinical condition.

1.3 METHODS OF NORMALISATION

1.3.1 During static exercise tasks

Various techniques of normalisation have been developed to reduce variability and standardise the EMG signal. The most commonly used method for EMG normalisation remains an isometric maximal voluntary contraction (MVC). The isometric MVC is used to obtain the highest force from a voluntary contraction. The use of the isometric MVC as a method of normalisation is standard for static tasks [128]. The EMG activity at maximum effort or the highest EMG amplitude obtained during the MVC is selected as value used for the denominator in the normalisation equation;

$$\text{Normalised EMG} = \frac{\text{Trial EMG}}{\text{Reference EMG (MVC)}} \times 100\ \%$$

EMG recordings during static maximal contractions are generally considered to be reproducible [2; 20; 103; 142; 187]. However, few studies have actually demonstrated this reproducibility [86]. Howitson et al.[86] investigated intra-subject reliability and group repeatability of EMG amplitude during isometric MVCs repeated over 5 days. They studied the EMG signal produced by the biceps brachii of 15 subjects who were unfamiliar with performing isometric MVCs. During evaluation of the group data, they found that EMG displayed good reproducibility (CV = 4 % and ICC R = 0.92). However, the intra-subject reproducibility was more variable (CV = 18 %). Therefore individuals can show large variation in EMG during MVCs, however the variation is less when the data are expressed as a group.

Kollmitzer et al. [105] investigated the reliability of EMG signal of the vastii muscles over short (3 min), intermediate (90 min) and long term (6 weeks) measurements. During this study 18 subjects performed isometric MVC's at 100 % and 50 % MVC over the various time intervals. 50 % MVC had greater reliability over the 3 min (for all muscles), the 90 min and the 6 week measurement for RF, VM and by combining the vastii data (RF + VM + VL). This study demonstrated that EMG measured during 50 % MVC had greater reliability than EMG measured during 100 % MVC.

The alternative to using maximal voluntary contractions for EMG normalisation is the use of submaximal voluntary contractions. Submaximal MVC's appear more reliable than maximal MVC's [105; 124; 197]. Mathur [124] showed EMG amplitudes of RF and VM to be more repeatable at 20 % MVC (ICC R = 0.91 and 0.88 respectively) than at 80 % MVC (ICC R= 0.66 and 0.83 respectively). Kollmitzer et al. [105] also found RF activity to have greater repeatability during submaximal sustained contractions of 50 % MVC (ICC R = 0.74) than at 100 % MVC (ICC R = 0.45). Yang & Winter [197] demonstrated the repeatability of measuring EMG of the triceps brachii muscle during 30 %, 50 % and 100 % MVC during elbow extensions within and between 3 days of testing. The EMG showed high repeatability at the submaximal contractions of 30 % and 50 % MVC (ICC ranged R = 0.80 - 0.95 and 0.78 - 0.93 respectively), whereas the repeatability of triceps activity at 100 % MVC ranged between R = 0.52 - 0.81.

1.3.2 During dynamic exercise tasks

More contentious are studies that have utilised surface EMG during dynamic exercise. Maximal exercise to exhaustion is commonly used to investigate fatigue and has contributed to the generation of theories regarding the origin of fatigue during dynamic exercise.

Researchers have thus acknowledged the limitations of a static MVC for normalising data and have improved the type of normalisation techniques by developing techniques used for dynamic activities. These techniques have used normalisation procedures which have involved the same movement as the movement being examined [198]. Since in dynamic contractions, such as walking, cycling and running the use of isometric MVC as a reference is debatable [31; 89; 198]. Several investigators have found that EMG activity recorded during dynamic activities can exceed measurements from the maximal isometric efforts [29; 122; 179]. Therefore the use of MVC normalisation could possibly be an inappropriate method for dynamic activities, since it may not represent the maximum activation capacity of the muscle either during non-isometric contractions or at muscle lengths other than those muscle lengths at which the MVC was performed [24]. However, despite these limitations the MVC method of normalisation has still been used for normalisation in research involving both static and dynamic muscle activity [9; 44; 120; 158; 162].

It is logical that the same type of movement used for the muscle activity should also be used for the method of normalisation. This method controls for the experimental errors discussed previously during dynamic movement, including changes in muscle length, electrode shifts and conductivity of tissues. This matter has been investigated in studies of gait analysis. Normalisation procedures for gait EMG have been comprehensively investigated [25]. Different methods of normalisation have been developed to lessen the variability in EMG during gait analysis [43; 24; 192]. The following are key methods used currently in gait research, however each method's criteria as an appropriate method of normalisation is still debatable.

i) Isometric MVC Method

This method divides each data point in gait EMG by the peak EMG amplitude from an isometric MVC of the same muscle [44]. This method is recommended when the goal is to determine the level of muscle activity that is required to perform the task, in relation to its maximal static capacity [10; 35]. This method has also been recommended for use in the clinical population until alternative methods are established [172].

ii) Isokinetic MVC Method (which is an MVC derived from a fixed movement pattern)

This method divides each data point in gait EMG by the peak EMG amplitude from an isokinetic MVC of the same muscle [98]. This method also allows for determination of level of muscle activity required to perform a task, in relation to its maximal isokinetic capacity.

iii) Peak Dynamic Method

This method normalises EMG from each stride in gait to the peak EMG amplitude found during that gait cycle [104]. This method allows for the determination of muscle activity level in relation to maximal activity obtained within a gait cycle. It has been proposed that this method is appropriate for examining a change in muscle activity recorded on different days.

iv) Mean Dynamic Method

This method normalises EMG from each stride in the gait cycle to the mean value recorded during that gait cycle. This method was more repeatable than the Peak Dynamic method described above [24]. This method allows for muscle activity level determination in relation to average muscle activity obtained within a gait cycle. It has been proposed that this method is appropriate for examining a change in muscle activity over days and across interventions. Furthermore, this method is recommended for use in the clinical population [21].

Yang & Winter [198] revealed that both the peak and mean dynamic method of normalising EMG data reduced inter - subject variability, compared to the 50 % MVC method during gait analysis. Shiavi et al. [168; 169] also investigated peak and mean dynamic methods, and showed that mean dynamic method was slightly better at reducing inter-subject variability particularly during muscle quiescence because of relatively lower standard deviations during these periods. However, this method has been criticised by Knutson et al. [103], who argues that these methods remove true biological variation within the group, presumably due the additional experimental factors that contribute to the EMG signal 'noise' during dynamic activity.

A comprehensive study by Burden et al. [24] compared inter-subject and intra-subject variability using all the above EMG normalising methods during the gait cycle. They reported that the Isokinetic MVC method produced the greatest intra-subject variability. EMG amplitude has been shown to have high repeatability during isokinetic knee extensions [70; 91; 115], which has been attributed to the highly standardised range of motion of the knee extension [115]. However, Burden et al. [24] concluded from their study that Isokinetic method should not be used as a method of normalisation in gait studies. The authors argue that the Isokinetic method does not reduce intra or inter-subject variability, nor does it provide a more representative measure of muscle activation during gait than the isometric MVC Method. They specified that Mean and Peak Dynamic methods serve as a measure of level muscle activation throughout the gait cycle in relation to the average and maximum activity recorded during the gait cycle. However, these methods are unable to provide information on the maximal degree of muscle activation (in relation to the muscles' maximal capabilities) that is required to perform a specific task. In contrast, the isometric and isokinetic MVC methods of normalisation are able to demonstrate the maximal muscle activity achieved in producing a specific task.

There is much debate in the literature regarding the use of appropriate methods of normalisation in studies involving dynamic muscle activities, such as using lactate threshold as a measure of exercise intensity and a metabolic marker to which EMG should be normalised. More research needs to be undertaken to find the most appropriate method of normalisation best suited to conduct various investigations. Particularly, it is important to be able to determine a change in muscle activity over

various days following an intervention. Another application for normalising EMG is to be able to interpret a once off measurement of maximal muscle activity during a dynamic activity.

The normalisation debate can also be extended into research performed in the clinical population, where the measurement of surface EMG has become more popular because it is non-invasive. Recently Bolgla & Uhl [21] investigated reliability of methods of normalisation for the evaluation of hip musculature during rehabilitation exercises. The study found the MVC method had ICC values exceeding R = 0.93 for all six rehabilitation exercises, whereas the dynamic methods exceeded R = 0.85 for all exercises except one. The researchers concluded that even though the MVC method had higher repeatability, the application of this method in the symptomatic population would be questionable since these patients might not elicit a valid MVC because of pain and muscle inhibition. Bolgla & Uhl [21] highlighted that the difficulty with previous studies investigating EMG amplitudes using MVC method, was that they used healthy populations and inferred similar findings to symptomatic subject populations. There are few clinical studies in the literature that have conducted research on the appropriateness of the methods of normalisation and thus Soderberg & Knutson [172] have advised researchers to continue using the MVC method for normalisation in the clinical population until alternative methods are established. There is therefore a need to establish a more appropriate method of normalisation in clinical research.

1.4 CONCLUSION

The interpretation of EMG activity measured during dynamic exercise remains contentious, primarily due to differences between methods used for normalisation and issues relating to the reliability of the measured signal.

The present review has described the contributions of intrinsic and extrinsic factors to the variability of the EMG measurement. Since the intrinsic factors cannot be controlled, the review has focused on different techniques used in minimising the EMG variability caused by extrinsic factors. In particular, the use of the appropriate method of normalisation is important to minimise EMG variability. The appropriate

method of normalisation is defined as a method that is capable of showing repeatability, reliability (low intra and inter-subject variation) and sensitivity to changes in EMG amplitude that is due to biological change and not the contribution of experimental factors.

The use of an isometric maximal voluntary contraction (MVC) as a method of normalisation has been standard for both static and dynamic exercises. However, researchers have recently improved the type of normalisation techniques by developing alternative strategies for normalising EMG during dynamic activities. By using the same type of movement for normalisation, experimental errors, such as changes in muscle length, electrode shifts and conductivity of tissues have the potential to be reduced. However, most of the research testing dynamic methods of normalisation has involved testing the reliability and repeatability of EMG amplitude during gait cycle analysis. It remains important to identify the limitations in the measurement of surface EMG and the ability of the method to provide information regarding the activity of muscle. This information provides better quality data and therefore more well-grounded interpretation of studies involving physiological models of fatigue. In addition, this information would enable better use of surface EMG in the clinical population, where invasive methods of EMG have been previously used due to better sensitivity.

1.5. OBJECTIVES OF THE THESIS

Given the identified need for minimising extrinsic risk factors through appropriate normalisation, the objective of the first part of the present thesis is to evaluate various normalisation techniques for use during dynamic exercise such as cycling and running. In determining a particular method for obtaining a reference value for normalisation, one must consider several issues. The important issues are the repeatability and reliability of the reference EMG value and the sensitivity of the method in detecting changes in exercise intensity. Moreover, the thesis expands on methods of normalisation for the clinical population, where the interpretation of EMG data is used diagnostically.

This thesis thus focuses on identifying the most appropriate methods of normalisation that can be used in testing dynamic muscle activity. Specifically the

thesis aims to investigate the repeatability, reliability and sensitivity of the following methods of normalisation in measuring muscle activity in cycling and running to exhaustion;

- MVC method
- Modified Peak Dynamic method - to be used in maximal sprints of cycling and running
- Modified Mean Dynamic method - to be used in submaximal exercise of performing at 70 % of maximal cycling power and running speed.

Based on the findings of these studies the appropriate method of normalisation are used to address questions on muscle activity at exhaustion in cycling and running as well as in patients with Peripheral Vascular Disease. The clinical application has the potential to determine how data can be interpreted given that the appropriate methods of EMG analysis are used.

CHAPTER 2

CRITICAL ANALYSIS OF METHODS FOR NORMALISATION OF SURFACE EMG ACTIVITY DURING CYCLING

2.1 INTRODUCTION

In Chapter 1, an overview of EMG activity was provided, and some of the experimental factors influencing EMG amplitude were discussed and described. In particular, the factors that influence the measured EMG signal were divided into intrinsic and extrinsic factors. Intrinsic factors include a) the electrode shift; b) the lengthening of muscle and possible shift of innervation zones, c) changing of conductivity of the muscle due to angular displacement about the joint causing changes in muscle fiber diameter, length and orientation [137]. These factors may influence the measured EMG signal during a dynamic activity like cycling. In addition to these intrinsic factors, extrinsic or experimenter-related factors can be controlled for through the correct application and placement of electrodes supported by a thorough understanding of electrophysiology and anatomy.

To measure biological variation in the EMG signal, which is important in studies where surface EMG is used to gain understanding of physiological regulation, it is important to minimise the variation caused by these factors (described in detail in Chapter 1). This is in part achieved through the appropriate method of normalisation.

Methods of normalisation for EMG activity measured during both static and dynamic exercise have been reviewed in detail in Chapter 1. However, normalisation of EMG data during dynamic activities such as cycling is relatively poorly understood, and there are presently few alternatives to the use of the isometric maximal voluntary contraction (MVC) method [119; 121; 162]. The use of the MVC as a method of normalisation has theoretical constraints, since, during cycling there are variations in actions of the muscles involved. For example, changes in joint angle, joint angle velocity, and muscle lengthening and shortening all occur during cycling, raising

questions about the efficacy of the MVC method as a normalisation tool for EMG data measured during cycling [88]. This is particularly the case when the EMG activity is being used in an attempt to quantify how much skeletal muscle may be active. Researchers have in the past made direct comparisons between the EMG signal measured during running or cycling and the EMG activity measured during an isometric MVC, expressing the resultant value as a percentage of maximum (for example, 45 % of the muscle was active at volitional exhaustion). Quite clearly, this comparison is questionable on the grounds that the two muscle contractions are very different in nature. This creates the need to evaluate other possible means for normalisation during activities such as cycling and running.

Few studies however, have found alternative methods for normalisation of EMG activity during cycling [88; 113; 119; 165; 178] with most using the MVC method. As discussed in Chapter 1, this method of normalisation involves a movement of muscle that is different from the movement and recruitment patterns employed during cycling.

Furthermore, the MVC typically involves an isometric knee extension movement at an angle of 60 degrees from full extension, a movement which does not occur during cycling and can be argued to be non-functional. Hunter et al. [88] investigated EMG methods of normalisation in cycling by using two angles (60º and 180º) with four types of pedal contractions. The investigation found that the isometric MVC produced a higher integrated EMG (iEMG) value than the protocols using knee angles at 60º, 180º or one dynamic maximal cycle pedal revolution. This finding suggests that muscle activity during cycling does not provide the highest EMG amplitude. This study however did not investigate the repeatability of the various normalisation protocols but rather only investigated which of the four protocols produced the greatest iEMG activity, and thus suffers from the same potential limitation with regards to the comparison between cycling exercise and static, isometric contractions. Therefore the most repeatable method for normalising muscle activity during cycling has yet to be established.

For a method of normalisation to be considered optimal, the following three factors have been identified;

a) Repeatability: Defined as the within and between day variation in a group. The intra-class correlation (ICC) is the statistical method used to identify repeatability. The method represents how closely the values of one trial track the values of another trial from individual to individual [84].

b) Reliability: refers to the reproducibility of values of the same individual performing repeated trials [84]. The coefficient of variation (CV) is the statistical method used to evaluate variability.

$$\text{The CV equation} = \frac{\text{Standard deviation}}{\text{Mean}} \times 100$$

c) Sensitivity: The method should be able to identify changes in muscle activity due to the change in workload or exercise intensity.

These factors are described in detail in Chapter 1 (Section 1.2.4.2.9), but briefly, it is important that the EMG measured during the normalisation procedure is consistent from one day to the next (repeatability), and also that when the EMG activity measured during dynamic activity is normalised against the chosen procedure, the value obtained must reflect changes in EMG that occur as the exercise workload increases (sensitivity).

The present chapter therefore aims to examine and evaluate possible methods for normalisation of EMG measured during cycling, according to the requirements for a good normalisation technique which are described above. Four methods for normalising EMG during cycling were evaluated and their variability and sensitivity to change was compared to the method of normalisation using isometric MVC. Therefore the methods of normalisation used in this study were;

1) MVC method - using the peak EMG amplitude of 5 second isometric MVC (Maximal static normalisation). This is the current widely-used “standard” method.
2) Active period MVC method – isolating each active contraction of cycling and normalising to the mean EMG amplitude of 5 second isometric MVC (Maximal static normalisation).
3) Sprint method - using the peak amplitude averaged over a 10 second cycling sprint activity (Maximal Dynamic normalisation).

4) 70 % Peak Power Output Method - using the averaged peak amplitude during 5 minute cycling at 70 % PPO at fixed cadence (Submaximal Dynamic Normalisation).

2.2 METHODOLOGY

2.2.1 Subject selection

Thirteen well-trained cyclists were recruited from local cycling clubs to participate in this study. Subjects were included if they were currently between the ages of 18-35 years old and if they were able to complete the Cape Argus cycle race (109 km) under 3h 30 min. The study was approved by the Research and Ethics Committee of the Faculty of Health Sciences within the University of Cape Town. The study was performed in accordance with the principles of the Declaration of Helsinki (October, 2000), Good Clinical Practice (GCP) and the laws of South Africa. All subjects signed the informed consent forms and were asked to maintain their same physical activity pattern over the course of the study and not to begin any new training or recreational programs. Subjects performed the same experimental protocol on each of the three testing days. Each testing day was separated by 5-7 days of normal training.

2.2.2 Experimental design

Subjects were required to visit the laboratory on four separate occasions. During the first visit, a complete anthropometrical assessment was performed on each subject. Each subject was also familiarised with all the equipment on their first visit. A Physical Activity Readiness Questionnaire (PAR-Q) was administered to all subjects prior to participation to ensure that they represented a group at low risk for exercise. On trial days, subjects were firstly prepared for the placement of the electrodes on six muscles (Vastus medialis (VM); Vastus lateralis (VL), Rectus femoris (RF), Biceps femoris (BF) and Medial and Lateral gastrocnemius (MG and LG respectively)) on the right leg. Following this each subject performed three maximum voluntary contractions (MVC) using the Biodex Dynamometer 3 (Biodex Medical Systems, New York). Subjects were then allowed a 10 minute warm-up on the electronically braked cycle ergometer (Lode, Groningen, Holland), after which they

performed a 10 second maximal sprint, where each subject started the sprint from a power output of 200 Watts (W). After a 30 minute rest period, subjects performed their Peak Power Output (PPO) test until exhaustion on the Lode bike [80]. Subjects were asked to inform the researcher just prior to anticipating having to stop as a result of exhaustion, so that EMG could be recorded during that time period. After another 30 minute rest period, each subject then cycled at 70 % of their PPO for 5 minutes at 90 revolutions per minute. Heart rate was measured continuously throughout the PPO test until exhaustion using Polar Accurex NV heart rate monitor (Polar Electro OY, Kempele, Finland). For analysis, heart rate was averaged and reported as the average for a 5 second interval. A summary of the experimental design is shown in Figure 2.1.

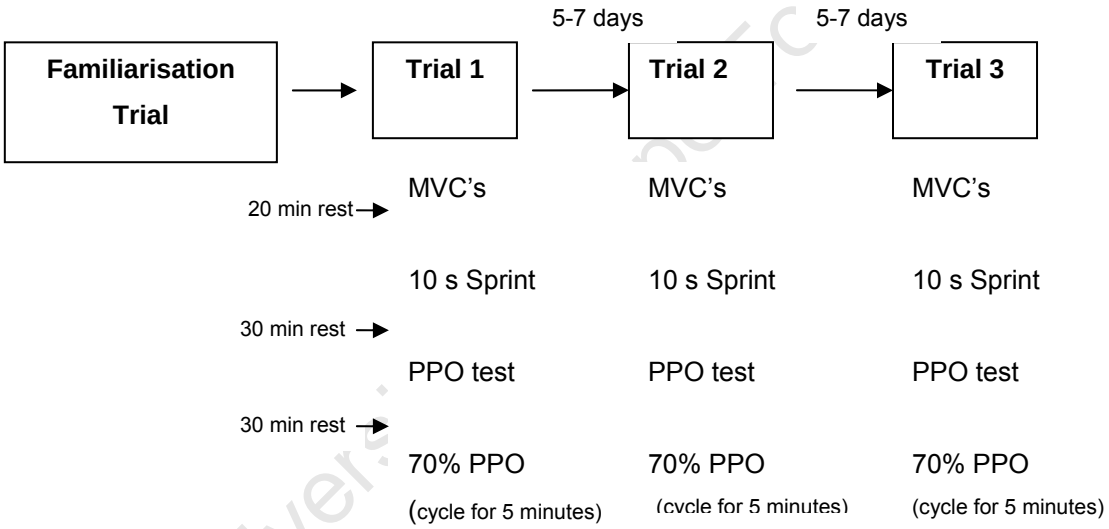


Figure 2.1: Experimental design

2.2.3 Body composition

Body mass and stature of each subject was measured when subjects reported to the laboratory for the first time. Skinfold thickness of each subject was measured at the biceps, triceps, subscapular, supra-iliac, abdominal, mid-thigh and calf sites [163]. Percent body fat was calculated using the equations of Durnin & Womersly [45] from the sum of seven skinfold measurements.

2.2.4 Maximal voluntary contractions

Before the start of each trial, each subject's peak isometric force of the lower limb was measured using the Biodex Dynamometer, with simultaneous EMG recordings captured for muscle activity measurement. The subject's hips, thighs and upper bodies were firmly strapped to the seat of the dynamometer. In this position, each subject's hip angle was at 100° flexion. The subjects' crossed their arms over their chests for each test. All isometric tests were conducted with the knee at as angle of 60° from full leg extension for the VM, RF and VL as well as BF muscles. The lateral condyle of the femur was carefully aligned with the axis of rotation of the dynometer and the distal end to the calf was secured to the lever arm of the dynamometer using a padded Velcro strap. When measuring the force output for the MG and LG, the right leg was elevated with the thigh resting on a holder and the foot firmly strapped against a foot-plate, setting a knee angle of 30°. The ankle was carefully aligned to the axis of rotation of the dynamometer. This test was conducted with the ankle at a -15° angle from full vertical (reference plane at 0°), from where the subjects had to push against the foot-plate using the gastrocnemius muscles. A standardised warm up included four isometric contractions of the knee and calf muscle extensors at 50 %, 60 %, 70 % followed by 80 % for each subject's subjective maximum. The isometric test included three maximum voluntary contractions (MVC) of 5 seconds each separated by 60 second intervals. Subjects were verbally motivated to encourage them to achieve their maximum potential. The seating position and length of leg attachments of subjects were standardised over all three days.

2.2.5 Peak power output test

During the PPO, EMG activity was measured from the six muscles described above. Subjects performed their PPO test on the Lode bike starting at workload of 3.33 $W.kg^{-1}$ body mass. After 150 s, the workload increased by 50 W and then increased by 25 W every 150 s until exhaustion. Exhaustion was defined as the inability to maintain pedalling frequency above 60 revolutions per minute (rpm). PPO was defined as the highest power output the subject completed for 150 s, plus the fraction of time spent in the final work rate multiplied by 25 W. The vertical and horizontal

position of the seat, handle bar length and height were standardised for each subject over the three days of testing. Subjects were also required to remain seated throughout the PPO trials.

2.2.6 EMG data collection

The EMG activity of the muscles was recorded using the telemetric EMG system (Telemyo 900, Noraxon, USA, Inc., Arizona, USA). Two electrodes (Blue Sensor, Medicotest, Denmark) were placed on the belly of the following lower limb muscles during all the tests; Vastus lateralis (VL), Vastus medialis (VM), Rectus femoris (RF), Biceps femoris (BF) and Medial and Lateral gastrocnemius (MG and LG respectively). Prior to placing the electrodes on the skin, the skin over the muscle was shaved and cleaned with ethanol. The placement and location of the electrodes are according to the recommendations by SENIAM (Surface EMG for Non-invasive Assessment of Muscles) [82]. Therefore, two electrodes were carefully taped to the belly of each muscle, parallel to the muscle fibers with an inter-electrode distance of 20 mm. A telemetric signal was relayed to an antenna connected to an online computer and captured at 2000 Hz. The wire-leads connected to the electrodes were well secured with tape to avoid artefacts from lower limb movements during cycling. Before recording the EMG, each subject was asked to contract their muscles to verify the absence of crosstalk in the EMG signal. Since the trials were approximately 15 minutes of cycling, the influence of sweat on the EMG signal was assumed to be negligible.

2.2.7 EMG analysis

The raw digital EMG signal was processed using Noraxon's Myoresearch software (Version 2.11).The raw EMG data was filtered using a 50 Hz notch filter to remove any electrical interference from external sources (MyoResearch 2.02). The signal was then filtered a second time using a 15-500 Hz band pass filter. This allowed noise or movement interference below 15 Hz and other non-physiological signals above 500 Hz to be removed. The data were smoothed using route mean squared analysis (RMS), which was calculated for a 50 ms window.

EMG analysis of PPO trial:

EMG was recorded for 10 s halfway through each workload and at exhaustion during the PPO trials. From this recording 3 s of EMG data were extracted and analysed (used for MVC, Sprint and 70 % PPO method of normalisation). The method of sampling of the PPO trial changed when the data were normalised to the Active Period MVC. For this method, 3 contractions (with similar peak amplitudes) were isolated within each 10 s recording. The EMG value ($\mu v \cdot s$) was calculated for each contraction and then time-normalised to one second [$(\mu v \cdot s) \cdot s^{-1}$] (referred to as mean amplitude).

2.2.8 Methods of normalisation

Four methods of normalisation were investigated in this study.

2.2.8.1 MVC Method

When normalising using the MVC method, 3 s of EMG data were extracted from the middle period of the 5 s MVC which produced the highest force. The amplitude of the 3 s periods taken from each workload measurement during the PPO was normalised to the 3 s period obtained during the MVC. EMG data from the PPO trials were expressed as a percentage of MVC. Subjects were verbally motivated to encourage them to achieve their maximum potential (during both the PPO and MVC protocols).

2.2.8.2 Sprint Method

When normalising to the 10 s sprint, three 3 s period within the 10 s measurement (taken in the sprint) were isolated and averaged. The averaged amplitudes of these sections were used for normalisation. EMG data from the PPO trials are expressed as a percentage of averaged EMG amplitude during sprint (Figure 2.2). Subjects were verbally motivated to encourage them to achieve their maximum potential.

2.2.8.3 70 % PPO Method

When normalising to 70% PPO, EMG was captured for 10 seconds at the end of each minute (of cycling at 70 % PPO for five minutes). 3 s periods were then analysed from each 10 s measurement, and the resulting amplitude was then averaged (Figure 2.2). EMG data from the PPO trials are expressed as a percentage of the average EMG amplitude during the 70 % PPO cycle.

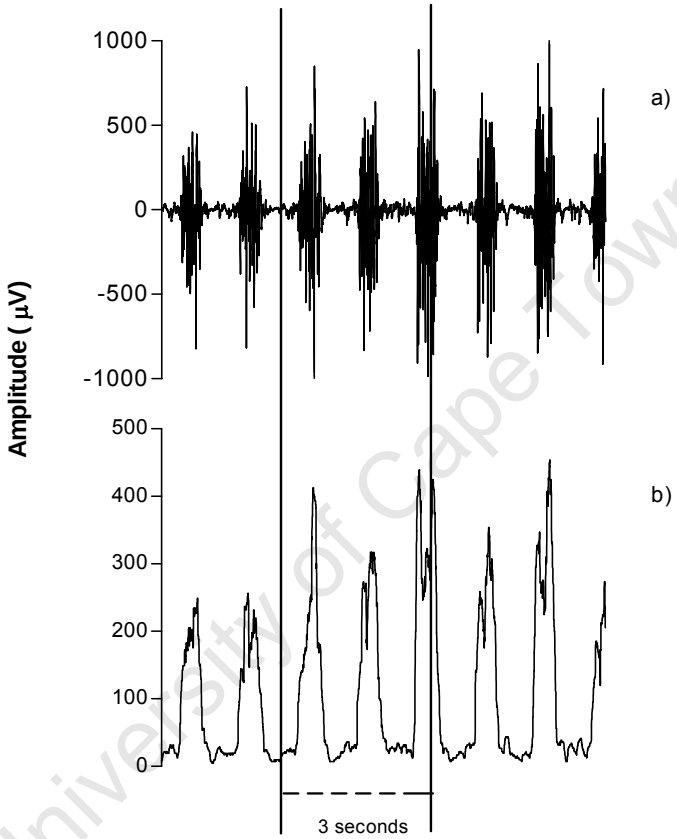


Figure 2.2: The method used to analyse the PPO trials, Sprint and 70 % PPO methods of normalisation. (a) *Raw EMG data of 10 second period from the PPO trial and the methods of normalisation;* ***(b)*** *Rectified EMG of 10 second period, showing the 3 second epoch starting at the beginning of muscle contraction, from the PPO trial and the methods of normalisation.*

2.2.8.4 Active Period MVC Method (AP MVC Method)

The mean amplitudes from the PPO trial were normalised to the mean amplitude of the pre-trial MVC (obtained from three seconds out of the five seconds of EMG recorded) which produced the highest force. The EMG data from the PPO trial is expressed as a percentage of MVC (Figure 2.3).

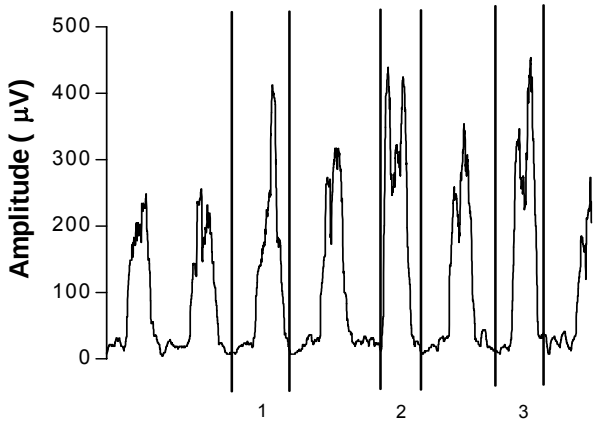


Figure 2.3: The method used to analyse the PPO trials for AP MVC method of normalisation. *From rectified EMG, isolation of 3 active periods (contractions) within the 10 second recording and obtaining the mean amplitude values.*

2.2.9 Statistical analysis

All results were analysed using a statistical software programme (Statistica 7, StatSoft, Tulsa, OK, USA). Results were expressed as means ± standard deviation (SD) The repeatability was investigated using the Intraclass Correlation Coefficient (ICC). The ICC scores ranged between R = 0.80 and 1.00 were defined as representing "good" reproducibility, scores between R = 0.60 and 0.79 a "fair" reproducibility and less than R = 0.60 a "poor" reproducibility [170]. The 95 % confidence intervals were calculated for the ICC using software downloaded from www.newstats.org [85]. Analysis of Variance (ANOVA) with repeated measures was used to detect significant differences between the methods of normalisation. Sometimes there was a negative ICC meaning the with-in subject variance exceeded the between subject variance, thus ICC R = 0 which indicates no repeatability [115]. A Tukey *post hoc* test was used to detected differences in muscle activity between the 6 stages of the PPO test. The method of normalisation variability over 3 days for each subject (Intra-subject variability) was assessed using coefficient of variation (CV) analysis. Intra-subject variability for each method of normalisation was calculated by dividing the overall standard deviation by the overall mean over 3 trials and expressing this as a percentage. Acceptable variability has been defined as CV values less than 10 % [127; 194], however Taylor & Bronks [178] have shown CV values between 9 – 12 % for the quadriceps muscles. Thus, as a

cautionary measure, the definition for an “acceptable” CV value is regarded as less than 12 % in this and the subsequent chapters. P< 0.05 has been considered as significant in all statistical tests.

2.3 RESULTS

2.3.1 Descriptive characteristics

The descriptive characteristics of the cyclists are shown in Table 2.1. Based on these data the subjects can be considered trained cyclists.

Table 2.1: Subject characteristics (n=13)

Variable	Mean ± SD
Age (yrs)	27.3 ± 8.0
Height (m)	1.8 ± 0.1
Mass (kg)	72.6 ± 6.1
Max Heart Rate (bpm)	183 ± 9
% Body Fat	10.5 ± 2.7
PPO (W)	370.0 ± 28.7
Predicted $VO_{2\ max}$ (ml/kg/min)	64.1 ± 4.5
P/W ratio ($W.kg^{-1}$)	5.2 ± 0.5

Predicted VO_2max derived from equation of Hawley and Noakes [80]

2.3.2 Repeatability

Table 2.2 shows the ICC values for each of the four methods of normalisation. The data are displayed for every load increase in power output for each muscle (n=3 trials). The quadriceps muscles (VM) have “poor” repeatability using most methods of normalisation during the load 6 (which is nearing the end of the trials for most subjects) and the calf muscles (MG and LG) are highly repeatable over the different power output loads.

Table 2.2: Intra-class correlations of EMG normalised to MVC, Sprint, 70% PPO and AP MVC methods for each load during PPO trials.

Muscle	Method	Load 1	Load 2	Load 3	Load 4	Load 5	Load 6
VM	MVC	0.68 (0.31-0.91)	0.74 (0.41-0.93)	0.73 (0.36-0.93)	**0.86 (0.58-0.97)**	**0.83 (0.52-0.97)**	0.39 (0.00-0.93)
	AP MVC	**0.82 (0.49-0.96)**	0.76 (0.38-0.95)	0.76 (0.38-0.95)	**0.81 (0.47-0.96)**	**0.84 (0.54-0.97)**	0.38 (0.00-0.86)
	Sprint	0.56 (0.04-0.91)	0.70 (0.34-0.91)	0.77 (0.28-0.97)	**0.91 (0.56-0.99)**	**0.94 (0.68-1.00)**	0.62 (0.00-0.97)
	70%PPO	**0.96 (0.88-0.99)**	**0.94 (0.83-0.98)**	0.76 (0.44-0.93)	**0.87 (0.61-0.97)**	**0.95 (0.83-0.99)**	**0.85 (0.37-0.99)**
RF	MVC	*0.00 (0.00-1.00)*	0.08 (0.00-0.58)	0.64 (0.25-0.89)	0.65 (0.27-0.90)	0.71 (0.29-0.94)	0.83 (0.41-0.98)
	AP MVC	*0.39 (0.02-0.75*	0.24 (0.00-0.65)	0.54 (0.17-0.83)	0.65 (0.31-0.88)	**0.82 (0.57-0.95)**	0.74 (0.38-0.93)
	Sprint	*0.71 (0.40-0.90)*	0.32 (0.00-0.71)	0.78 (0.52-0.93)	0.55 (0.17-0.84)	0.68 (0.31-0.91)	0.34 (0.00-0.81)
	70%PPO	***0.83 (0.59-0.95)***	**0.86 (0.65-0.96)**	**0.92 (0.79-0.98)**	0.77 (0.46-0.94)	**0.91 (0.73-0.98)**	0.64 (0.14-0.93)
VL	MVC	0.72 (0.46-0.94)	**0.85 (0.61-0.94)**	**0.85 (0.61-0.94)**	0.79 (0.49-0.94)	**0.85** (0.59-0.94)	**0.84** (0.44-0.95)
	AP MVC	0.68 (0.31-0.91)	0.74 (0.41-0.93)	0.72 (0.37 -0.92)	0.67 (0.30-0.90)	0.62 (0.22-0.88)	0.61 (0.21-0.88)
	Sprint	0.55 (0.17-0.84)	0.66 (0.31-0.89)	0.73 (0.41-0.92)	0.77(0.46-0.94)	0.75 (0.41-0.93)	0.74 (0.28-0.93)
	70%PPO	**0.95 (0.87-0.98)**	**0.94 (0.85-0.98)**	**0.96 (0.90-0.99)**	**0.94 (0.84-0.98)**	**0.85 (0.63-0.96)**	**0.83 (0.55-0.96)**
BF	MVC	**0.88 (0.70-0.97)**	**0.90 (0.74-0.97)**	**0.89 (0.72-0.97)**	**0.87 (0.66-0.97)**	**0.81 (0.53-0.95)**	**0.88 (0.54-0.99)**
	AP MVC	**0.87 (0.70-0.96)**	**0.88 (0.72-0.96)**	**0.85 (0.66-0.95)**	**0.90 (0.75-0.97)**	**0.84 (0.59-0.96)**	0.66 (0.01-0.97)
	Sprint	**0.90 (0.75-0.97)**	0.77 (0.50-0.92)	**0.87 (0.69-0.96)**	**0.80 (0.53-0.94)**	0.76 (0.44-0.93)	0.71 (0.33-0.93)
	70%PPO	**0.89 (0.74-0.97)**	**0.83 (0.62-0.94)**	**0.90 (0.76-0.97)**	**0.88 (0.70-0.96)**	**0.81 (0.55-0.94)**	**0.92 (0.74-0.98)**
MG	MVC	**0.84 (0.65-0.94)**	**0.86 (0.69-0.95)**	**0.84 (0.65-0.94)**	**0.93 (0.83-0.98)**	**0.88 (0.70-0.97)**	**0.83 (0.55-0.96)**
	AP MVC	0.63 (0.30-0.86)	0.68 (0.37-0.88)	0.69 (0.39-0.89)	0.69 (0.39-0.89)	0.69 (0.39-0.89)	**0.81(0.55-0.94)**
	Sprint	**0.90 (0.76-0.97)**	**0.91 (0.78-0.97)**	**0.91 (0.78-0.97)**	**0.96 (0.89-0.99)**	**0.90 (0.75-0.97)**	**0.85 (0.57-0.97)**
	70%PPO	**0.88 (0.72-0.96)**	**0.94 (0.85-0.98)**	**0.94 (0.85-0.98)**	**0.81 (0.55-0.94)**	**0.84 (0.63-0.95)**	**0.90 (0.68-0.98)**
LG	MVC	0.75 (0.48-0.91)	**0.83 (0.62-0.94)**	**0.81 (0.58-0.93)**	**0.85 (0.66-0.95)**	**0.90 (0.74-0.97)**	**0.85 (0.67-0.87)**
	AP MVC	**0.80 (0.58-0.93)**	**0.82 (0.61-0.94)**	**0.85 (0.67-0.95)**	**0.84 (0.65-0.94)**	**0.83 (0.63-0.94)**	**0.92 (0.80-0.98)**
	Sprint	0.79 (0.55-0.93)	**0.81 (0.58-0.93)**	**0.81 (0.58-0.93)**	**0.92 (0.80-0.98)**	**0.85 (0.63-0.96)**	0.78 (0.41-0.95)
	70%PPO	**0.96 (0.90-0.99)**	**0.92 (0.81-0.97)**	**0.92 (0.81-0.97)**	**0.97 (0.92-0.99)**	**0.89 (0.72-0.97)**	**0.90 (0.68-0.98)**

Intra-class correlations with 95% confidence intervals for methods of normalisation for each muscle, Vastus medialis VM), Rectus femoris (RF), Vastus lateralis (VL), Biceps femoris (BF), Medial gastrocnemius (MG) and Lateral gastrocnemius (LG), over 3 separate days of cycling. Values highlighted in bold represent what are considered to be "good" ICC values. The section represented in the block is graphically displayed in Figure 2.4.

Table 2.3 depicts the mean ICC values of the six power output loads of Table 2.2 for each method of normalisation.

Table 2.3: Mean Intra-class correlations for Peak Power Output EMG normalised to MVC, Sprint, 70 % PPO and AP MVC methods

Muscle	MVC	Sprint	70 % PPO	AP MVC
VM	0.70 (0.52-0.88)	0.75 (0.59-0.91)	**0.88 (0.80-0.97)**	0.72 (0.55-0.91)
RF	0.47 (0.11-0.85)	0.56 (0.35-0.77)	**0.82 (0.71-0.93)**	0.56 (0.33-0.79)
VL	0.82 (0.76-0.87)	0.70 (0.61-0.79)	**0.91 (0.85-0.97)**	0.67 (0.62-0.73)
BF	0.87(0.84-0.91)	0.80 (0.72-0.88)	**0.87 (0.83-0.92)**	0.83 (0.74-0.93)
MG	0.86 (0.82-0.92)	**0.90 (0.86-0.94)**	0.88 (0.83-0.94)	0.70 (0.64-0.76)
LG	0.83 (0.78-0.88)	0.82 (0.77-0.88)	**0.92 (0.89-0.96)**	0.84 (0.80-0.89)

Mean ICC values of methods of normalisation for each muscle; Vastus medialis (VM), Rectus femoris (RF), Vastus lateralis (VL), Biceps femoris (BF), Medial gastrocnemius (MG) and Lateral gastrocnemius (LG), over 3 separate days of cycling. Values presented as mean (of 6 work loads) with 95 % confidence intervals. Bolded values indicate the highest ICC value for each muscle.

The 70 % PPO method was the only method of normalisation to yield "good" ICC values for all muscles, with average ICC values for all muscles being greater than 0.82 (Table 2.3). The MVC method was repeatable for MG, LG, VL and BF as seen by average ICC values greater than 0.80. This method however, showed "poor" repeatability for RF (0.47 (0.11-0.85)). The Sprint method showed similar repeatability patterns as the MVC method, where MG and LG had "high" average ICC values, (ICC R = 0.90 (0.86 - 0.94) and 0.82 (0.77 - 0.88) respectively), and VM, VL and BF had only "fair" repeatability (Average ICC between 0.70 and 0.75) but slightly less ICC values than that derived from the MVC method . Repeatability for the AP MVC method was "fair to good" except for RF, where average ICC was R = 0.56 (0.33 - 0.79).

To further highlight the variation in muscle activity using different methods of normalisation, a subsection of ICC values from Table 2.2 (highlighted in the block) are graphically presented. ICC values for RF activity at Load 1 (subject body weight x 3.33 W) were randomly chosen, as this load showed the most variation in ICC values of each method of normalisation, thus highlighting the individual variation in muscle

activity at this power output. In an attempt to clarify the results the graphs in Figure 2.4 are arranged so that the first 6 subjects are represented on the left panel and the remaining subjects on the right panel. The greatest variation in muscle activity over the 3 trials was found for the MVC Method (ICC R = 0.00 (0.00-1.00)), while the sprint (Figure 2.4 c) and 70 % PPO (Figure 2.4 d) methods showed less variation in muscle activity from day-to-day and thus show greater ICC values.

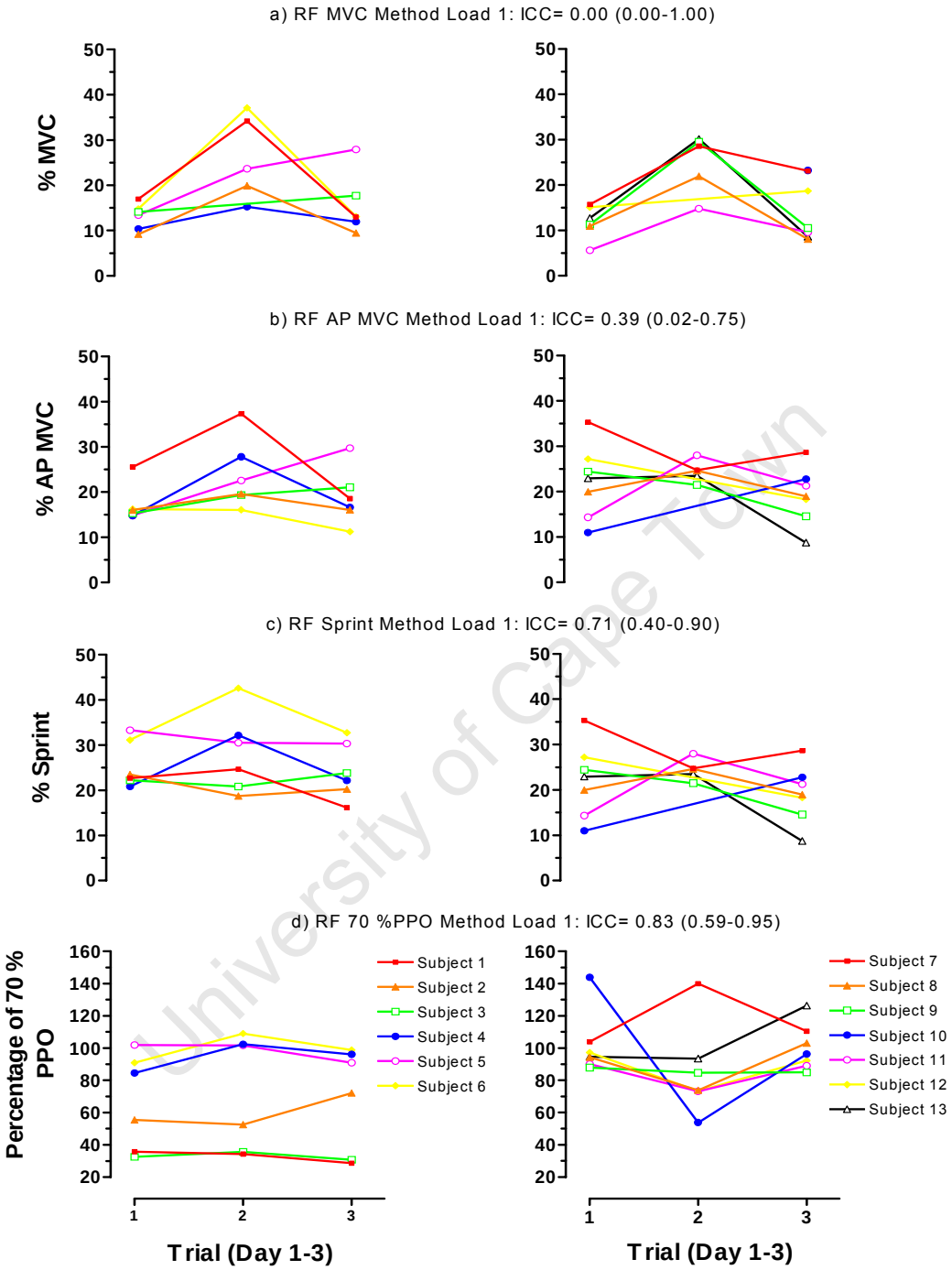


Figure 2.4: Variability of Rectus femoris (RF) muscle activity at Load 1 over three trials normalised to a) MVC method, b) Active Period MVC, c) Sprint method and d) 70% PPO method. *These graphs illustrate the intra-class correlation with 95% confidence intervals highlighted in the block in Table 2.2. The first 6 subjects are represented on the left panel and the remaining subjects on the right panel*

2.3.3 Intra-subject reliability

The intra-subject coefficient of variation (CV) of each subject muscle activity over the 3 trials provides an indication of which method of normalisation produced more reproducible results. Figure 2.5, provides a graphical display of the CV for each method normalisation for each subject. The graphs showed various degrees of variation for each subject. To simplify the intra-subject CV's in Figure 2.5 and to emphasize which method showed the lowest intra-subject CV's, the graphs were divided into 3 zones; Zone 1(CV values ≤ 12 %), Zone 2 (CV values 12 % > and ≤ 20 %), Zone 3 (CV values > 20 %).

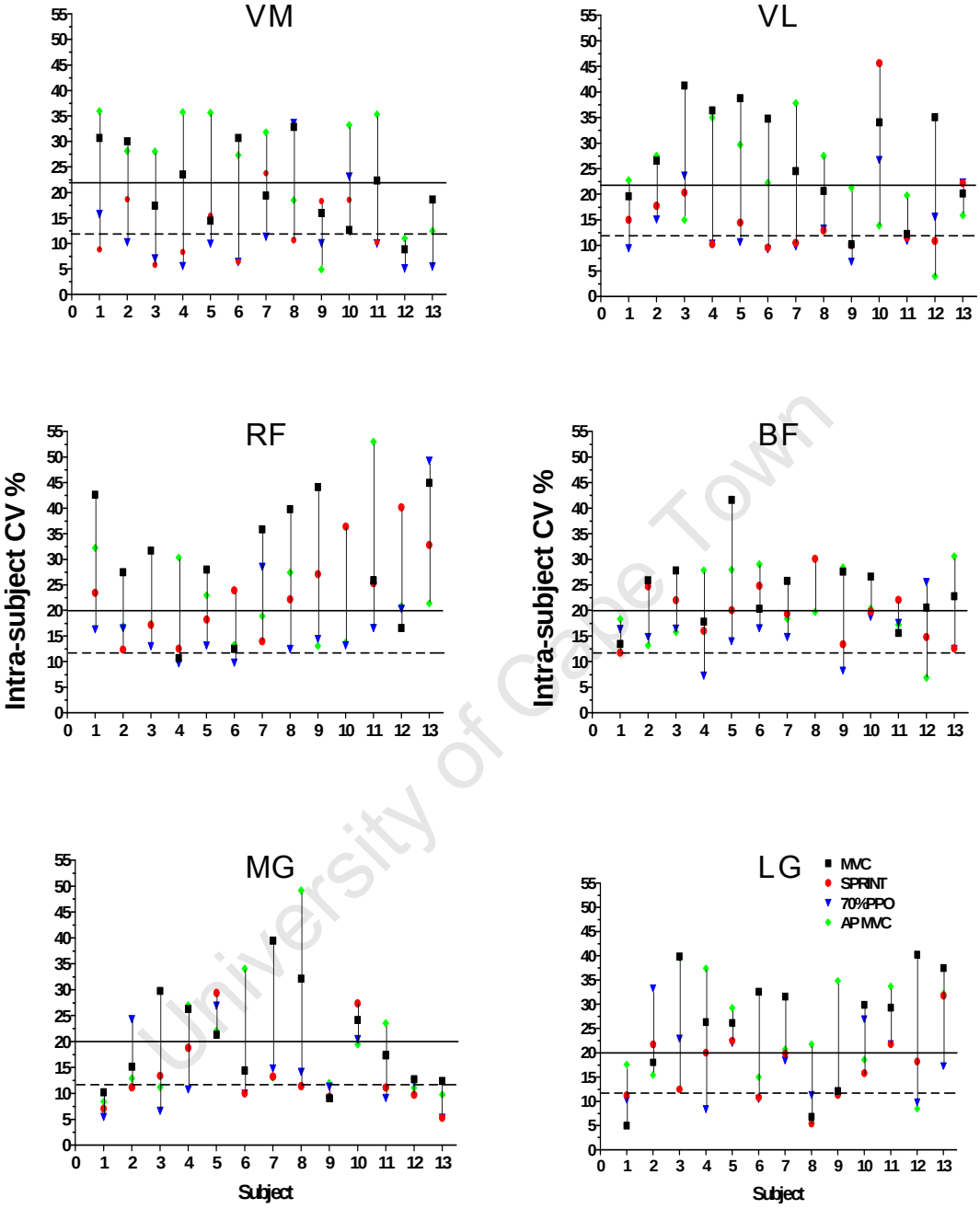


***Figure 2.5**: **Intra-subject coefficient of variation (CV) for the four methods of normalisation for Vastus medialis (VM), Vastus lateralis (VL), Rectus femoris (RF), Biceps femoris (BF), Medial and Lateral gastrocnemius (MG and LG respectively) recorded over three trials.** Dashed line represents the range of "low" variation, between 0 - 12 %. Solid Line representing "medium" variation (12-20 %).*

Since there are different subject numbers for the different methods, the CV values were expressed as a percentage of the subject number. For example for VM 70 % PPO method, there are 10 CV values in Zone 1, therefore 10/13 subjects = 77 %, 1 CV value in Zone 2 therefore 1/13 = 7 % and 2 CV values in Zone 3 therefore 2/13 = 15 %.The method of normalisation with the highest percentage of CV values in Zone 1 is seen to show the least intra-subject variability over 3 trials. Furthermore, the method of normalisation with the highest percentages in Zone 3, has the most intra-subject variability.

Table 2.4: Percentage of the summed scores for the intra-subject coefficient of variation zones

Muscles	Norm Method	Zone 1 %	Zone 2 %	Zone 3 %
VM	MVC	8	**46**	46
	AP MVC	15	15	**69**
	Sprint	58	33	8
	70%PPO	**77**	8	15
VL	MVC	8	15	**77**
	AP MVC	8	**31**	62
	Sprint	46	**31**	23
	70%PPO	**54**	23	23
RF	MVC	8	17	**75**
	AP MVC	0	46	54
	Sprint	0	38	61
	70%PPO	**15**	**61**	23
BF	MVC	0	25	**75**
	AP MVC	8	46	46
	Sprint	8	54	39
	70%PPO	**17**	**75**	8
MG	MVC	15	**38**	**46**
	AP MVC	38	23	38
	Sprint	**61**	23	15
	70%PPO	54	23	23
LG	MVC	15	15	**69**
	AP MVC	8	**31**	62
	Sprint	31	**31**	38
	70%PPO	**47**	15	38

Percentage of CV values in each zone for each method of normalisation and muscle; Vastus medialis (VM), Vastus lateralis (VL), Rectus femoris (RF), Biceps femoris (BF), Medial and Lateral gastrocnemius (MG and LG respectively). CV values summed in each zone calculated as a percentage of the subject number *per Zone 1(CV values ≤ 12 %), Zone 2 (CV values 12 % > and ≤ 20 %), Zone 3 (CV values > 20 %). The methods with highest percentages in each zone for each muscle, is highlighted in bold.*

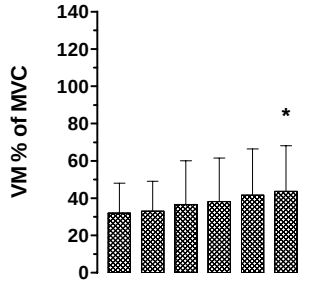
This calculation highlighted that 70 % PPO method showed the least intra-subject variability for five out of six muscles (VL, RF, BF and LG) (Table 2.4). The Sprint

method produced the least intra-subject variability for MG, where 61 % of the CV values were in Zone 1. The MVC method showed the highest percentage intra-subject variability for all muscles except VM, where most of the CV values were in Zone 3 (ranging between 46 % - 77 %).The AP MVC method also showed most of its CV values to be in Zone 3 (ranging between 38 % - 69 %).

2.3.4 Sensitivity

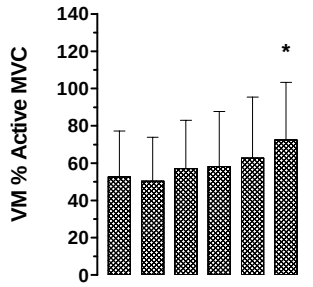
The PPO trial consisted of increasing workloads. For the sensitivity phase of the experiment the PPO trial was divided into 5 loads and a final load. The muscle activity was measured at each load and the data were normalised using the four methods. The assumption of this experimental approach was that a sensitive method of normalisation would detect the changes in muscle activity as the workload increased.

For the purpose of this analysis the EMG measurement should detect differences in EMG activity with increasing workload. This however does not take into account peripheral factors, associated with fatigue, which may affect the amplitude of the EMG signal. Only the results of one of the three trials is shown (we randomly chose to present Trial 2 data). This approach was chosen for the reason that by averaging the data over three trials would cause a 2^{nd} degree error by reducing the standard deviations. To clarify the data analysis a graph of EMG amplitude is shown for each muscle at different power output loads, where load 1 is the first power output load cycled and F is the final power output cycled by each subject. The graphs/tables are interpreted as follows; in Figure 2.5, the 70 % PPO method, the muscle activity in VM load 1 compared to that in VM final resulted in a significant difference of $p< 0.00$ and the muscle activity in VM load 2 compared to that in VM final resulted in a significant difference of $p< 0.01$.



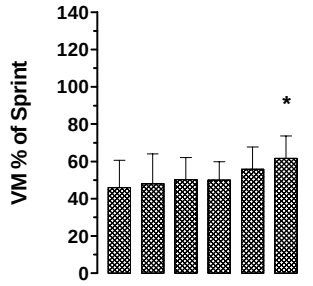
MVC method ($F_{5;40}$=8.6; p < 0.001)

STAGES	{1} 31.5	{2} 32.5	{3} 37.5	{4} 38.2	{5} 41.5	{6} 45.5
VM 1 {1}		1.00	0.19	0.12	0.00	0.00
VM 2 {2}	1.00		0.38	0.26	0.01	0.00
VM 3 {3}	0.19	0.38		1.00	0.64	0.04
VM 4 {4}	0.12	0.26	1.00		0.78	0.07
VM 5 {5}	0.00	0.01	0.64	0.78		0.62
Mfinal {6}	0.00	0.00	0.04	0.07	0.62	



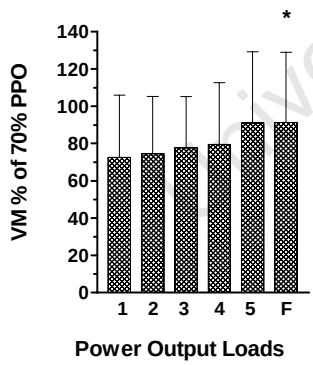
Active Period MVC method ($F_{5;40}$=11.6; p < 0.001)

STAGES	{1} 52.6	{2} 50.4	{3} 57.1	{4} 58.0	{5} 62.6	{6} 72.4
VM 1 {1}		0.98	0.75	0.56	0.04	0.00
VM 2 {2}	0.98		0.34	0.21	0.01	0.00
VM 3 {3}	0.75	0.34		1.00	0.54	0.00
VM 4 {4}	0.56	0.21	1.00		0.72	0.00
VM 5 {5}	0.04	0.01	0.54	0.72		0.05
VMfinal {6}	0.00	0.00	0.00	0.00	0.05	



Sprint method ($F_{5;30}$= 9.8; p < 0.005)

STAGES	{1} 42.1	{2} 42.9	{3} 48.9	{4} 50.7	{5} 55.7	{6} 62.3
VM 1 {1}		1.00	0.38	0.16	0.01	0.00
VM 2 {2}	1.00		0.53	0.25	0.01	0.00
VM 3 {3}	0.38	0.53		0.99	0.39	0.01
VM 4 {4}	0.16	0.25	0.99		0.71	0.03
VM 5 {5}	0.01	0.01	0.39	0.71		0.43
VMfinal {6}	0.00	0.00	0.01	0.03	0.43	



70% PPO method ($F_{5;40}$= 7.2; p < 0.001)

STAGES	{1} 72.7	{2} 73.6	{3} 80.8	{4} 83.8	{5} 90.9	{6} 96.4
VM 1 {1}		1.00	0.59	0.25	0.01	0.00
VM 2 {2}	1.00		0.70	0.33	0.01	0.00
VM 3 {3}	0.59	0.70		0.99	0.34	0.03
VM 4 {4}	0.25	0.33	0.99		0.71	0.13
VM 5 {5}	0.01	0.01	0.34	0.71		0.87
VMfinal {6}	0.00	0.00	0.03	0.13	0.87	

Figure.2.6: Vastus medialis (VM) mean muscle activity at the 6 power output loads of PPO for Trial 2, when normalised to MVC, Active Period MVC, Sprint and 70 % PPO method. *Muscle activity significantly different between stages was shown as *P<0.05. The table provides the post hoc repeated measures significant differences between the stages. The bold value heading each column is the average EMG at each load. The column heading {1} represents VM load 1, {2} represents VM load 2 etc.*

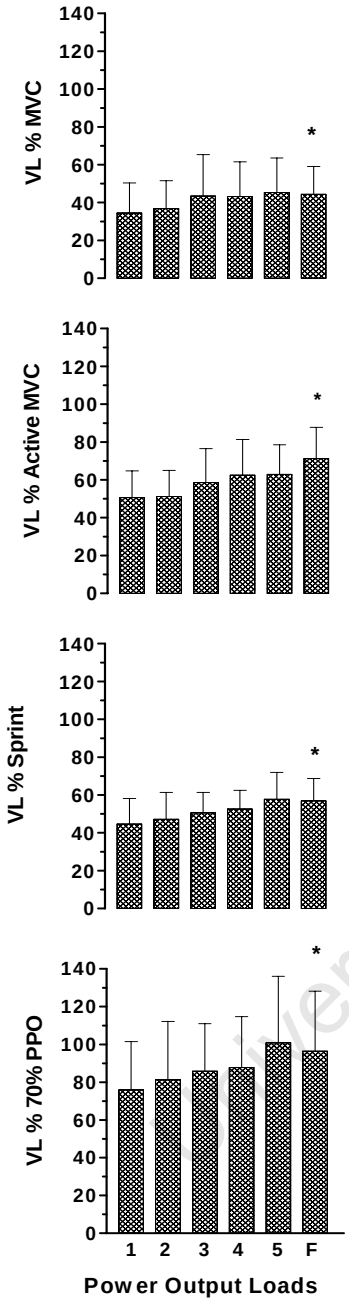
For VM over 6 loads (Figure 2.6), the Sprint and AP MVC methods showed muscle activity to be significantly different over more loads than the MVC and 70 % PPO methods. These two methods found muscle activity in loads 1 - 4 to be significantly lower than the final load, whereas MVC and 70 % PPO methods were only able to detect loads 1 - 3 to be significantly lower than the final load.

Figure 2.7 shows AP MVC method to have the highest number of significant changes over loads for VL, where loads 1-5 were significantly lower than the final load. In addition loads 1 and 2 were significantly lower than loads 4 - final. The Sprint and 70% PPO methods only showed loads 1 and 2 to be significantly lower than the final load. The MVC method did show significant differences between loads using repeated measures ANOVA, however the post hoc analysis was unable to detect any specific significances between loads.

AP MVC method showed the most significant differences over loads for RF (Figure 2.8), where muscle activity in loads 3 and 4 were significantly lower than the activity in the final load. In addition, muscle activity in loads 1 and 2 were significantly lower than loads 4, 5 and final. MVC and 70 % PPO methods showed similar findings where loads 1 and 2 were significantly lower than 4, 5 and final. The MVC method showed the 5th and final load to be significantly higher than load 3 ($p=0.03$ and $p=0.02$ respectively) whereas the 70 % PPO method only showed load 3 to be tending towards significance compared to load 5 ($p=0.07$) and the final load ($p=0.06$).

For BF (Figure 2.9), the Sprint method showed the greatest sensitivity in detecting change in muscle activity over the workloads, where load 1 was significantly lower than loads 4, 5 and final. 70 % PPO and MVC method were only able to detect significant differences of load 1 compared to the 5^{th} and final load. Significant differences between loads were found for AP MVC, however the *post hoc* analysis was unable to identify which loads were significantly different. None of the methods were able to detect significant differences in muscle activity between the workloads for MG and LG (Figure 2.10 and 2.11 respectively), even though the MVC and Sprint methods showed a

significant stage effect throughout the trial for MG ($p = 0.029$ and $p = 0.032$, respectively).



MVC Method ($F_{5;45}$=3.4; p < 0.010)

STAGES	{1} 35.9	{2} 37.6	{3} 45.3	{4} 43.0	{5} 45.3	{6} 45.1
VL 1		1.00	0.06	0.27	0.06	0.07
VL 2	1.00		0.18	0.57	0.18	0.21
VL 3	0.06	0.18		0.98	1.00	1.00
VL 4	0.27	0.57	0.98		0.98	0.99
VL 5	0.06	0.18	1.00	0.98		1.00
VL final	0.07	0.21	1.00	0.99	1.00	

Active Period MVC Method ($F_{5;55}$=15.7; p < 0.000)

STAGES	{1} 50.6	{2} 51.2	{3} 58.6	{4} 62.4	{5} 62.9	{6} 71.3
VL 1		1.00	0.06	0.00	0.00	0.00
VL 2	1.00		0.10	0.00	0.00	0.00
VL 3	0.06	0.10		0.76	0.64	0.00
VL 4	0.00	0.00	0.76		1.00	0.03
VL 5	0.00	0.00	0.64	1.00		0.05
VLfinal	0.00	0.00	0.00	0.03	0.05	

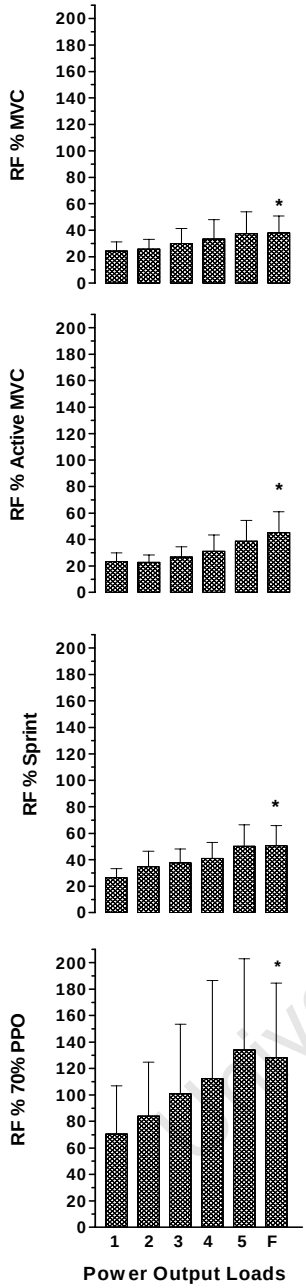
Sprint Method ($F_{5;40}$=4.2; p < 0.003)

STAGES	{1} 46.1	{2} 46.9	{3} 51.5	{4} 53.7	{5} 57.6	{6} 57.9
VL 1		1.00	0.63	0.27	0.02	0.02
VL 2	1.00		0.76	0.39	0.04	0.03
VL 3	0.63	0.76		0.99	0.52	0.47
VL 4	0.27	0.39	0.99		0.87	0.84
VL 5	0.02	0.04	0.52	0.87		1.00
VLfinal	0.02	0.03	0.47	0.84	1.00	

70 %PPO Method ($F_{5;45}$=6.1; p < 0.001)

STAGES	{1} 77.6	{2} 79.9	{3} 87.9	{4} 91.9	{5} 100.7	{6} 98.7
VL 1		1.00	0.51	0.11	0.00	0.00
VL 2	1.00		0.77	0.26	0.01	0.02
VL 3	0.51	0.77		0.95	0.16	0.31
VL 4	0.11	0.26	0.95		0.60	0.82
VL 5	0.00	0.01	0.16	0.60		1.00
VLfinal	0.00	0.02	0.31	0.82	1.00	

Figure 2.7: Vastus lateralis (VL) mean muscle activity at the 5 power output loads of PPO for Trial 2, when normalised to MVC, Active Period MVC, Sprint and 70 % PPO method. *Muscle activity significantly different between stages as shown as *P<0.05. The table provides the post hoc repeated measures significant differences between the stages. The bold value heading each column is the average EMG at each load. The column heading {1} represents VL load 1, {2} represents VL load 2 etc.*



MVC Method ($F_{5;35}$=11.6; p < 0.001)

STAGES	{1} 24.6	{2} 26.1	{3} 29.9	{4} 33.3	{5} 37.3	{6} 37.9
RF 1 {1}		0.99	0.25	0.01	0.00	0.00
RF 2 {2}	0.99		0.56	0.04	0.00	0.00
RF 3 {3}	0.25	0.56		0.69	0.03	0.02
RF 4 {4}	0.01	0.04	0.69		0.51	0.37
RF 5 {5}	0.00	0.00	0.03	0.51		1.00
RFfinal {6}	0.00	0.00	0.02	0.37	1.00	

Active Period MVC Method ($F_{5;45}$=27.9; p < 0.000)

STAGES	{1} 23.9	{2} 23.3	{3} 27.3	{4} 31.8	{5} 38.6	{6} 47.7
RF 1 {1}		1.00	0.75	0.04	0.00	0.00
RF 2 {2}	1.00		0.60	0.02	0.00	0.00
RF 3 {3}	0.75	0.60		0.50	0.00	0.00
RF 4 {4}	0.04	0.02	0.50		0.10	0.00
RF 5 {5}	0.00	0.00	0.00	0.10		0.01
RFfinal {6}	0.00	0.00	0.00	0.00	0.01	

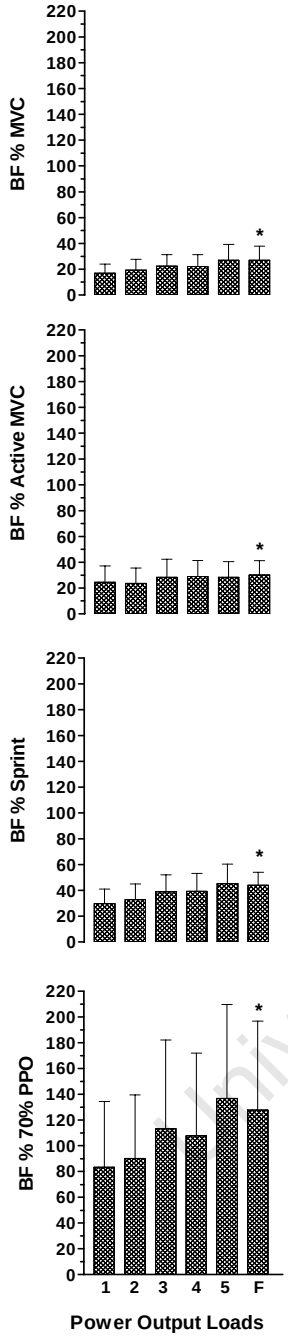
Sprint Method ($F_{5;40}$=10.6; p < 0.000)

STAGES	{1} 27.4	{2} 34.4	{3} 38.1	{4} 42.3	{5} 50.1	{6} 52.1
RF 1 {1}		0.53	0.12	0.01	0.00	0.00
RF 2 {2}	0.53		0.95	0.39	0.01	0.00
RF 3 {3}	0.12	0.95		0.90	0.05	0.02
RF 4 {4}	0.01	0.39	0.90		0.41	0.19
RF 5 {5}	0.00	0.01	0.05	0.41		1.00
RFfinal {6}	0.00	0.00	0.02	0.19	1.00	

70% PPO Method ($F_{5;35}$=11.3; p < 0.000)

STAGES	{1} .7	{2} .8	{3} 1.0	{4} 1.2	{5} 1.3	{6} 1.3
RF 1 {1}		0.97	0.11	0.00	0.00	0.00
RF 2 {2}	0.97		0.42	0.02	0.00	0.00
RF 3 {3}	0.11	0.42		0.63	0.07	0.07
RF 4 {4}	0.00	0.02	0.63		0.79	0.77
RF 5 {5}	0.00	0.00	0.07	0.79		1.00
RFfinal {6}	0.00	0.00	0.07	0.77	1.00	

Figure 2.8: Rectus femoris (RF) mean muscle activity at the 5 power output loads of PPO for Trial 2, when normalised to MVC, Active Period MVC, Sprint and 70 % PPO method. *Muscle activity significantly different between stages was shown as *P<0.05. The table provides the post hoc repeated measures significant differences between the stages. The values in blue are tending towards significance. The bold value heading each column is the average EMG at each load. The column heading {1} represents RF load 1, {2} represents RF load 2 etc.*



MVC Method ($F_{5;45}$=6.6; p < 0.000)

STAGES	{1} 16.9	{2} 18.9	{3} 21.7	{4} 22.2	{5} 27.1	{6} 27.1
BF 1 {1}		0.95	0.31	0.21	0.00	0.00
BF 2 {2}	0.95		0.84	0.72	0.01	0.01
BF 3 {3}	0.31	0.84		1.00	0.19	0.18
BF 4 {4}	0.21	0.72	1.00		0.28	0.27
BF 5 {5}	0.00	0.01	0.19	0.28		1.00
BFFinal {6}	0.00	0.01	0.18	0.27	1.00	

Active Period MVC Method ($F_{5;50}$=2.4; p < 0.045)

STAGES	{1} 23.8	{2} 22.5	{3} 27.3	{4} 28.0	{5} 28.2	{6} 29.2
BF 1 {1}		0.99	0.72	0.52	0.48	0.25
BF 2 {2}	0.99		0.38	0.22	0.19	0.08
BF 3 {3}	0.72	0.38		1.00	1.00	0.97
BF 4 {4}	0.52	0.22	1.00		1.00	1.00
BF 5 {5}	0.48	0.19	1.00	1.00		1.00
BFFinal {6}	0.25	0.08	0.97	1.00	1.00	

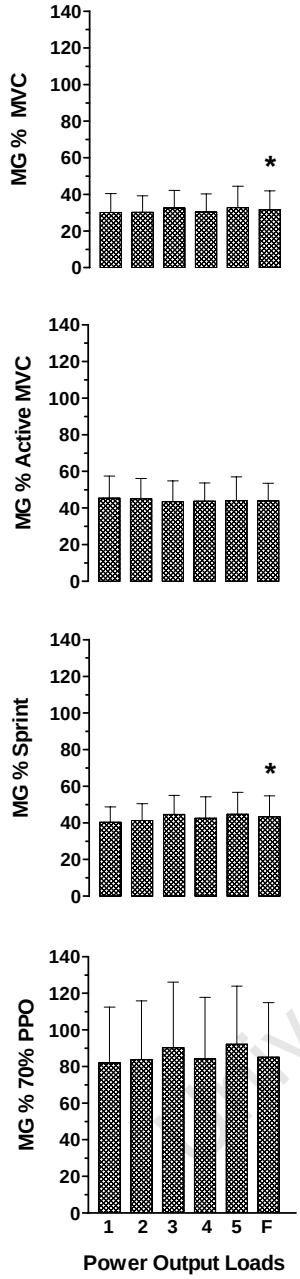
Sprint Method ($F_{5;45}$=8.0; p < 0.000)

STAGES	{1} 29.0	{2} 31.9	{3} 38.3	{4} 38.7	{5} 45.2	{6} 44.6
BF 1 {1}		0.95	0.07	0.05	0.00	0.00
BF 2 {2}	0.95		0.39	0.32	0.00	0.00
BF 3 {3}	0.07	0.39		1.00	0.30	0.40
BF 4 {4}	0.05	0.32	1.00		0.37	0.47
BF 5 {5}	0.00	0.00	0.30	0.37		1.00
BFFinal {6}	0.00	0.00	0.40	0.47	1.00	

70%PPO Method ($F_{5;45}$=5.2; p < 0.000)

STAGES	{1} 86.8	{2} 91.0	{3} 114.6	{4} 114.9	{5} 136.7	{6} 132.0
BF 1 {1}		1.00	0.26	0.25	0.00	0.01
BF 2 {2}	1.00		0.44	0.42	0.01	0.03
BF 3 {3}	0.26	0.44		1.00	0.50	0.74
BF 4 {4}	0.25	0.42	1.00		0.52	0.75
BF 5 {5}	0.00	0.01	0.50	0.52		1.00
BFFinal {6}	0.01	0.03	0.74	0.75	1.00	

Figure 2.9: Biceps femoris (BF) mean muscle activity at the 5 power output loads of PPO for Trial 2, when normalised to MVC, Active Period MVC, Sprint and 70 % PPO method. *Muscle activity significantly different between stages was shown as *P<0.05. The table provides the post hoc repeated measures significant differences between the stages. The bold value heading each column is the average EMG at each load. The column heading {1} represents BF load 1, {2} represents BF load 2 etc.*



MVC Method ($F_{5:50}$=2.7; p < 0.029)

STAGES	{1} .3	{2} .3	{3} .3	{4} .3	{5} .3	{6} .3
MG 1 {1}		1.00	0.21	0.76	0.12	0.95
MG 2 {2}	1.00		0.12	0.60	0.07	0.86
MG 3 {3}	0.21	0.12		0.93	1.00	0.71
MG 4 {4}	0.76	0.60	0.93		0.81	1.00
MG 5 {5}	0.12	0.07	1.00	0.81		0.54
MGfinal {6}	0.95	0.86	0.71	1.00	0.54	

Active Period MVC Method ($F_{5;60}$= 0.1; p < 0.970)

STAGES	{1} 45.4	{2} 44.9	{3} 43.6	{4} 43.8	{5} 44.1	{6} 43.9
MG 1 {1}		1.00	0.98	0.99	1.00	0.99
MG 2 {2}	1.00		1.00	1.00	1.00	1.00
MG 3 {3}	0.98	1.00		1.00	1.00	1.00
MG 4 {4}	0.99	1.00	1.00		1.00	1.00
MG 5 {5}	1.00	1.00	1.00	1.00		1.00
MGfinal {6}	0.99	1.00	1.00	1.00	1.00	

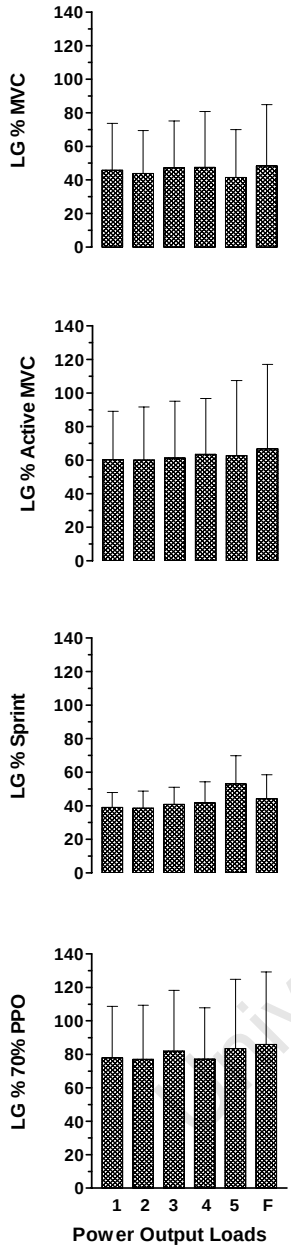
Sprint Method ($F_{5;50}$= 2.6; p < 0.032)

STAGES	{1} .4	{2} .4	{3} .4	{4} .5	{5} .4	{6} .4
MG 1 {1}		1.00	0.08	0.44	0.10	0.70
MG 2 {2}	1.00		0.17	0.67	0.21	0.89
MG 3 {3}	0.08	0.17		0.94	1.00	0.77
MG 4 {4}	0.44	0.67	0.94		0.96	1.00
MG 5 {5}	0.10	0.21	1.00	0.96		0.82
MGfinal {6}	0.70	0.89	0.77	1.00	0.82	

70%PPO Method ($F_{5;50}$= 2.2; p < 0.065)

STAGES	{1} 83.1	{2} 84.1	{3} 92.7	{4} 88.6	{5}92.1	{6} 85.5
MG 1 {1}		1.00	0.16	0.71	0.21	0.99
MG 2 {2}	1.00		0.25	0.84	0.32	1.00
MG 3 {3}	0.16	0.25		0.90	1.00	0.45
MG 4 {4}	0.71	0.84	0.90		0.95	0.96
MG 5 {5}	0.21	0.32	1.00	0.95		0.54
MGfinal {6}	0.99	1.00	0.45	0.96	0.54	

Figure 2.10: Medial gastrocnemius (MG) mean muscle activity at the 5 power output loads of PPO for Trial 2, when normalised to MVC, Active Period MVC, Sprint and 70 % PPO method. *Muscle activity significantly different between stages was shown as *P<0.05. The table provides the post hoc repeated measures significant differences between the stages. The bold value heading each column is the average EMG at each load. The column heading {1} represents MG load 1, {2} represents MG load 2 etc.*



MVC Method ($F_{5;45}$=0.2; p < 0.970)

STAGES	{1} .4	{2} .4	{3} .4	{4} .4	{5} .4	{6} .4
LG 1 {1}		0.99	1.00	1.00	1.00	0.98
LG 2 {2}	0.99		1.00	1.00	0.99	1.00
LG 3 {3}	1.00	1.00		1.00	1.00	1.00
LG 4 {4}	1.00	1.00	1.00		1.00	0.99
LG 5 {5}	1.00	0.99	1.00	1.00		0.99
LGFinal {6}	0.98	1.00	1.00	0.99	0.99	

Active Period MVC Method ($F_{5;60}$= 0.4; p < 0.800)

STAGES	{1} 79.4	{2} 76.7	{3} 79.1	{4} 79.4	{5} 83.4	{6} 81.6
LG 1 {1}		1.00	1.00	1.00	0.99	1.00
LG 2 {2}	1.00		1.00	1.00	0.93	0.98
LG 3 {3}	1.00	1.00		1.00	0.99	1.00
LG 4 {4}	1.00	1.00	1.00		0.99	1.00
LG 5 {5}	0.99	0.93	0.99	0.99		1.00
LGFinal {6}	1.00	0.98	1.00	1.00	1.00	

Sprint Method ($F_{5;55}$=1.0; p < 0.420)

STAGES	{1} .4	{2} .3	{3} .4	{4} .4	{5} .3	{6} .4
LG 1 {1}		1.00	1.00	1.00	0.51	1.00
LG 2 {2}	1.00		1.00	1.00	0.51	1.00
LG 3 {3}	1.00	1.00		1.00	0.51	1.00
LG 4 {4}	1.00	1.00	1.00		0.52	1.00
LG 5 {5}	0.51	0.51	0.51	0.52		0.52
LGFinal {6}	1.00	1.00	1.00	1.00	0.52	

70%PPO Method ($F_{5;50}$=0.2; p < 0.950)

STAGES	{1} 79.4	{2} 76.7	{3} 79.1	{4} 79.4	{5} 83.4	{6} 81.6
LG 1 {1}		1.00	1.00	1.00	0.99	1.00
LG 2 {2}	1.00		1.00	1.00	0.93	0.98
LG 3 {3}	1.00	1.00		1.00	0.99	1.00
LG 4 {4}	1.00	1.00	1.00		0.99	1.00
LG 5 {5}	0.99	0.93	0.99	0.99		1.00
LGFinal {6}	1.00	0.98	1.00	1.00	1.00	

Figure 2.11: Lateral gastrocnemius (LG) mean muscle activity at the 5 power output loads of PPO for Trial 2, when normalised to MVC, Active Period MVC, Sprint and 70 % PPO method. *Muscle activity significantly different between stages was shown as *P<0.05. The table provides the post hoc repeated measures significant differences between the stages. The bold value heading each column is the average EMG at each load. The column heading {1} represents LG load 1, {2} represents LG load 2 etc.*

An alternative method for showing the sensitivity of each method of normalisation to track muscle activity to power output changes, was to calculate the ratio of power output to EMG amplitude. The results of this analysis are graphically displayed in Figure 2.12. The slope of the lines is an indication of the sensitivity of the methods ability to track changes in power output, where a slope close to zero suggests that the method is more sensitive. This however does not take into account peripheral factors and fatigue that may affect the amplitude of the EMG signal. Therefore the data between load 5 and the final load should be interpreted with caution due to the increase in fatigue.

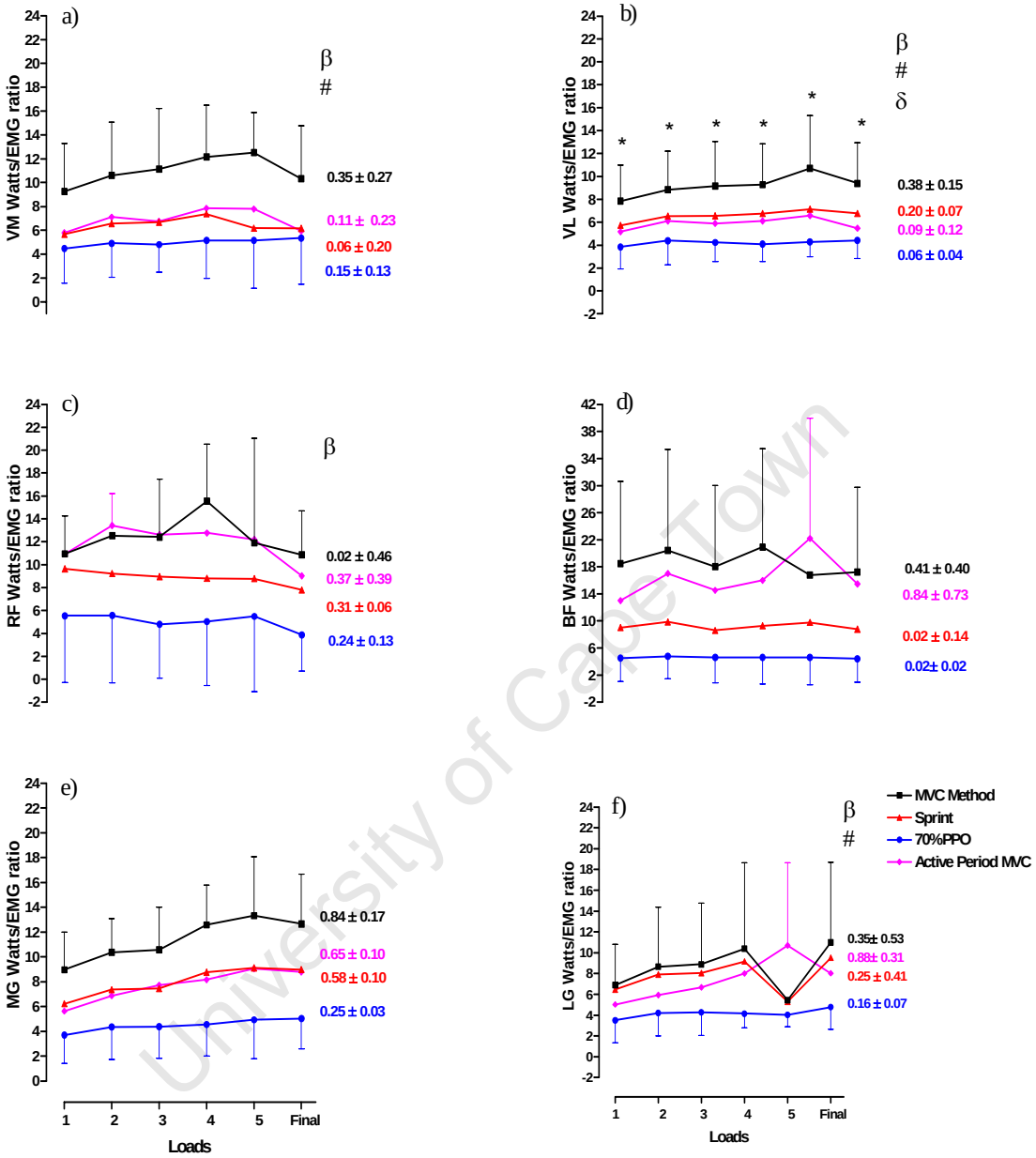


Figure *2.12: Power output to EMG ratio for 6 power output loads of Trial 2, when normalised to MVC, Active Period MVC, Sprint and 70 % PPO method for a) Vastus medialis (VM), b) Vastus lateralis (VL), c) Rectus femoris (RF), d) Biceps femoris (BF, e) Medial gastrocnemius (MG), f) Lateral gastrocnemius (LG).* *The slope and standard deviations of each method are presented.*

Significant load effect: # (P<0.05);

Significant method effect: β (P<0.05);

Significant interaction effect: δ (P<0.05);

*Significantly different to Sprint, AP MVC and 70 % PPO method: *(P<0.05)*

There was no significant difference in the Power/EMG ratio between the various methods of normalisation except for VL, where ratio of the MVC method was significantly greater than the other methods at each power output load (Figure 2.12. b); and LG, where the Power/EMG ratio for 70 % PPO method was significantly lower than the AP MVC method (Figure 2.12.f). The 70 % PPO method had the most horizontal slopes for most of the muscles except for VM, and was therefore the most capable method of normalisation for tracking EMG amplitude to changes in power output, assuming the absence of fatigue. For VM (Figure 2.12.a), the Sprint and AP MVC methods showed the most horizontal slopes closest to zero of 0.06 ± 0.20 and 0.11 ± 0.23 respectively. The MVC method had the highest slope for this VM (0.35 ± 0.27). For VL, 70 % PPO and AP MVC methods had the most horizontal slope with similar values of (0.06 ± 0.04) and (0.09 ± 0.12) respectively. The MVC (0.02 ± 0.46) and 70% PPO method (0.24 ± 0.13) had the most horizontal slopes for RF, the MVC method however had the greatest standard deviation. The Sprint and 70 % PPO method showed similarly horizontal slopes for BF (0.02 ± 0.02 and 0.02 ± 0.14 respectively). For MG and LG, the 70 % PPO method had the most horizontal slope (0.25 ± 0.03) and (0.16 ± 0.07) respectively. It is important to note that these slope values are relatively high compared to the lowest slope values for VM, VL and BF.

To summarise which method is most sensitive in representing corresponding power output change with change in EMG amplitude, the results of the two methods used were tabulated, namely; sensitivity of EMG amplitude to increasing power output workloads (Figure 2.6-2.11) and power output to EMG ratio (Figure 2.12). The results are presented in Table 2.5. In summary, the AP MVC method demonstrated the greatest ability in detecting changes in EMG amplitude over increasing power output stages for most muscles. However, when analysing the data with regards to power output/EMG ratio, the 70 % PPO method showed the greatest ability in tracking EMG amplitude to power output changes. Important to note was that the MVC method was not seen to be ‘usable’ in detecting changes between workloads for VM and VL (including the gastrocnemius muscles which none of the methods were able to detect). This method was also not ‘usable’ for tracking EMG amplitude to power output changes for VM, VL and BF. The AP MVC was also found to be not ‘usable’ in detecting changes

in power output for BF as well as EMG/power output ratio for the gastrocnemius muscles.

Table 2.5: The recommended and 'usable' methods of normalisation found for each muscle using the sensitivity analysis.

Muscle	Sensitivity between workloads	Sensitivity of power output to EMG ratio
VM	Sprint , AP MVC (70 % PPO)	Sprint, AP MVC (70 % PPO)
VL	AP MVC (70 % PPO & Sprint)	70 % PPO, AP MVC (Sprint)
RF	AP MVC, MVC (70 % PPO & Sprint)	70 % PPO, MVC (Sprint)
BF	Sprint (70 % PPO & MVC)	Sprint and 70 % PPO (AP MVC)
MG	None	70 % PPO (Sprint & MVC)
LG	None	70 % PPO (Sprint & MVC)

Most sensitive methods of normalisation found from sensitivity analysis in Figure 2.6 - 2.11 and Figure 2.12. VM, Vastus medialis; VL, Vastus lateralis; RF, Rectus femoris; BF, Biceps femoris; MG, Medial gastrocnemius and LG, Lateral gastrocnemius. The 'usable' methods are enclosed in brackets.

2.4 DISCUSSION

The aim of the study was to examine four different methods of normalising EMG activity measured during incremental cycling to exhaustion, with specific emphasis on identifying the most repeatable and suitable one for dynamic cycling exercises. These methods included two methods with normalisation to an isometric MVC (MVC method and Active Period MVC method) and two methods with normalisation to cycling (Sprint method and 70 % PPO method). According to the previously described requirements for a method of normalisation to be considered appropriate, it must display repeatability,

reliability and must be sensitive to changes in exercise workload during the dynamic cycling trial. These three criteria are evaluated individually in the following sections.

2.4.1 Repeatability of the methods of normalisation

Repeatability is an indication of reproducibility, thus the method should produce similar results over different testing trials (within day and between days). The primary finding of this study was that while there are variations between the specific muscles and with specific loads, the highest repeatability of EMG activity of the lower limb muscles was found when using the 70 % PPO Method (Tables 2.2 and 2.3). Since the 70 % PPO method utilises a submaximal exercise profile, this finding is consistent with earlier research that showed submaximal exercise to more repeatable than maximal efforts in isometric contractions [105; 124; 197]. Mathur et al. [124] studied the repeatability of RF, VL and VM during sustained isometric contractions at 80 % and 20 % MVC maintained to exhaustion. EMG amplitude was reliable (moderate to high) during 20 % MVC where ICC ranged from 0.58 - 0.99. In contrast, the 80 % MVC trial had a greater degree of variability and produced low ICC values. Likewise, Kollmitzer et al. [105] showed EMG to be more repeatable for 50 % MVC knee extensions than 100 % MVC, where RF was more reliable than VL and VM.

With respect to specific muscles, Mathur et al. [124] found no differences in the repeatability of VM, VL and RF EMG activity during the 20 % MVC, which they attributed to the lower force requirement whilst performing the task. The present study showed that VM, RF and VL had high ICC (averaged over 6 loads, R = 0.88, 0.82 and 0.91 respectively) when normalised to the submaximal dynamic 70 % PPO method (Table 2.3). Laplaud et al. [113], whose method of normalisation involved expressing root mean square (RMS) values as a percentage of the maximal RMS value recorded during the incremental cycling trial, found RF to be the least repeatable. It is important to note that the present study showed RF to have low ICC values when normalised to the 'maximal' methods of normalisation of MVC, Sprint and AP MVC (Table 2.3). However, the submaximal 70 % PPO method did show RF to have good repeatability where ICC was R = 0.82 (0.71 - 0.93). The fact that RF is a biarticulate muscle which is involved in

both leg extension and flexion cannot be the sole reason for the variability found in the measurements. If this was the reason, then the BF which is involved in leg flexion and extension would show similar trends. Yet in the present study, BF had good repeatability for all 4 methods of normalisation (Table 2.3). Another possible reason for RF variability could be that RF comprises a higher percentage of fatigable fast twitch muscle fibers [108; 124], which would explain the high variability found using the 'maximal' and fatiguing methods of normalisation used in the present study and in the study conducted by Laplaud et al. [113]. The use of the submaximal 70 % PPO method may reduce this variability by activating fewer fast twitch fibers and not fatiguing these fibers to the same degree as maximal methods of normalisation.

Moreover, the fact that the 70 % PPO Method is dynamic in nature may result in a more relevant representation of muscle activity during the trial. This method is representative of regular neural muscle control strategy [63] and takes into account the change in muscle length during cycling. Kellis & Baltzopoulos [98] found dynamic method of normalisation to be more appropriate because this method considers the effects of muscle activation, muscle length and angular velocity on EMG. The study of Kellis & Baltzopoulos [98] was one of the first studies to use EMG from MVC during an isokinetic muscle action that used the same joint angle and joint angle velocity as the task EMG for the denominator in the normalisation equation.

Few studies have investigated the suitability of using isometric MVC methods to normalise EMG measured during non-isometric tasks. A study by Dubo et al. [44], where EMG during gait was normalised to 100 % MVC, found large inter-subject variability. Knutson & Richards [104] continued this research by normalising each stride in the gait cycle to the peak EMG amplitude during that stride. Their findings showed a lower variability than that reported by Dubo et al. [44], where EMG was normalised to 100 % MVC. Yang & Winter [198] subsequently investigated both methods of normalisation during gait. They normalised gait EMG to isometric methods; 50 % MVC (since they previously showed 100 % MVC to be less reliable) and mean EMG per unit isometric force ($\mu v.Nm^{-1}$). They also used dynamic methods, peak and mean within-subject ensemble averages. This study found the dynamic methods reduced inter-subject variability.

The result of the present study and previous research pertaining to the repeatability of the methods indicates that the use of static isometric methods is not as appropriate for normalisation in dynamic cycling exercise. The dynamic methods of normalisation, predominantly the 70 % PPO method, were found to be 'highly' repeatable for most muscles.

2.4.2 Intra-subject reliability of the methods of normalisation

The reliability tested in this chapter refers to the reproducibility of EMG activity of the same individual performing repeated trials [84]. This study found the best intra-subject reliability of EMG amplitude when normalising the cycling trials to 70 % PPO method. For most muscles (VM, VL, RF, BF and LG) (Table 2.4), summed percentage scores ranged from 14 % to 21 %. The Sprint method produced similar intra-subject CV values to that of the 70 % PPO method, and the MVC and AP MVC methods had the highest variability for all muscles (as seen by the highest scores produced). These findings suggest that EMG amplitude during cycling is most reliable when normalised to dynamic methods, preferably the submaximal methods.

This finding is similar to that of Bolgla & Uhl [21]. They investigated the repeatability and reliability of three methods of normalisation; MVC, Mean dynamic (m-Dyn) and Peak Dynamic (pk-Dyn) methods for analysing hip abductor rehabilitation exercises on healthy subjects. Subjects performed 3 open kinetic and 3 closed kinetic chain hip abductor exercises. Intra-subject CV's ranged between 11 - 22 % for all methods; however the m-Dyn method provided the lowest intra-subject CVs for 5 out of the 6 exercises performed. Interestingly the pk-Dyn method had the highest CV values. Based on these results, Bolgla & Uhl [21] agreed with other studies [25; 196; 198], that the m-Dyn method provides greater measurements of reliability.

It is of interest to note is that the maximal methods of normalisation (MVC, AP MVC and Sprint methods) show the highest variability, which could possibly be explained by the following reasoning. An inverse relationship exits between force production and force variability [124] where higher forces show greater variability and hence increased intra-

subject variability [142]. By performing a maximal task/contraction, we assume the person is performing and providing a maximal effort, which adds to the variability of the method [197] as well as the motivational factors [106]. In addition, EMG measurements from MVC's have previously been shown to be less reliable than submaximal contractions [105; 124; 198], and because some individuals are able to activate their muscles closer to the maximal level of activation than others [69]. Felici [64] explained that MVC was a poor representation of muscle function during normal activity since it represents a simplified version of movement. This could be a possible reason for the high intra-subjects CV's found for the MVC methods.

The finding of the present study is in agreement with previous research that has found dynamic methods of normalisation to have less variability than the maximal static isometric method.

2.4.3 Sensitivity of the methods of normalisation

Sensitivity of the method of normalisation refers to the methods' ability to track changes in workload or exercise intensity. For this analysis, the EMG measurement was able detect differences in EMG activity with increasing workload. This however does not take into account the presence of fatigue and the affect this has on the EMG amplitude. It is reasonable to expect muscle force and EMG amplitude to have a linear relationship (in the absence of fatigue), since they both depend on the number of motor units recruited [59]. For this reason, EMG amplitude increases with increasing power output/workload [18; 51; 52; 119]. Taylor & Bronks [178] found during incremental cycling, the relationship between EMG amplitude and exercise intensity to be linear. Similarly, in the present study the PPO trial consisted of increasing workloads. For the sensitivity phase of the experiment the PPO trial was divided into 5 loads and a final load cycled by each subject. The muscle activity was measured at each load and the data were normalised using the four methods. The assumption of this experimental approach was that a sensitive method of normalisation would detect the changes in muscle activity as the workload increased.

Since muscle activity has been shown to be repeatable during incremental cycling [68; 113], it could be assumed that the changes found in muscle activity during each cycling load was highlighted more by the type of method of normalisation used and not entirely due to the variation in muscle activity caused by fatigue or from the intrinsic and extrinsic influences on the EMG signal. In this study the aim was to measure the ability of each method to detect changes in muscle activity over 5 workloads of the PPO test and also during the final stage of the PPO trial. The AP MVC and Sprint methods (Figures 2.6 - 2.11 and Table 2.5) were the most sensitive in detecting changes in muscle activity over all the 6 stages for all muscles except MG and LG. However, the AP MVC and Sprint methods were not highly repeatable for RF, VL (Table 2.2). By interpreting these data in an alternative manner (Figure 2.12), i.e. where the data are presented as a ratio of power output to EMG amplitude, it was found that the 70 % PPO method showed the lowest/most horizontal linear slope for all muscles except VM. This technique represents the ability of the method to track changes in power output with that of EMG amplitude.

It is important to note that no method was able to detect changes in EMG amplitude over the 6 power out stages for MG and LG. This could possibly be answered by studies investigating the effects of cycling cadences on muscle activity. A study conducted by Marsh et al. [121] showed that an increase in cadence resulted in a significant increase in average EMG amplitude of the gastrocnemius muscles despite the cyclists' cycling experiences. This study was in agreement with study conducted by Goto et al. [72], which found gastrocnemius EMG amplitude increased linearly with an increase in cadence. More interestingly, Gregor et al. [74] showed gastrocnemius activation to be associated more strongly with cadence changes than changes in power output. They found similar gastrocnemius EMG amplitude when cycling at 90 rpm for power outputs of both 90 W and 270 W, despite significant power output differences; for instance when EMG amplitude was lower when cycling at a cadence of 60 rpm at 180 W. Sarre et al. [165], found that muscle activity of the knee extensor muscles (VM and VL) were not significantly influenced by cadence manipulations. Even though the cadence was not recorded during the trials in the present study, it can thus be assumed that all the cyclists maintained their cadence until exhaustion, since no method of

normalisation showed any significant changes in gastrocnemius activity throughout the PPO trials.

In summary, the most appropriate method of normalisation is one that reflects reproducibility over different testing times, has low variation and the ability to detect changes in EMG amplitude with changes in exercise intensity.

It is important to point out that the specific, practical application of measured EMG data is also dependent on the research question and the nature of the study being performed. For example, for a once-off trial in which the absolute EMG activity or percentage of total active muscle is to be estimated using surface EMG measurements, repeatability is less important than intra-individual CV. Rather, in this situation, it is important to use a method of normalisation that represents what may be considered a true maximum of skeletal muscle activation. In contrast, if the study is designed to measure physiological/biological changes as a result of some intervention or fatigue process, then the reliability, repeatability and sensitivity become more important. Therefore, in considering the optimal normalisation technique, the three previously identified factors (repeatability, reliability and sensitivity) must be interpreted in the context of the overall research question.

Accordingly, the present study has shown that normalising EMG during cycling to the 70 % PPO method meets the three requirements and is therefore recommended for studies that are investigating changes in muscle activity over different days, where reliability and repeatability of measurements becomes more important.

However, for investigating the maximal muscle activity during a trial, the Sprint method of normalisation is recommended, since it provides a reliable method that is similar in nature to the subsequent cycling trial. Further, the Sprint method was sensitive to changes in workload. However, it is important to note that this method showed poor reproducibility for RF. Nevertheless, for a once-off trial where the absolute level of EMG activity (and hence muscle activity) is to be indirectly measured, it is still recommended that researchers utilise the Sprint method as a method of normalisation for cycling activity.

With regards to the MVC methods of normalisation, both MVC methods (MVC and AP MVC) are questionable. Normalising to the MVC method was unable to detect changes in EMG amplitude over increasing power output stages as that shown by the AP MVC. Furthermore, the AP MVC displayed poor repeatability. If researchers choose to use the MVC methods, it is recommended that researchers analyse the data by taking 3 s epochs of trials and not 3 separate contractions (bursts) as done for AP MVC method, since this method is more repeatable. However it is important to note that by perhaps increasing the number of active periods analysed, repeatability could possibly be improved.

The findings of this study have important clinical laboratory implications with regards to the method of normalisation and which muscles are being measured, since certain methods of normalisation are found to be better for certain muscles only. It is important that researchers should use a method of normalisation that is suitable to their study design and research question and therefore allows them to measure true biological variation rather than incur experimental error as a result of an inappropriate methodology.

In conclusion, based on the results of the present study, the methods of normalisation most suited for use during cycling are the 70 % PPO method for trials to exhaustion and in repeated trial study designs, while the Sprint method is recommended for use when the absolute amount of muscle activity (relative to a maximum) is desired as an outcome. For running activity, however, these methods of normalisation may differ. There are a few factors that distinguish running from cycling:

a) The presence of an eccentric part of contraction, during which muscle lengthens. This is absent during cycling.
b) Greater range of motion around the joints, since cycling is constrained to a specific range through the cyclical pedal action.
c) Movement in three planes during running, whereas in cycling, movement and muscle action occurs across two dimensions only.

Therefore, it is considered necessary to evaluate the optimal methods of normalisation for running in a separate study. This study is performed as Chapter 3 of the present thesis.

CHAPTER 3

CRITICAL ANALYSIS OF METHODS FOR NORMALISATION OF SURFACE EMG ACTIVITY DURING RUNNING

3.1 INTRODUCTION

Running requires a complex sequence and combination of muscle activation which includes an interplay of muscular forces and external forces (inertial, gravitational and reaction forces) [63]. Running also involves the shortening (concentric) and lengthening (eccentric) of muscle action known as the stretch shortening cycle (SSC) [73]. Effective storage and release of elastic energy during SSC exercises plays an important role in force production. This ability to utilise stored elastic energy is influenced by the level of preactivation of active muscles as well as the increase in muscle stiffness, velocity and magnitude of stretch, including the time delay between eccentric and concentric phases [149]. Muscle preactivation, which represents a central driven feedforward, anticipatory mechanism [43; 71] during walking and running, regulates muscle stiffness and transition time between pre-stretch and shortening components of SSC.

EMG has been widely used during running studies [110; 134; 135; 171; 189], and has contributed to the understanding of the running gait and neuromuscular causes of fatigue during running activity. Furthermore it is known that EMG amplitude of leg muscles increases with increased running speed [43]. Karamindis et al. [94] investigated reproducibility of EMG and ground reaction forces during various running techniques, however, they only studied at 3 different running speeds (3.5, 3.0 and 2.5 $m.s^{-1}$). A study conducted by Guidetti et al. [75] investigated the inter and intra-subject variability of EMG in normal running gait profiles. This study found that intra-subject CV for peak amplitudes were low and resulted in repeatable waveforms near the peaks of activity, however the inter-subject CV's were high since there appeared to be differences in peak amplitude location between subjects. This study highlighted that individual peculiarities exists in the EMG pattern for a given muscle in running gait. It is clear from these studies that

quantifying the repeatability and error associated with the measurement of EMG obviously has important implications for interpreting and applying the data.

During running activity an individual adjusts their stride frequency and length by greater or lesser amounts [143]. According to Hanon et al. [78], EMG activity cannot be considered as a steady parameter, because even during the same stage, the characteristic of each burst or active period can vary concurrently with changes in stride length and frequency. This contributes to the variability in EMG activity during running. As explained in Chapter 2, there is no reliable alternative to normalising EMG activity during dynamic exercise; more specifically running studies have not shown many alternatives to the use of isometric MVC method as a normalisation tool. The only studies involving the investigation of dynamic activity has been in walking gait analysis, which was reviewed in Chapter 1. The studies reviewed showed that the mean dynamic method of normalisation of EMG activity was the most repeatable and reliable form of normalisation in gait analysis [169; 198].

This chapter therefore aims to find the most appropriate method of normalisation for EMG activity during running. This ideal method of normalisation is required to show "good" repeatability, reliability and sensitivity to changes in exercise intensity (which have been previously discussed in Chapters 1 and 2). In accordance, three methods for normalising EMG during running were evaluated and their variability and sensitivity to change were compared to the MVC method of normalisation. The methods of normalisation used in this study were;

1) MVC method- using the peak EMG amplitude of a 5 second isometric MVC (Maximal static normalisation). This is the current widely-used method.
2) Sprint method- using the averaged peak amplitude over a 20 m sprint (Maximal Dynamic normalisation).
3) 70 % Peak Running Speed Method- using the averaged peak amplitude during 5 minutes of running at 70 % PRS at fixed speed (Submaximal Dynamic Normalisation).

3.2 METHODOLOGY

3.2.1 Subject selection

Twelve well-trained runners were recruited from local running clubs to participate in this study. Subjects were included if they were currently between the ages of 18-35 years old and if they were able to complete the Two Oceans half marathon race (21 km) under 1h 30 min. Once they had signed the informed consent, subjects were required to visit the laboratory on four separate occasions. The study was approved by the Research and Ethics Committee of the Faculty of Health Sciences within the University of Cape Town. The study was performed in accordance with the principles of the Declaration of Helsinki (October, 2000), ICH Good Clinical Practice (GCP) and the laws of South Africa. Subjects were asked to maintain their same physical activity pattern over the course of the study and not to begin any new training or recreational programs. In addition, subjects were asked to not consume caffeine within 3 hours of testing and to maintain the same diet on the morning of each trial. Subjects performed the same experimental protocol on each of the three testing days. Each testing day was separated by 5 - 7 days of normal training.

3.2.2 Experimental design

During the first visit to the laboratory, subjects underwent an anthropometrical assessment and answered the PAR-Q as described in Chapter 2 (Section 2.2.3). Each subject was then familiarised with the equipment used and the testing protocol. On the trial days, subjects were prepared for the placement of the electrodes on 6 muscles (Vastus lateralis (VL), Vastus medialis (VM), Rectus femoris (RF), Biceps femoris (BF) and Medial and Lateral gastrocnemius (MG and LG respectively) on the right leg. Following this each subject performed three maximum voluntary contractions (MVC) of the quadriceps, Biceps femoris and gastrocnemius muscles using the Biodex Dynamometer 3 (Biodex Medical Systems, New York). Subjects were then allowed a self-paced 10 minute warm-up on the gym track, after which they performed a two 20 m maximal sprints. After a 30 minute resting period, subjects then performed their Peak

Running Speed (PRS) test until exhaustion. After a 30 minute resting period, each subject then ran at 70 % of their obtained PRS for 5 laps (700 m). Heart rate was measured continuously throughout the PRS test using Polar Accurex NV heart rate monitor (Polar Electro OY, Kempele, Finland). Subjects were required to wear the same running shoes for all three trials. A schematic of the study design is shown in Figure 3.1.

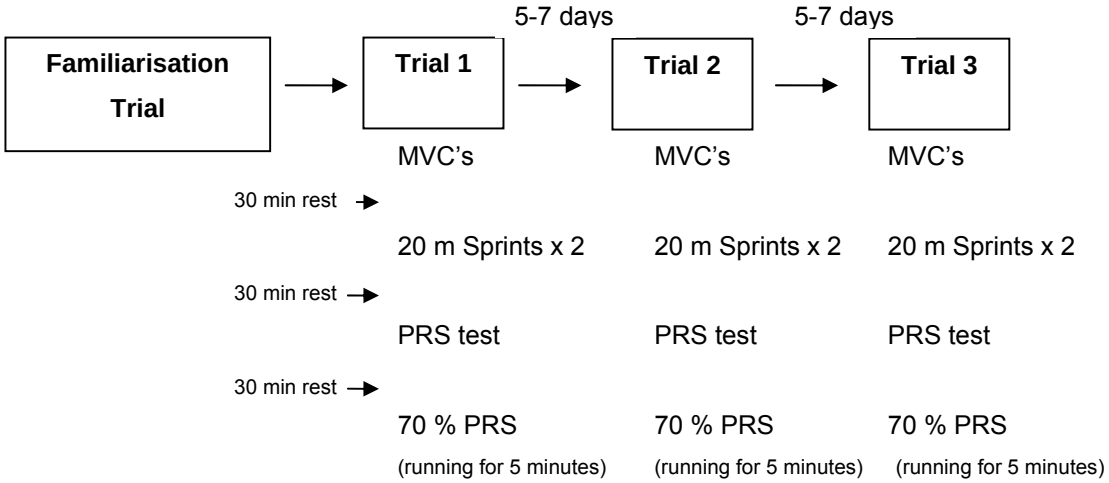


Figure 3.1: Experimental design

3.2.2.1 Familiarisation trial

During their first visit subjects were familiarised with the Biodex by performing maximal voluntary contractions of the quadriceps, Biceps femoris and calf muscles of the right leg. Thereafter, they performed a peak treadmill running speed test on the treadmill (which is a validated method of incremental running speed to exhaustion, explained in Section 3.2.5). By using this preliminary test on the treadmill, subjects' peak track running speed (which were performed in the subsequent three trials) were compared to the validated treadmill method. Once the subjects completed the PTRS test, they were familiarised with the pacing required when running on the indoor gym track. Maintaining the required speed set by the pacing lights is a novel experience for many runners, therefore it was important that they familiarised themselves efficiently prior to the actual experimental trial days.

3.2.3 Maximal voluntary contractions

The same protocol was used as described in Chapter 2 (Section 2.2.4).

3.2.4 20 m sprints

Subjects performed two maximal 20 m sprints on an indoor running track. They were given a 20-30 m running start to ensure a normal and maximal running gait throughout the 20 m distance and to exclude gait changes associated with acceleration during the sprint. The 10 m and 20 m running time was recorded using four photocell gates connected to an electronic timer (Newtest, LTD, Oulu, Finland). The muscle activity during the fastest 20 m was used for EMG normalisation.

3.2.5 Peak running speed trial

The subjects performed the peak running speed test on an indoor gym track. The track had pacing lights which were used to control the running speed of each subject. The indoor track was 140 m with the pacing lights situated on the inner circumference of the track at intervals of 7 m. The lights were set to blink sequentially to coincide with a given running velocity. The pacing lights were set to mimic the Peak Treadmill Running Speed test, where the speed started at 10 $km.h^{-1}$ [167]. Every 30 s, the pacing light speed increased by 0.5 $km.h^{-1}$ until the subject reached exhaustion. The PRS was defined as the fastest running speed the subject could maintain for 30 s. Following a 30 minute resting period the subjects performed a submaximal run on the track at 70 % of PRS for 5 laps. Heart rate (Polar ® Heart Rate Monitors, Polar Electro OY, Kempele, Finland) was measured continuously during the PRS trials. Importantly, all subjects ran around the track in the same direction, where the bend of the track was on the left side for all subjects for all trials.

3.2.6 EMG data collection

The EMG activity of the muscles was recorded using the telemetric EMG system (Telemyo 900, Noraxon, USA, Inc., Arizona, USA), which has been stated to provide scientifically reliable data from the developers. Two electrodes (Blue Sensor, Medicotest, Denmark) were placed on the belly of the lower limb muscles described above during all the tests. Prior to placing the electrodes on the skin, the skin over the muscle was shaved and cleaned with ethanol. Two electrodes were placed on the belly of each muscle with an inter-electrode distance of 20 mm, and carefully taped. A telemetric signal was relayed to an antenna connected to an online computer and captured at 2000 Hz. The wire-leads connected to the electrodes were well secured to the subjects' leg with elastoplast tape to avoid artefacts from lower limb movements during running. The transmitter unit was placed in a halter which was strapped to the subject's back; this technique contributed to minimising the movement artefact. The mass of the halter (± 400 grams) was assumed to have a negligible effect on each subject's running technique. EMG was recorded on one straight of the track (± 35 m) as the track had a slight sharp bend. Thus each subject's data were captured whilst running upright on the straight of the track, thereby eliminating any contribution of weight bearing when running around the bend. Before recording the EMG, each subject was asked to contract their muscles to verify the absence of crosstalk in the EMG signal. Since the trials were approximately 15 minutes of running, the influence of sweat rate was considered as negligible

3.2.7 EMG analysis

The same filtering procedures were performed as described in Chapter 2 Section 2.2.7.

EMG analysis of PRS trial:

EMG data ($\mu v.s$) were calculated for each step (active contraction) and then time-normalised to one second $(\mu v \cdot s) \cdot s^{-1}$ (referred to as mean amplitude). EMG was recorded on one straight of the track (± 35 m) at each running speed and at exhaustion during the

PRS trials. A decision was taken to analyse three strides of EMG data as Arsenault et al. [10] showed that three strides of EMG data per subject provided information as reliable as that obtained from twelve strides during gait trials. Therefore three strides with similar peak amplitudes in the EMG recording were analysed and the derived mean amplitude at each speed was averaged. The EMG data could not be time normalised into the four phases of the running profile i.e. a) preactivation (100 ms before ground contact) b) ground contact c) braking phase and d) propulsion phase, due to problems experienced with the footswitch.

3.2.8 Methods of normalisation

There were three methods of normalisation which were investigated in this study.

3.2.8.1 MVC Method

The EMG from the PRS trial were normalised to the mean amplitude of the MVC (obtained from 3 s out of the 5 s of EMG recorded) which produced the highest force. EMG data from the PRS trials were expressed as a percentage of MVC.

3.2.8.2 Sprint Method

The EMG from the fastest sprint was analysed by isolating three peak amplitude contractions from the middle of the sprint recording. The resultant mean amplitudes were averaged and used for normalisation. The EMG data from PRS trials were expressed as a percentage of the fastest sprint.

3.2.8.3 70 % PRS Method

EMG was recorded for 10 s in each of the five laps run at 70 % PRS. Three peak amplitudes were then isolated from each of the five measurements and the resulting mean amplitude was then averaged. The EMG data from PRS trials were expressed as a percentage 70 % PRS.

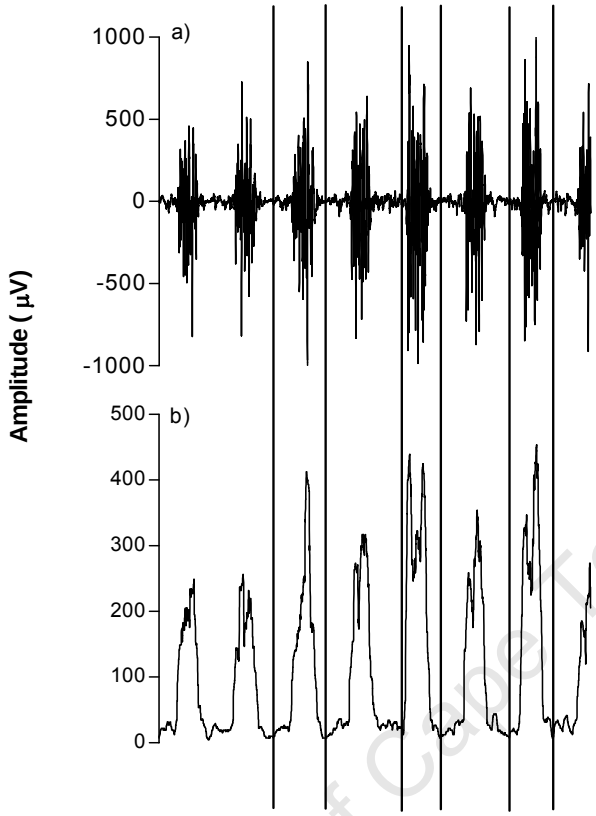


Figure 3.2: The method used to analyse EMG from the PRS trials, Sprint and 70 % PRS methods of normalisation. (a) *Raw EMG data from the PRS trial, Sprint, 70 % PRS;* ***(b)*** *Rectified EMG of 10 second period, isolating the 3 contraction within the recording and taking the mean amplitude values from the PRS trial, Sprint, 70 % PRS.*

The reason for not investigating the MVC method, as in the cycling trial in Chapter 2 (by taking 3 s epochs), was due to the EMG data in the running trials being slightly noisy between active contractions for certain subjects. This would have added false amplitude to the filtered and rectified signal, resulting in the possible misinterpretation of the EMG data. The EMG data were thus analysed by isolating the active periods and not by analysing 3 s epochs as described in Chapter 2.

3.2.9 Statistical analysis

All results were analysed using a statistical software programme (Statistica 7, StatSoft, Tulsa, OK, USA.). Results were expressed as means ± standard deviation (SD).

Correlation analysis was performed to measure relationship between the peak running speeds during the PTRS and the PRS protocols. Repeatability was investigated using the Intraclass Correlation Coefficient (ICC). The ICC values of between R = 0.80 and 1.00 were defined as representing "good" reproducibility, scores between R = 0.60 and 0.79 a "fair" reproducibility and scores less than R = 0.60 a "poor" reproducibility [170]. The 95 % confidence intervals were calculated for the ICC using software downloaded from www.newstats.org [85]. Analysis of Variance (ANOVA) with repeated measures was used to detect significant differences between the methods of normalisation. Sometimes there was a negative ICC, meaning the with-in subject variance exceeded the between subject variance, thus ICC R = 0 indicates no repeatability [115]. A Tukey *post hoc* test was used to detect differences in muscle activity between the running speeds during the PTR test. Reliability of method of normalisation over 3 days for each subject (Intra-subject reliability) was assessed using coefficient of variation (CV) analysis. This was calculated by dividing the overall standard deviation by the overall mean over 3 trials and expressing this as a percentage. As in Chapter 2, CV values less than 12 % were defined as "acceptable". P< 0.05 has been considered as significant in all statistical tests.

3.3 RESULTS

3.3.1 Descriptive characteristics

The descriptive characteristics of the runners are shown in Table 3.1. Based on the data, subjects can be considered well trained.

Table 3.1: General subject characteristics (n=12)

Variable	Mean ± SD
Age (yrs)	25.0 ± 5.7
Height (m)	1.7 ± 0.1
Mass (kg)	72.3 ±10.8
Max Heart Rate (bpm)	199 ± 5
% Body fat	13.1 ± 4.2
Peak Treadmill Running Speed (PTRS) ($km.h^{-1}$)	19.2 ± 1.3
Peak Track Running Speed (PRS) ($km.h^{-1}$)	18.8 ± 1.2

The relationship between the PTRS and PRS was defined as r = 0.86 resulting in a r^2 = 0.74, which suggests that about 74 % of the change in the PRS test could be explained by the PTRS test (Figure 3.3). Since the PRS test was performed on an indoor track of 135 m and the presence of two sharp bends whereas the PTRS was performed on a treadmill, this could possibly contribute to the 26 % variance in data.

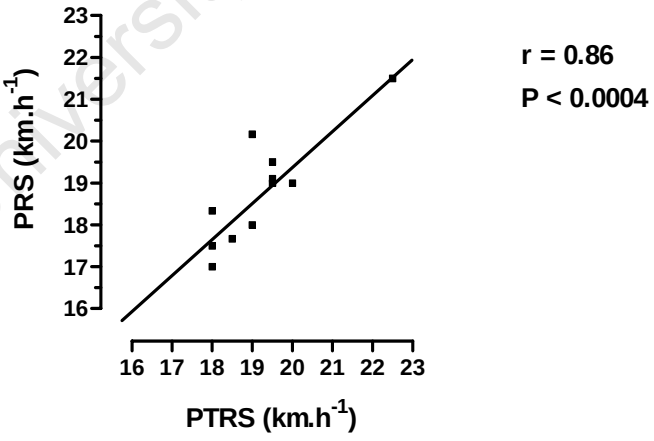


Figure 3.3: Relationship between the Peak Treadmill Running Speed (PTRS) and Peak Track Running Speed (PRS).

3.3.2 Repeatability

Table 3.2 shows the ICC values for every 2 $km.h^{-1}$ increase in running speed for each muscle, normalised to the three methods for three trials. The repeatability of muscle activity with increasing running speed is seen to be variable for all methods of normalisation. However, the 70 % PRS method was the least repeatable with increasing running speed.

Table 3.2: Intra-class correlations for EMG normalised to MVC, Sprint and 70 % PRS methods for every 2 km.h^{-1} running speed during PRS trials. Values highlighted in bold represent what are considered to be "good" ICC values

Muscle	Method	10 km.h^{-1}	12 km.h^{-1}	14 km.h^{-1}	16 km.h^{-1}	18 km.h^{-1}
VM	MVC	**0.89** (0.68-0.97)	**0.87** (0.64-0.97)	**0.90** (0.68-0.98)	**0.92** (0.72-0.99)	**0.93**(0.63-1.00)
	Sprint	**0.94** (0.84-0.98)	**0.89** (0.72-0.97)	**0.90** (0.73-0.97)	**0.87** (0.66-0.97)	0.75 (0.31- 0.96)
	70%PPO	0.10 (0.00-0.57)	0.50 (0.11-0.82)	0.78 (0.50-0.93)	0.76 (0.41-0.94)	0.45 (0.00-0.88)
RF	MVC	0.53 (0.00-0.93)	0.47 (0.00-0.91)	0.59 (0.00-0.96)	0.78(0.20-0.98)	**0.96**(0.66-1.00)
	Sprint	**0.80** (0.24-0.98)	0.73 (0.11-0.98)	**0.83** (0.31-0.99)	0.73 (0.13-0.98)	**0.85** (0.19-1.00)
	70%PPO	0.57 (0.01-0.94)	0.62 (0.05-0.95)	**0.86** (0.39-0.99)	0.60 (0.00- 0.96)	0.75 (0.00-0.99)
VL	MVC	0.66 (0.33-0.88)	0.79 (0.53-0.93)	0.67(0.34-0.89)	0.57 (0.19-0.85)	0.73(0.39-0.92)
	Sprint	0.71 (0.36-0.92)	0.78 (0.48-0.94)	0.65 (0.27-0.90)	0.68 (0.28-0.92)	**0.81**(0.47-0.96)
	70%PPO	0.00 (0.00-1.00)	0.41 (0.00-0.79)	0.41 (0.00-0.79)	0.58 (0.15-0.88)	0.36 (0.00-0.82)
BF	MVC	**0.86** (0.54-0.98)	**0.85** (0.52-0.98)	**0.87** (0.57-0.98)	0.65 (0.16-0.93)	0.70(0.16-0.96)
	Sprint	**0.80** (0.34-0.97)	**0.83** (0.41-0.98)	**0.83** (0.41-0.98)	0.77 (0.28-0.97)	0.00 (0.00-1.00)
	70%PPO	0.77(0.40-0.95)	0.74 (0.34-0.94)	0.69 (0.26-0.93)	0.78 (0.41-0.95)	0.52 (0.04-0.88)
MG	MVC	**0.85** (0.65-0.95)	**0.92** (0.79-0.98)	**0.91** (0.76-0.97)	***0.90*** *(0.73-0.97)*	**0.89**(0.66-0.98)
	Sprint	0.11 (0.00-0.58)	0.71 (0.38-0.91)	0.69 (0.35-0.90)	*0.71 (0.36-0.92)*	**0.80** (0.51-0.96)
	70%PPO	0.75 (0.59-0.96)	0.70 (0.37-0.91)	0.45 (0.06-0.80)	*0.11 (0.00-0.60)*	0.00 (0.00-1.00)
LG	MVC	**0.84** (0.54-0.97)	0.34 (0.00-0.81)	0.00 (0.00-1.00)	**0.93** (0.70-0.99)	**0.97**(0.73-1.00)
	Sprint	**0.85** (0.52-0.98)	0.79 (0.39-0.96)	0.79 (0.39-0.96)	0.78 (0.67-0.97)	0.13 (0.00-0.87)
	70%PPO	0.79 (0.43-0.96)	0.70 (0.28-0.93)	0.60 (0.09-0.92)	0.69 (0.21-0.94)	0.00 (0.00-1.00)

Intra-class correlations with 95% confidence intervals for methods of normalisation for each muscle, Vastus medialis (VM), Rectus femoris (RF), Vastus lateralis (VL), Biceps femoris (BF), Medial gastrocnemius (MG) and Lateral gastrocnemius (LG), over 3 separate days running. Values highlighted in bold represent what are considered to be "good" ICC values. The section represented in Figure 3.4 is highlighted in the block.

Table 3.3: Average Intra-class correlations (ICC) for Peak Running Speed test EMG normalised to MVC, Sprint and 70 % PRS methods

Muscle	MVC	Sprint	70 % PRS
Vastus medialis	**0.90 (0.87-0.93)**	0.87 (0.78 -0.96)	0.51(0.17-0.86)
Rectus femoris	0.66 (0.42-0.92)	**0.79 (0.72-0.86)**	0.68 (0.53-0.83)
Vastus lateralis	0.68 (0.58-0.79)	**0.73 (0.64-0.81)**	0.27 (0.01-0.52)
Bicep femoris	**0.84 (0.78-0.90)**	0.65 (0.20-1.00)	0.70 (0.57-0.83)
Medial gastrocnemius	**0.89 (0.86-0.93)**	0.60 (0.26-0.95)	0.40 (0.00-0.82)
Lateral gastrocnemius	0.61 (0.09-1.00)	**0.67 (0.30-1.00)**	0.56 (0.16-0.95)

Values presented as mean ICC (10 km.h^{-1} to 18 km.h^{-1}) with 95 % confidence intervals. ICC for methods of normalisation, of each muscle, over 3 trials. Bolded values indicate the highest mean ICC value for each muscle.

The MVC and Sprint methods were the most repeatable for normalising EMG activity during running. The MVC method had the highest average ICC values for VM, BF and MG ("good" repeatability where ICC R > 0.80). The MVC method had "fair" repeatability for RF, VL and LG, where ICC values were between R = 0.61 - 0.68. The Sprint method had the highest repeatability for RF, VL and LG normalisation. In addition, this method had "good" repeatability when normalising VM (0.87(0.78 - 0.96)) and "fair" repeatability for normalising BF and MG (0.65 (0.20 - 1.00) and 0.60 (0.26 - 0.95) respectively).The 70 % PRS method had "poor" repeatability for most muscles except for BF (0.70 (0.57 - 0.83)) and RF (0.68 (0.53 - 0.83)), which was "fairly" repeatable.

To further highlight the variation in muscle activity using different methods of normalisation a subsection of ICC values from Table 3.2 (highlighted in the block) have been graphically represented. The ICC values for MG activity at 16 km.h^{-1} were randomly chosen, as this speed showed the most variation in ICC values of each method of normalisation, thus highlighting the individual variation in muscle activity at this running speed. In an attempt to clarify the results the graphs in Figure 3.4 are arranged so that the first 6 subjects are represented on the left panel and the remaining subjects on the right panel. When normalised to the MVC method (Figure 3.4.a), ICC R = 0.90 (0.73 - 0.97), only subjects' 1 and 11 showed "high" variations in MG muscle activity over 3 days. The Sprint method (Figure 3.4.b) showed more subjects to have distinct variation in MG muscle activity and thus the ICC was lower

at R = 0.71 (0.36 - 0.92) (subjects 3, 8, & 9). Most individuals showed variation in MG muscle activity when normalised to 70 % PRS method, where ICC R = 0.17(0.00 - 0.60) (subjects 1, 3, 5, 8, 9, 10 & 11).

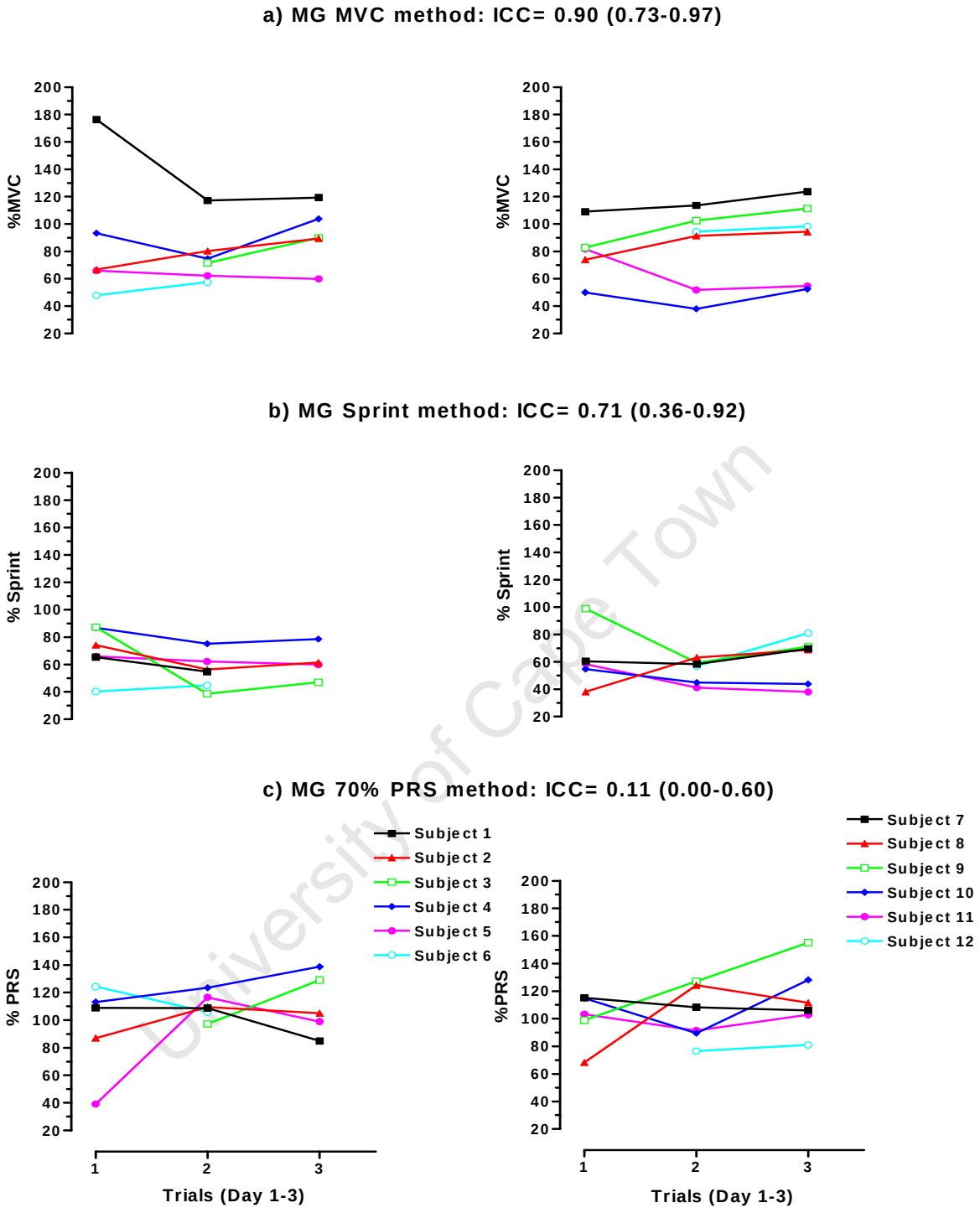


Figure 3.4: Variability of Medial gastrocnemius (MG) muscle activity at 16 km.km^{-1} over the three trials normalised *to* ***a)*** *MVC method,* ***b)*** *Sprint method and* ***c)*** *70% PRS method. The graphs illustrate the intra-class correlation with 95 % confidence intervals highlighted in the block in Table 3.2. The first six subjects 6 subjects are represented on the left panel and the remaining subjects on the right panel.*

3.3.3 Reliability

The intra-subject coefficient of variation (CV) of each subject muscle activity over the 3 trials provides an indication of which method of normalisation produced more reproducible results. Figure 3.5 provides a graphical display of the CV of each method of normalisation for each subject. The graphs showed various degrees of variation for each subject. To simplify the intra-subject CV's in Figure 3.5 and to emphasise which method showed the lowest intra-subject CV's, the graphs were divided into 3 zones; Zone 1(CV values ≤ 12 %), Zone 2 (CV values 12 % > and ≤ 20 %), Zone 3 (CV values > 20 %).

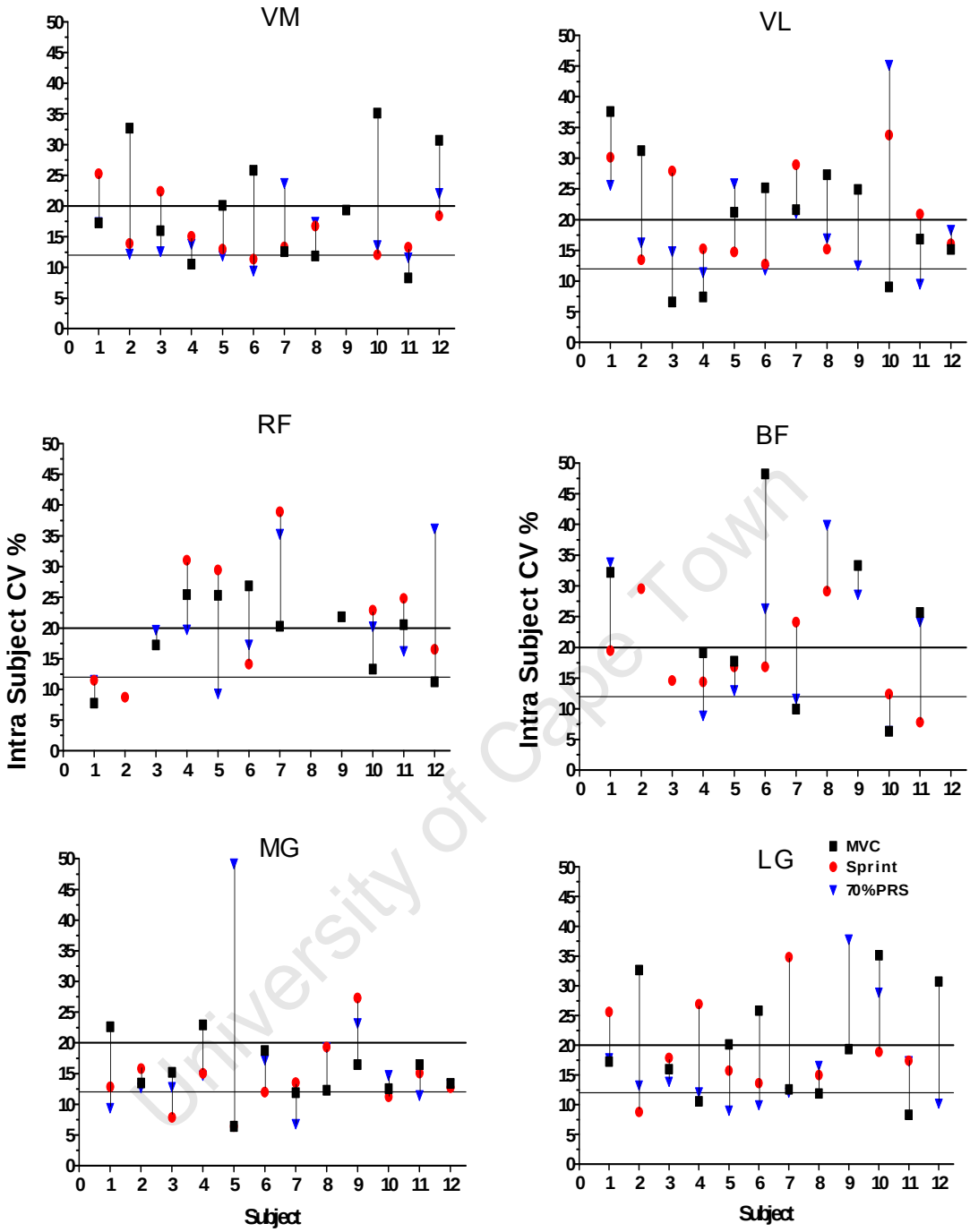


Figure 3.5: Intra-subject coefficient of variation (CV) of the three methods of normalisation for Vastus medialis (VM), Vastus lateralis (VL), Rectus femoris (RF), Biceps femoris (BF), Medial and Lateral gastrocnemius (MG and LG respectively) recorded over three trials. *Dashed line represents the range of low variation, between 0 - 12 %. The solid line represents the range of high variation, above 20 %. Note that for Subject 3, 2, 8 and 1; RF and BF muscle activity was not captured, for 2 trials, thus unable to calculate CV value.*

The percentages were calculated for each zone as described in Chapter 2 Section 2.3.3. The method of normalisation with the highest percentage of CV values in Zone 1 and 2 would have the least intra-subject variability over 3 trials.

Table 3.4: Percentage of the summed scores for the intra-subject coefficient of variation zones

Muscles	Norm Method	Zone 1 %	Zone 2 %	Zone 3 %
VM	MVC	25	33	**42**
	Sprint	18	**64**	18
	70%PPO	**27**	55	18
VL	MVC	**25**	25	**50**
	Sprint	0	**55**	45
	70%PPO	**25**	42	33
RF	MVC	20	20	**60**
	Sprint	**22**	22	56
	70%PPO	**22**	**45**	33
BF	MVC	25	25	**50**
	Sprint	9	**54**	27
	70%PPO	**33**	12	45
MG	MVC	17	**66**	**17**
	Sprint	**33**	58	9
	70%PPO	25	58	**17**
LG	MVC	25	33	**42**
	Sprint	10	**60**	30
	70%PPO	**42**	42	16

Percentage of CV values in each Zone for each method of normalisation and muscle; Vastus medialis (VM), Vastus lateralis (VL), Rectus femoris (RF), Biceps femoris (BF), Medial and Lateral gastrocnemius (MG and LG respectively). CV values summed in each zone calculated as a percentage of the subject number per Zone 1(CV values ≤ 12 %), Zone 2 (CV values 12 % > and ≤ 20 %), Zone 3 (CV values > 20 %). The methods with highest percentages in each zone for each muscle, is highlighted in bold.

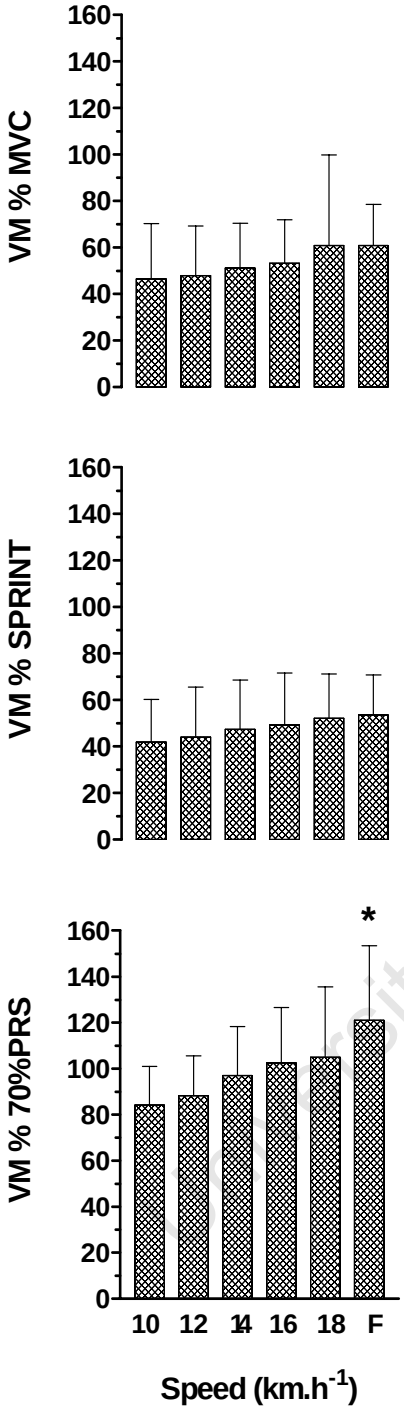
The calculation highlighted that 70 % PRS method had the highest CV values for most muscles, amongst the methods of normalisation, in Zone 1 (except MG) (Table 3.4). This method showed to have most of its CV values in Zone 2, where the percentages ranged from 11 % - 58 %. Important to note is that this method also has the least CV values in Zone 3 ranging between 17 % - 45 %. Whereas, the MVC

method had the highest CV values amongst the methods of normalisation in Zone 3 for all muscles (17 % - 60 %), resulting in the highest intra-subject variability. This method does however show to have high CV values in Zone 2 (20 % - 66 %).

The Sprint method had the highest CV values (amongst the methods of normalisation) for MG (33 %) and RF (22 %) in Zone 1. The greater CV values for this method were found in Zone 2, ranging between 22 % - 64 %.

3.3.4 Sensitivity

The same method that was used in Chapter 2 Section 2.3.4 was used to determine the sensitivity of each method of normalisation in detecting changes in muscle activity as the running speed increased. For the purpose of this analysis the EMG measurement should detect differences in EMG activity with increasing running speed. This however does not take into account peripheral factors, associated with fatigue, which may affect the amplitude of the EMG signal. Only the results of one of the three trials is shown (Trial 2 data were randomly chosen). To clarify the data analysis a graph of EMG amplitude is shown for each muscle at different running speeds. A table showing the p-values for differences between running speeds are also shown (Tukey *post hoc* analysis).



MVC METHOD ($F_{5;35}$=1.98; p < 0.110)

STAGES	{1} 50.1	{2} 49.8	{3} 50.6	{4} 53.4	{5} 60.9	{6} 64.0
VM10 {1}		1.00	1.00	0.99	0.51	0.24
VM12 {2}	1.00		1.00	0.99	0.48	0.22
VM14 {3}	1.00	1.00		1.00	0.56	0.27
VM16 {4}	0.99	0.99	1.00		0.82	0.53
VM18 {5}	0.51	0.48	0.56	0.82		1.00
VM Final {6}	0.24	0.22	0.27	0.53	1.00	

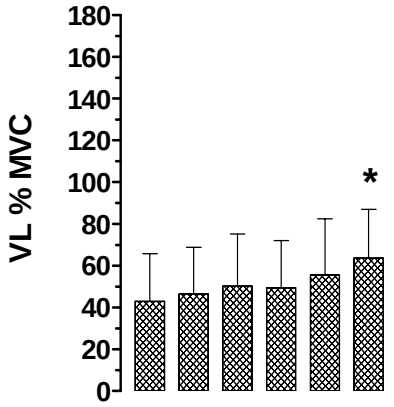
SPRINT METHOD ($F_{5;30}$= 0.96; p < 0.450)

STAGES	{1} 47.2	{2} 48.1	{3} 49.6	{4} 51.4	{5} 52.1	{6} 57.8
VM10 {1}		1.00	1.00	0.97	0.95	0.40
VM12 {2}	1.00		1.00	0.99	0.98	0.49
VM14 {3}	1.00	1.00		1.00	1.00	0.67
VM16 {4}	0.97	0.99	1.00		1.00	0.85
VM18 {5}	0.95	0.98	1.00	1.00		0.90
VM Final {6}	0.40	0.49	0.67	0.85	0.90	

70% PRS METHOD ($F_{5;35}$=3.33; p < 0.015)

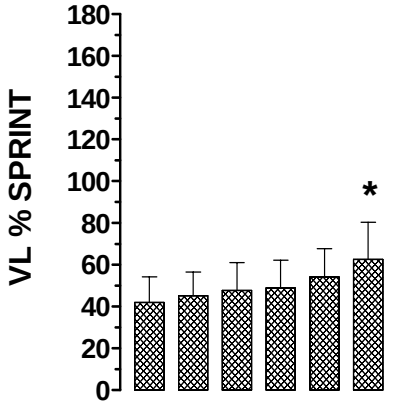
STAGES	{1} 87.0	{2} 88.2	{3} 92.8	{4} 98.5	{5} 105.0	{6} 119.9
VM10 {1}		1.00	0.99	0.84	0.44	0.02
VM12 {2}	1.00		1.00	0.89	0.52	0.03
VM14 {3}	0.99	1.00		0.99	0.80	0.08
VM16 {4}	0.84	0.89	0.99		0.98	0.26
VM18 {5}	0.44	0.52	0.80	0.98		0.64
VM Final {6}	0.02	0.03	0.08	0.26	0.64	

Figure 3.6: Vastus medialis (VM) muscle activity in Trial 2, normalised to MVC, Sprint and 70 % PRS, methods. *F indicates the final speed that each subject reached during Trial 2. Differences between running speeds was shown as * p<0.05. The corresponding table indicates the significant difference in muscle activity between the 6 running speeds using Tukey post hoc analysis (highlighted in red). The bold value heading each column is the average EMG at each running speed. The column heading {1} represents VM 10, {2} represents VM 12 etc.*



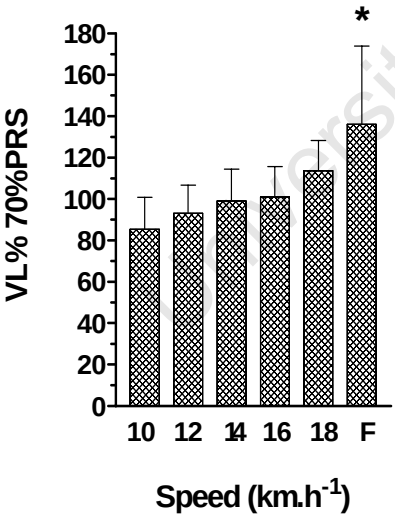
MVC METHOD ($F_{5;45}$=6.35; p < 0.000)

STAGES	{1} 42.8	{2} 45.6	{3} 50.2	{4} 48.9	{5} 55.6	{6} 64.1
VL10 {1}		0.99	0.53	0.71	0.05	0.00
VL12 {2}	0.99		0.90	0.97	0.21	0.00
VL14 {3}	0.53	0.90		1.00	0.81	0.03
VL16 {4}	0.71	0.97	1.00		0.64	0.01
VL18 {5}	0.05	0.21	0.81	0.64		0.37
VL Final {6}	0.00	0.00	0.03	0.01	0.37	



SPRINT METHOD ($F_{5;40}$= 5.92; p < 0.000)

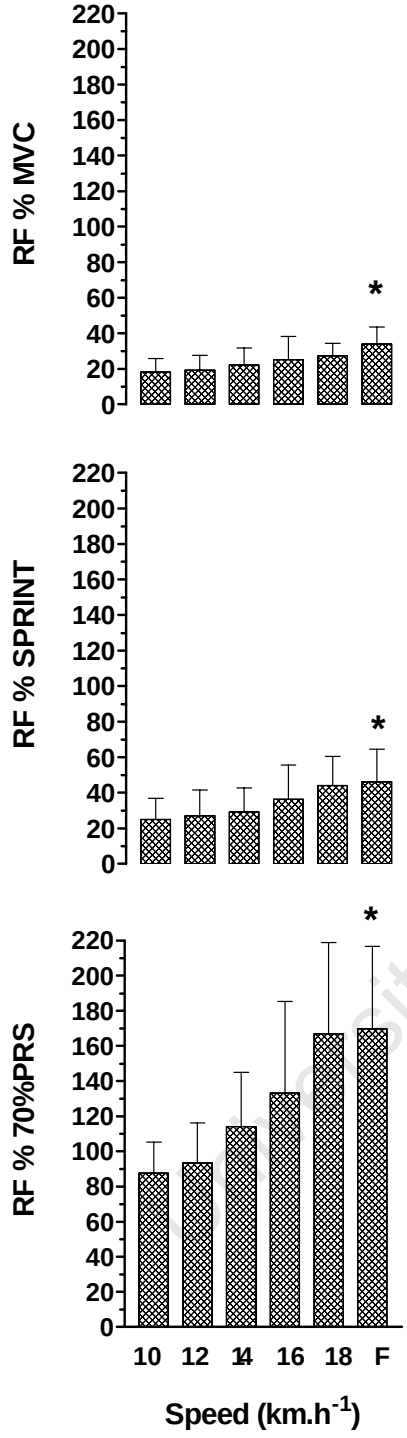
STAGES	{1} 42.5	{2} 45.2	{3} 48.3	{4} 49.5	{5} 54.2	{6} 64.0
VL10 {1}		0.99	0.78	0.63	0.12	0.00
VL12 {2}	0.99		0.98	0.93	0.35	0.00
VL14 {3}	0.78	0.98		1.00	0.77	0.01
VL16 {4}	0.63	0.93	1.00		0.89	0.03
VL18 {5}	0.12	0.35	0.77	0.89		0.26
VL Final {6}	0.00	0.00	0.01	0.03	0.26	



70% PRS METHOD ($F_{5;45}$=8.96; p < 0.000)

STAGES	{1} 86.4	{2} 92.9	{3} 99.9	{4} 101.6	{5} 113.6	{6} 139.9
VL10 {1}		0.98	0.66	0.54	0.04	0.00
VL12 {2}	0.98		0.97	0.93	0.22	0.00
VL14 {3}	0.66	0.97		1.00	0.65	0.00
VL16 {4}	0.54	0.93	1.00		0.77	0.00
VL18 {5}	0.04	0.22	0.65	0.77		0.06
VL Final {6}	0.00	0.00	0.00	0.00	0.06	

Figure 3.7: Vastus lateralis (VL) muscle activity in Trial 2, normalised to 70% PRS, Sprint and MVC methods. *F indicates the final speed that each subject reached during Trial 2. Differences between running speeds was shown as * p<0.05. The corresponding table indicates the significant difference in muscle activity between the 6 running speeds using Tukey post hoc analysis (highlighted in red).The blue highlighted values are the values tending towards significance. The bold value heading each column is the average EMG at each running speed. The column heading {1} represents VL 10, {2} represents VL 12 etc.*



MVC METHOD ($F_{5;25}$=19.04; p < 0.000)

STAGES	{1} 15.3	{2} 16.8	{3} 18.4	{4} 19.8	{5} 27.2	{6} 34.6
RF10 {1}		0.99	0.78	0.45	0.00	0.00
RF12 {2}	0.99		0.98	0.81	0.00	0.00
RF14 {3}	0.78	0.98		0.99	0.01	0.00
RF16 {4}	0.45	0.81	0.99		0.05	0.00
RF18 {5}	0.00	0.00	0.01	0.05		0.05
RF Final {6}	0.00	0.00	0.00	0.00	0.05	

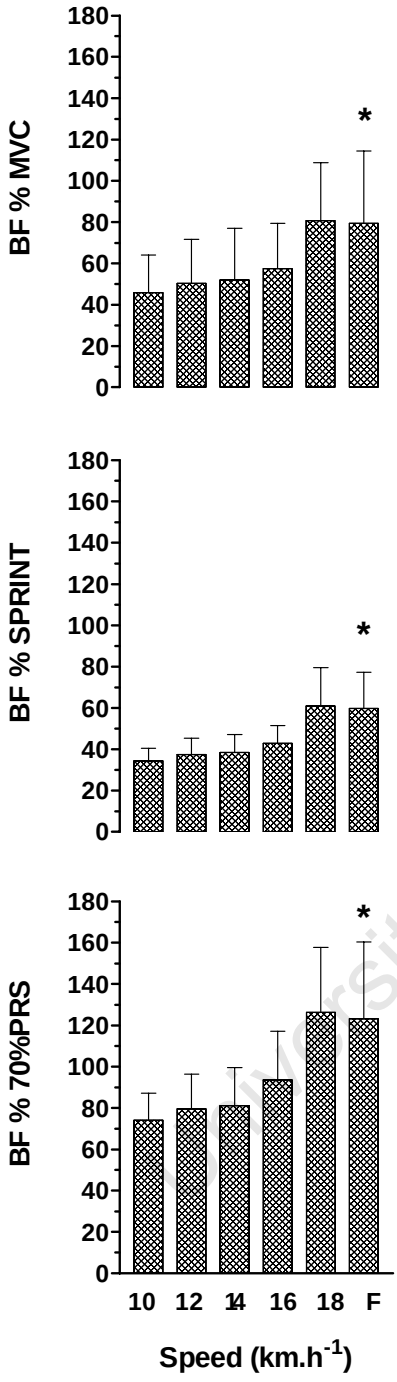
SPRINT METHOD ($F_{5;20}$=28.59; p < 0.000)

STAGES	{1} 25.9	{2} 29.4	{3} 30.5	{4} 32.2	{5} 44.1	{6} 53.8
RF10 {1}		0.83	0.60	0.27	0.00	0.00
RF12 {2}	0.83		1.00	0.91	0.00	0.00
RF14 {3}	0.60	1.00		0.99	0.00	0.00
RF16 {4}	0.27	0.91	0.99		0.01	0.00
RF18 {5}	0.00	0.00	0.00	0.01		0.03
RF Final {6}	0.00	0.00	0.00	0.00	0.03	

70% PRS METHOD ($F_{5;25}$= 16.93; p < 0.000)

STAGES	{1} 90.6	{2} 99.6	{3} 113.7	{4} 113.2	{5} 166.9	{6} 181.1
RF10 {1}		0.98	0.48	0.51	0.00	0.00
RF12 {2}	0.98		0.88	0.89	0.00	0.00
RF14 {3}	0.48	0.88		1.00	0.00	0.00
RF16 {4}	0.51	0.89	1.00		0.00	0.00
RF18 {5}	0.00	0.00	0.00	0.00		0.87
RF Final {6}	0.00	0.00	0.00	0.00	0.87	

***Figure 3.8**: **Rectus femoris (RF) muscle activity in Trial 2, normalised to 70% PRS, Sprint and MVC methods.** F indicates the final speed that each subject reached during Trial 2. Differences between running speeds was shown as * p<0.05. The corresponding table indicates the significant difference in muscle activity between the 6 running speeds using Tukey post hoc analysis (highlighted in red). The bold value heading each column is the average EMG at each running speed. The column heading {1} represents RF10, {2} represents RF 12 etc.*



MVC METHOD ($F_{5;40}$=19.20; p < 0.000)

STAGES	{1} 44.7	{2} 48.8	{3} 53.0	{4} 58.5	{5} 80.7	{6} 84.7
BF10 {1}		0.97	0.65	0.14	0.00	0.00
BF12 {2}	0.97		0.97	0.49	0.00	0.00
BF14 {3}	0.65	0.97		0.92	0.00	0.00
BF16 {4}	0.14	0.49	0.92		0.00	0.00
BF18 {5}	0.00	0.00	0.00	0.00		0.98
BF Final {6}	0.00	0.00	0.00	0.00	0.98	

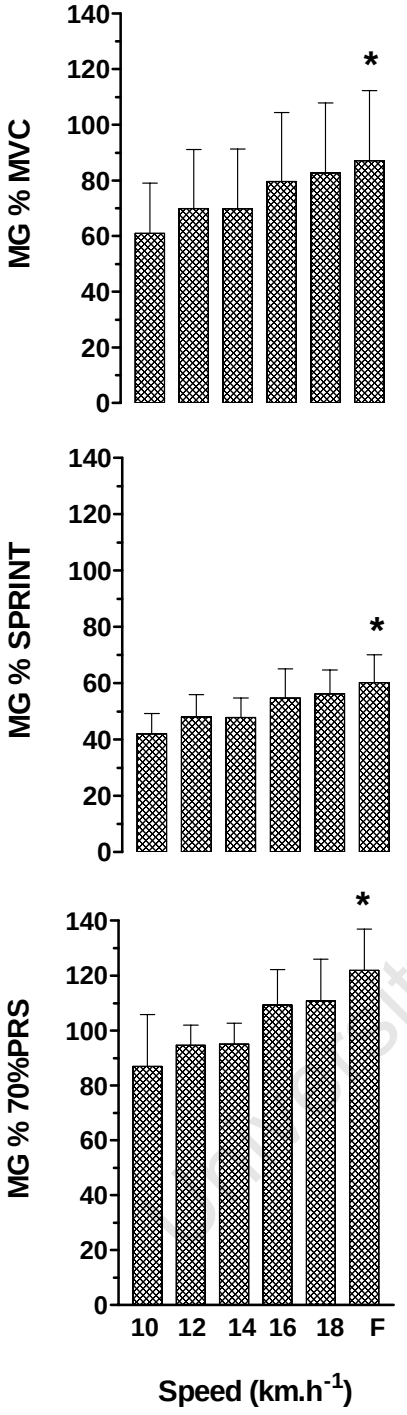
SPRINT METHOD ($F_{5;35}$=13.76; p < 0.000)

STAGES	{1} 32.3	{2} 34.9	{3} 38.3	{4} 42.4	{5} 60.9	{6} 63.0
BF10 {1}		1.00	0.84	0.37	0.00	0.00
BF12 {2}	1.00		0.98	0.69	0.00	0.00
BF14 {3}	0.84	0.98		0.97	0.00	0.00
BF16 {4}	0.37	0.69	0.97		0.01	0.00
BF18 {5}	0.00	0.00	0.00	0.01		1.00
BF Final {6}	0.00	0.00	0.00	0.00	1.00	

70% PRS METHOD ($F_{5;35}$=18.01; p < 0.000)

STAGES	{1} 73.7	{2} 78.2	{3} 83.9	{4} 96.5	{5} 126.3	{6} 132.8
BF10 {1}		0.99	0.83	0.10	0.00	0.00
BF12 {2}	0.99		0.98	0.28	0.00	0.00
BF14 {3}	0.83	0.98		0.67	0.00	0.00
BF16 {4}	0.10	0.28	0.67		0.01	0.00
BF18 {5}	0.00	0.00	0.00	0.01		0.97
BF Final {6}	0.00	0.00	0.00	0.00	0.97	

Figure 3.9: Biceps femoris (BF) muscle activity in Trial 2, normalised to 70% PRS, Sprint and MVC methods. *F indicates the final speed that each subject reached during Trial 2. Differences between running speeds was shown as * p<0.05. The corresponding table indicates the significant difference in muscle activity between the 6 running speeds using Tukey post hoc analysis (highlighted in red). The bold value heading each column is the average EMG at each running speed. The column heading {1} represents BF 10, {2} represents BF12 etc.*



MVC METHOD ($F_{5;45}$=10.93; p < 0.000)

STAGES	{1} 61.4	{2} 72.3	{3} 72.1	{4} 82.1	{5} 82.7	{6} 91.6
MG10 {1}		0.17	0.19	0.00	0.00	0.00
MG12 {2}	0.17		1.00	0.28	0.22	0.00
MG14 {3}	0.19	1.00		0.25	0.20	0.00
MG16 {4}	0.00	0.28	0.25		1.00	0.31
MG18 {5}	0.00	0.22	0.20	1.00		0.38
MG Final {6}	0.00	0.00	0.00	0.31	0.38	

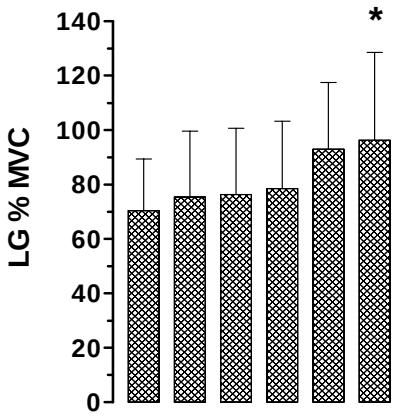
SPRINT METHOD ($F_{5;45}$=10.98; p < 0.000)

STAGES	{1} 41.8	{2} 49.2	{3} 48.9	{4} 55.4	{5} 56.1	{6} 62.7
MG10 {1}		0.18	0.22	0.00	0.00	0.00
MG12 {2}	0.18		1.00	0.36	0.25	0.00
MG14 {3}	0.22	1.00		0.31	0.21	0.00
MG16 {4}	0.00	0.36	0.31		1.00	0.19
MG18 {5}	0.00	0.25	0.21	1.00		0.29
MG Final {6}	0.00	0.00	0.00	0.19	0.29	

70% PRS METHOD ($F_{5;40}$=10.16; p < 0.000)

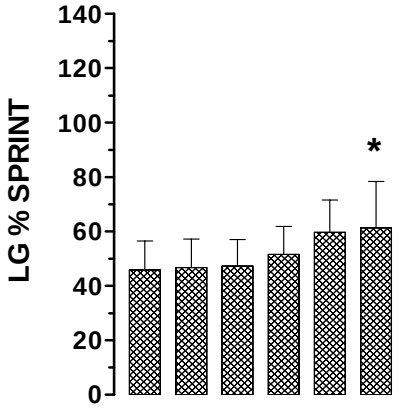
STAGES	{1} 85.6	{2} 95.5	{3} 95.9	{4} 109.8	{5} 110.8	{6} 126.4
MG10 {1}		0.64	0.61	0.01	0.00	0.00
MG12 {2}	0.64		1.00	0.26	0.19	0.00
MG14 {3}	0.61	1.00		0.28	0.21	0.00
MG16 {4}	0.01	0.26	0.28		1.00	0.13
MG18 {5}	0.00	0.19	0.21	1.00		0.17
MG Final {6}	0.00	0.00	0.00	0.13	0.17	

Figure 3.10: Medial gastrocnemius (MG) muscle activity in Trial 2, normalised to 70 % PRS, Sprint and MVC methods. *F indicates the final speed that each subject reached during Trial 2. Differences between running speeds was shown as * p<0.05. The corresponding table indicates the significant difference in muscle activity between the 6 running speeds using Tukey post hoc analysis (highlighted in red). The bold value heading each column is the average EMG at each running speed. The column heading {1} represents MG10, {2} represents MG 12 etc.*



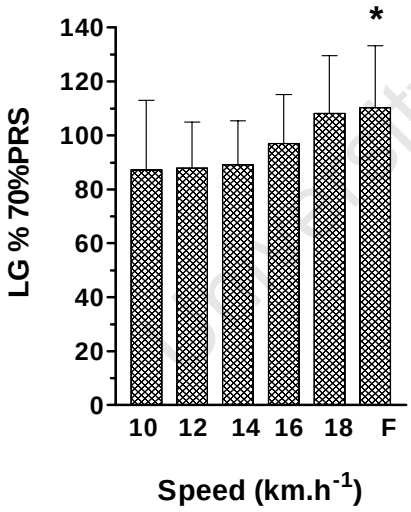
MVC METHOD ($F_{5;35}$=5.55; p < 0.001)

STAGES	{1} 72.8	{2} 72.9	{3} 74.9	{4} 84.	{5} 92.9	{6} 101.9
LG10 {1}		1.00	1.00	0.63	0.08	0.00
LG12 {2}	1.00		1.00	0.65	0.09	0.00
LG14 {3}	1.00	1.00		0.81	0.16	0.01
LG16 {4}	0.63	0.65	0.81		0.82	0.16
LG18 {5}	0.08	0.09	0.16	0.82		0.81
LG Final {6}	0.00	0.00	0.01	0.16	0.81	



SPRINT METHOD ($F_{5;35}$=6.42; p <0.000)

STAGES	{1} 46.9	{2} 47.8	{3} 48.4	{4} 53.2	{5} 59.6	{6} 65.6
LG10 {1}		1.00	1.00	0.68	0.05	0.00
LG12 {2}	1.00		1.00	0.80	0.08	0.00
LG14 {3}	1.00	1.00		0.86	0.11	0.00
LG16 {4}	0.68	0.80	0.86		0.65	0.06
LG18 {5}	0.05	0.08	0.11	0.65		0.71
LG Final {6}	0.00	0.00	0.00	0.06	0.71	



70% PRS METHOD ($F_{5;35}$=5.62; p < 0.001)

STAGES	{1} 86.5	{2} 86.9	{3} 88.0	{4} 97.2	{5} 108.2	{6} 114.0
LG10 {1}		1.00	1.00	0.66	0.04	0.01
LG12 {2}	1.00		1.00	0.70	0.05	0.01
LG14 {3}	1.00	1.00		0.79	0.07	0.01
LG16 {4}	0.66	0.70	0.79		0.63	0.19
LG18 {5}	0.04	0.05	0.07	0.63		0.96
LG Final {6}	0.01	0.01	0.01	0.19	0.96	

Figure 3.11: Lateral gastrocnemius (LG) muscle activity in Trial 2, normalised to 70% PRS, Sprint and MVC methods. *F indicates the final speed that each subject reached during Trial 2. Differences between running speeds was shown as * p<0.05. The corresponding table indicates the significant difference in muscle activity between the 6 running speeds using Tukey post hoc analysis (highlighted in red).The blue highlighted values are the values tending towards significance. The bold value heading each column is the average EMG at each running speed. The column heading {1} represents LG 10, {2} represents LG 12 etc.*

For VM over 6 running speeds (Figure 3.6), the 70 % PRS method showed muscle activity to be significantly different over more loads than the MVC and Sprint methods. The 70 % PRS method found muscle activity in loads 1 and 2 to be significantly lower than the final load, whereas MVC and Sprint methods were unable to detect significant differences between loads.

For VL (Figure 3.7), 70 % PRS and MVC methods both had the most sensitivity to increasing running speed, where muscle activity at 10 $km.h^{-1}$ was significantly lower than during 18 $km.h^{-1}$ and the final speed ran by all subjects. Muscle activity during 12, 14 and 16 $km.h^{-1}$ for both methods was significantly lower than the final load. However, the 70 % PRS method showed muscle activity during the 18 $km.h^{-1}$ tended towards a significant difference to that during the final speed ($p = 0.06$), resulting in this method possibly having a slighter greater sensitivity to increasing exercise intensity.

The Sprint method of normalisation showed the most significant differences between running speeds for RF (Figure 3.8). Muscle activity at 10, 12, 14 and 16 $km.h^{-1}$ was significantly lower than at 18 $km.h^{-1}$ and the final speed. Muscle activity during 18 $km.h^{-1}$ bout was significantly lower than muscle activity during the final speed. The 70 % PRS method was unable to highlight significant difference between 18 $km.h^{-1}$ and final speed. The MVC method was able to highlight this significance; however muscle activity during 16 $km.h^{-1}$ was not lower than muscle activity at 18 $km.h^{-1}$.

For BF (Figure 3.9), all the methods of normalisation showed the same significant difference between running speeds (10, 12, 14 and 16 $km.h^{-1}$ significantly lower than 18 $km.h^{-1}$ and final speed). For MG (Figure 3.10) all methods also had the same significant differences over various running speeds. All methods showed that muscle activity at 10 $km.hr^{-1}$ was significantly lower than at 16 $km.h^{-1}$, 18 $km.h^{-1}$ and final speeds, and that muscle activity at 12 $km.h^{-1}$ and 14 $km.h^{-1}$ was significantly lower than the final speed.

70 % PRS and MVC methods showed the most significant differences between running speeds for LG activity (Figure 3.11), where muscle activity during 10 $km.h^{-1}$ and 12 $km.h^{-1}$ was significantly lower than during 18 $km.h^{-1}$ and final speed;

14 km.h^{-1} was also significantly lower than muscle activity during the final speed. However, 70 % PRS method showed muscle activity at 14 km.h^{-1} tended towards being significantly lower than the activity during 18 km.h^{-1} ($p = 0.07$).

An alternative method for showing the sensitivity of each method of normalisation to track muscle activity to running speed changes, was to calculate the ratio of running speed to EMG amplitude. The results of this analysis are graphically displayed in Figure 3.12. As explained in Chapter 2, the slope of the line represents the ability of the method of normalisation to track changes in running speed. A slope of close to zero suggests that the method is more sensitive.

There was a load effect for the running speed/EMG ratios of VM, VL, MG and LG. The 70% PRS method had the most horizontal slopes and therefore was the most capable method of normalisation for EMG amplitude to changes in running speed for all muscles. For VM, VL and RF (Figure 3.12a), the Sprint and MVC methods had similar horizontal slopes. For BF, all the methods had similar slope values (70 % PRS = 0.003 ± 0.004; MVC = 0.002 ± 0.007 and Sprint = 0.004 ± 0.008) therefore it was not possible to differentiate methods of normalisation based on this analysis. For MG, the 70 % PRS method had the lowest slope (0.008 ± 0.002). For LG, 70 % PRS had the lowest slope of 0.009 ± 0.003; however MVC method's slope was similar at 0.012 ± 0.003.

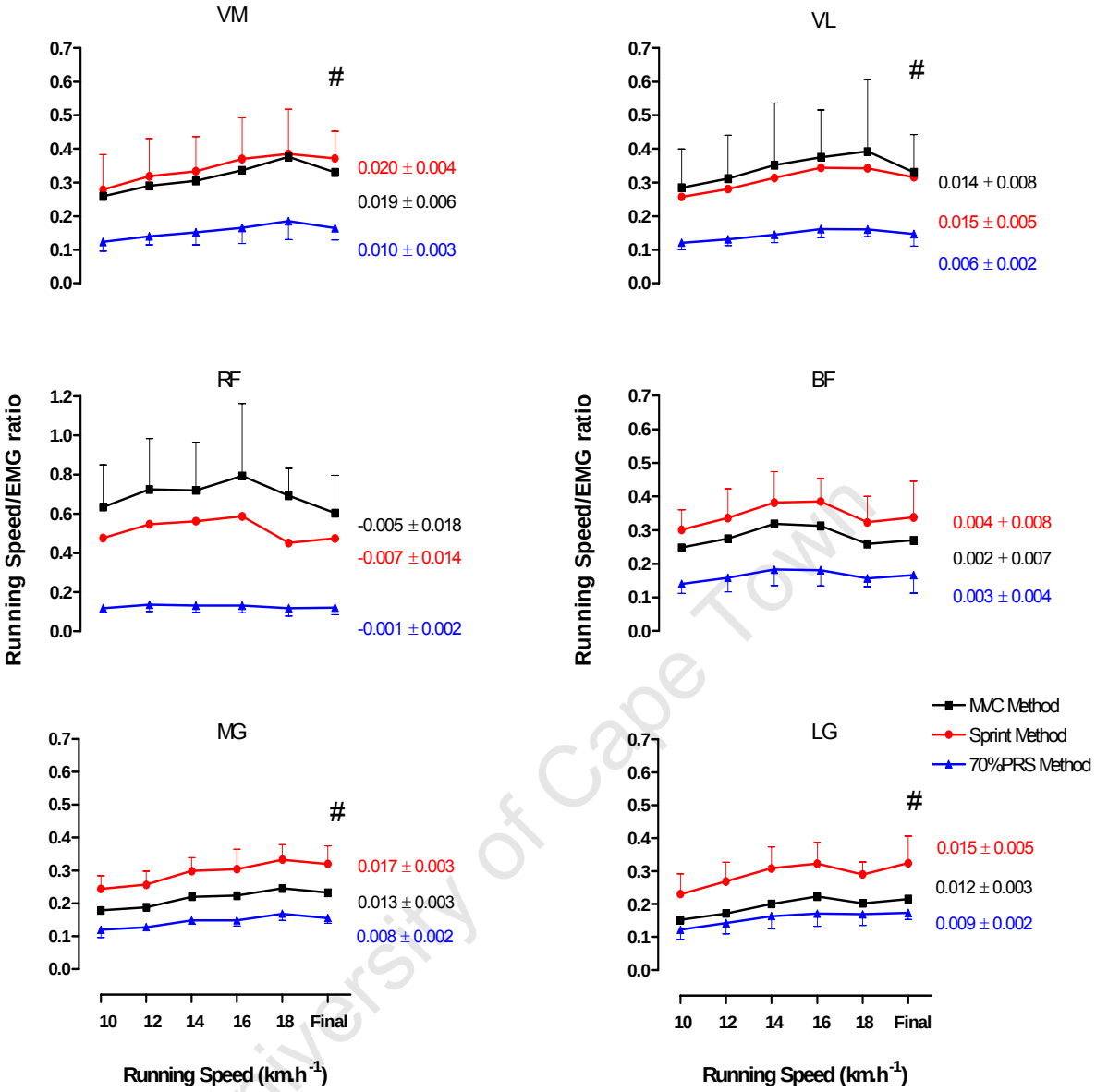


Figure 3.12: Running speed to EMG ratio for 6 power output loads of Trial 2, when normalised to MVC, Sprint and 70% PRS method *for* ***a)*** *Vastus medialis (VM),* ***b)*** *Vastus lateralis (VL),* ***c)*** *Rectus femoris (RF),* ***d)*** *Biceps femoris (BF,* ***e)*** *Medial gastrocnemius (MG),* ***f)*** *Lateral gastrocnemius (LG). The slope and standard deviations of each method are presented. Significant load effect # (P<0.05)*

To summarise which method was the most sensitive in representing corresponding running speed change with change in EMG amplitude, the results of the two sensitivity methods were reviewed for sensitivity of EMG amplitude to increasing running speed (Figures 3.6 - 3.11) and running speed to EMG ratio (Figure 3.12), the results represented in Table 3.5.

The 70 % PRS method had the greatest ability to detect changes in EMG amplitude over increasing running speed for all muscles. Moreover, when analysing the data with regards to running speed/EMG ratio, the 70 % PRS method also had the greatest ability to track EMG amplitude to running speed changes. The Sprint and MVC methods had similar running speed/EMG ratios for all muscles.

Table 3.5: The recommended and 'usable' methods of normalisation found for each muscle using the sensitivity analysis

Muscle	Sensitivity between workloads	Sensitivity of power output to EMG ratio
VM	70 % PRS	70 % PRS (Sprint & MVC)
VL	70 % PRS and MVC (Sprint)	70 % PRS (Sprint & MVC)
RF	Sprint (70% PRS & MVC)	70 % PRS (Sprint & MVC)
BF	All	All
MG	All	70 % PRS (Sprint & MVC)
LG	70% PRS and MVC (Sprint)	70 % PRS (Sprint & MVC)

Most sensitive methods of normalisation found from sensitivity analysis in Figures 3.6 - 3.12. VM, Vastus medialis; VL, Vastus lateralis; RF, Rectus femoris; BF, Biceps femoris; MG, Medial gastrocnemius and LG, Lateral gastrocnemius. The 'usable' methods are enclosed in brackets.

3.4 DISCUSSION

The aim of the study was to examine the reliability of three different methods of normalising EMG activity during incremental running to exhaustion. These methods included normalisation to an isometric MVC (MVC method) and two methods involving dynamic movement of running, namely the Sprint method and the 70 % PRS method. According to the previously described requirements (Chapter 1), for a method of normalisation to be considered appropriate, it should display "good" repeatability, reliability and sensitivity to changes in work output of the active muscles. These three criteria are evaluated individually in the following sections.

3.4.1 Repeatability of methods of normalisation

Repeatability is an indication of reproducibility, thus the method should produce similar results over different testing trials (within day and between days). The main finding of this chapter was that the MVC and Sprint methods were the most repeatable methods of normalisation for running EMG activity. The Sprint method was the most repeatable for the quadriceps and the LG muscles. The MVC method had the best repeatability for the VM, BF and MG muscles. This differs from the results of Chapter 2 which showed the submaximal dynamic activity method was the most repeatable during cycling trials.

Soderberg & Knutson [172] advised researchers to normalise their data using the MVC method, and to use proper subject training (for performing MVCs) since there is no clarification within the literature with regard to the most appropriate method of normalisation, and also because the use of dynamic contractions are "*confounded by the EMG/force velocity relationship and other factors such as change of muscle mass under the electrode site*". The magnitude of the EMG signal is likely to be affected by these variables, resulting in the questionable ability of using the dynamic contractions for normalisation. Other studies have found the MVC method of normalisation to be repeatable during dynamic exercise. For example, Earl et al. [46] studied quadriceps muscle activation during dynamic mini-squat exercises and reported ICC values of R = 0.99 when normalising to the MVC. Furthermore, Bolgla & Uhl [21] investigated the repeatability and reliability of three methods of

normalisation for analysing hip abduction activation during rehabilitation exercises (using the gluteus medias muscle). The MVC method in this study had ICC values exceeding R = 0.93 for all exercises, whereas the ICC values for the mean and peak dynamic methods exceeded R = 0.85.

Previously, studies have shown the MVC method to be repeatable for the normalisation of dynamic activities [103]. However, this study has shown that using maximal dynamic movement was as repeatable as the MVC method and possibly more appropriate, due to representing the same muscle movement for running. In this study, the submaximal dynamic method of 70 % PRS had 'poor' repeatability in muscle activity for most muscles measured except the biarticulate RF and BF (R = 0.68 (0.53 - 0.83) and 0.70 (0.57 - 0.83) respectively) muscles. This finding could possibly be explained by the study conducted by Shiavi et al. [169], which showed that at slower walking speeds, muscle activity was more variable than at faster speeds. In fact, as walking speed increased, all lower leg muscle patterns became more repeatable (66). Shiavi et al. [169] also stated that at slower speeds muscle activity patterns are more individualised and are reactive to individual motion requirements. The authors explain that at faster speeds, "*some type of motor programming is dominant and everyone tends to utilise the same patterns*". Therefore it may be concluded that muscle activity is affected by speed; at slower running speeds, there is greater ground contact time and less preactivation resulting in a decrease in muscle stiffness and duration of the transition between the lengthening and shortening phases of the stretch shortening cycle (SSC).

Changes in the preactivation may be associated with increased ground contact time and increased energy requirement during the propulsion phase of the running stride as well as muscle fatigue [11]. This increase in ground contact time could be greater during the 70 % PRS run than during the faster speeds achieved in the PRS, thus accounting for the variability in the data. For example, when calculating the average speed the subjects ran for the 70 % PRS (from Table 3.1, the mean peak track running speed was 18.8 $km.h^{-1}$) where 70 % of PRS equalled 13.2 $km.h^{-1}$ (therefore subjects ran the 70 % PRS between 12 $km.h^{-1}$ and 14 $km.h^{-1}$). The best repeatability between 12 $km.h^{-1}$ and 14 $km.h^{-1}$ for the 70 % PRS method was 'fair' to 'good' for all muscles except VL where ICC remained at R = 0.41 (0.00 - 0.79) (Table 3.2). This possibly indicates the effect of normalising EMG running activity to a slower running

speed than the speed the subjects ran in the trial, and could therefore explain the low repeatability which occurred for the submaximal method of normalisation. A study by Paavolainen et al. [149], found EMG amplitude to decrease significantly during a 20 m sprint after a constant speed 10 km run. The preactivation, which is affected by fatigue-induced changes reported in prolonged running, also decreased substantially in the 20 m maximal run [149]. This fatigue-induced change could explain the variability in the EMG amplitude normalised to 70 % PRS, since the 70 % PRS was measured after the PRS trial. However, the 30 min resting period after the PRS trial could be a sufficient fatigue recovery period for all the subjects. Unfortunately, due to methodological constraints, it was not possible to analyse preactivation in this study.

A study conducted by Karamindis et al. [94] investigated reproducibility of EMG amplitude and ground reaction forces during various stride frequencies and running speeds. Twelve subjects ran on the treadmill at 3.5, 3.0 and 2.5 $m.s^{-1}$ at 3 different stride frequencies; namely preferred and ± 10 % of preferred stride frequency, for 3 trials. They found that EMG amplitude was more repeatable in the calf muscles than the quadriceps muscles over the trials. Importantly, they found that the timing of preactivation was highly variable producing ICC values of $R < 0.60$, but the EMG amplitude of preactivation was reproducible (ICC $R > 0.70$ except for VL $R < 0.69$). Ground reaction forces parameters during all running conditions showed high ICC values averaging $R = 0.87$. The authors highlighted the fact that repeatability of EMG data does not depend primarily on running velocity or deliberate change in stride frequency, but rather how one analyses the data (with regards to timing of preactivation, ground contact phase etc). This emphasises the point that EMG parameters assigned for analysis should be carefully chosen. This could be an additional reason for the variability in the data of the present study, where the active periods were isolated from the start of activity to the end of activity and not distinguished between the contribution of preactivation and ground contact. Furthermore, it should be acknowledged that different gait patterns and economy of running of the subjects could possibly contribute to the variability in the data.

3.4.2 Reliability of the methods of normalisation

The reliability measured in this chapter refers to the reproducibility of EMG activity of the same individual performing repeated trials [84]. This study has similar findings to that of Chapter 2, where the submaximal dynamic method of normalisation had the least intra-subject variability for most muscles except MG. The 70 % PRS method displayed 22 % - 42 % of its CV values in Zone 1. The results of the present study are in agreement with Shiavi et al. [169], where the authors stated that at slower speeds muscle activity patterns are more individualised and are reactive to individual motion requirements. This has been demonstrated by the 70 % PRS method, where the intra-subject variation was the lowest (demonstrating the individualised EMG pattern), possibly due to the relatively slow running speed subjects ran in the 70 % PRS method.

Guidetti et al. [75] studied the inter and intra-subject reliability of running gait profile (normalised to peak amplitude of the running gait; thus Peak Dynamic method), where 5 subjects ran on a treadmill at 15 $km.h^{-1}$ (for 3 trials) and found that intra-subject variability was lower than inter-subject variability. However, the CV values were higher than those found in the present study, where their minimum CV ranged between 19 % - 40 % and the highest CV values ranged between 75 % - 122 %. Furthermore, EMG peak amplitudes times were highly repeatable among trials for a given subject. The authors infer that gait is a programmed (i.e. defined as high repeatability in neuromuscular output) process in running as suggested in walking [9]. However, it is important to note that the study conducted by Guidetti et al. **[75]** looked at the running profile (i.e. the four stages of the active contraction) whereas the current study analysed the entire active contraction.

The findings of the present study are also similar to the findings of Bolgla & Uhl [21]. They investigated the repeatability and reliability of three methods of normalisation when analysing six hip abductor rehabilitation exercises. The methods of normalisation used were MVC, Mean dynamic (m-Dyn) and Peak Dynamic (pk-Dyn) methods. Intra-subject CV's ranged between 11 - 22 % for all methods; however the m-Dyn method provided the lowest intra-subject CVs for 5 out of the 6 exercises performed. A possible reason for the high intra-subject variability found in the MVC method could be that each subjects' generation of MVC ultimately depends on the

level of motivation of the subject. Varying levels of motivation could be an explanation for the high intra-subjects CV's associated with the MVC methods. This explanation, however, does not explain or account for the lower intra-subject CV of the Sprint method. Sprinting is also a maximal activity and depends on motivation. However, since the muscle activity during the Sprint method is the same dynamic movement as the muscle activity during the trial measured, the extrinsic factors mentioned in Chapter 1 could possibly be lessened and not have influenced the data to the same extent that occurred for the MVC method of normalisation.

3.4.3 Sensitivity of the methods of normalisation

Sensitivity of the method of normalisation refers to the methods' ability to track changes in workload or exercise intensity. For this analysis, the EMG measurement was able detect differences in EMG activity with increasing workload. This however does not take into account the presence of fatigue and the affect this has on the amplitude of the EMG signal. The findings of this study are in accordance with previous research, where EMG activity increases with increasing running speed (Figures 3.6 - 3.11) [110; 134; 148]. The 70 % PRS method had the greatest ability to detect changes in EMG with increases in running speed for most muscles except RF (Table 3.7). All methods were 'usable' in detecting EMG amplitude change over increasing running speeds for most muscles, except for VM, where neither the MVC nor the Sprint method were able to detect any changes in EMG amplitude with running speed changes.

The hip and knee extensors and the plantar flexors, need higher muscle activity to tolerate the higher impact loads experienced with increasing running speed [111]. Hanon et al. [78] showed that RF and BF (biarticular and biphasic muscles) were influenced more by the increase in running speed than other leg muscles (running speed of 4, 5, 5.5, 6 and 7 $m.s^{-1}$ interspersed with resting periods). The level of activation in these muscles increased linearly with increasing running speed. The biarticulate muscles are seen as modulators in transferring power between articulations [79] and this could account for the increasing level of activation with running speed. Kyröläinen et al. [111] also displayed clear changes in RF and BF due to running speed. The present study concurred with the literature, where it was found that one could distinguish greater changes in muscle activity in RF and BF

with increasing running speed (Figure 3.8 and 3.9) than the other muscles measured, when using the 70 % PRS method for RF and all methods of normalisation for BF.

The 70 % PRS method was also the best method of normalisation for tracking changes in EMG amplitude to running speed (EMG/running speed ratio). Although, the Sprint and MVC methods also showed low slope values and thus could also be considered as good methods for tracking EMG to exercise intensity. Interestingly, the EMG/running speed slope values were much higher in cycling power output increase (in Chapter 2, Figure 2.12) where the slope values ranged from 0.02 ± 0.08 to 2.97 ± 1.26. This can be compared to the present study where the ranges were between 0.001 ± 0.002 - 0.020 ± 0.004.

In conclusion, as mentioned in Chapters 1 and 2, the most appropriate method of normalisation is one that reflects reproducibility over different testing times, has low variation and the ability to detect changes in EMG amplitude with changes in exercise intensity. This study has shown that normalising running EMG to the Sprint and MVC method displays the highest repeatability for VL, RF and LG; and VM, BF and MG respectively. Considering the practical application of measured EMG data (as mentioned in Chapter 1), the Sprint and MVC methods are recommended for researchers who are investigating maximal muscle activity over different days. However for investigating the maximal muscle activity during a trial, the Sprint method of normalisation is recommended, especially since it is sensitive in detecting EMG amplitude changes and is similarly capable of tagging changes in exercise intensity with EMG amplitude. The Sprint method of normalising also has lower intra-subject variation compared to MVC method.

Indeed, the Sprint and MVC methods also showed similar reproducibility. However, it is still recommended that researchers utilise the Sprint method as a method of normalisation for running activity. The reason for this is that this method best represents the dynamic muscle activity for the trial being investigated, by accounting for the experimental factors influencing EMG amplitude during dynamic exercise as described in Chapter 1. Furthermore, if one is using a once-off and repeated measurement (if wanting to investigate a change in muscle activity within a subject due to an intervention), the 70 % PRS method could also be recommended, since it

has the lowest intra-subject variability and the greatest ability in tracking changes in muscle activity with an increase in exercise intensity. This method was also found to be ‘fairly’ repeatable for RF and BF.

This study does present certain limitations such as the inability to analyse the EMG into the 4 running profile divisions of preactivation, ground contact phase, braking phase and propulsion phase. By analysing the data this way, the study would have possibly provided more clarity on why the repeatability of the 70 % PRS method was poor for most muscles, yet highly sensitive to changes in exercise intensity and having the least intra-subject variability.

In conclusion the data from this study shows it is important that researchers use a method of normalisation that is suitable to their study design and research question. Choosing the most appropriate method of normalisation reduces experimental error and increases the chance of detecting biological variation and hence improves the interpretation of the data.

CHAPTER 4

MODELS OF FATIGUE: EMG MEASUREMENT OF SKELETAL MUSCLE ACTIVITY AT EXHAUSTION

REVIEW OF THE LITERATURE

4.1 INTRODUCTION

In Chapters 2 and 3, the various methods of normalisation during dynamic exercise were evaluated. The appropriate use of these methods of normalisation will reduce the non-biological variability of the measured surface EMG signal and maximise the opportunity to detect those changes produced by biological systems. This will, in turn, improve the precision of using surface EMG as an investigative tool to evaluate skeletal muscle activation during exercise trials.

Specifically, one of the practical applications of surface EMG is to evaluate skeletal muscle activation during dynamic exercise trials to fatigue both in athletes and patients with a clinical condition. Numerous studies have evaluated fatigue during static, isometric tasks, but the uniform, reliable interpretation of EMG during dynamic exercise, is less well understood. There are numerous challenges with the use of surface EMG during dynamic exercise – these were described in Chapter 1. One of the key limitations is the method used for normalisation of the EMG signal. As a result of the findings of Chapter 2 and Chapter 3, the following sections of the present thesis addresses one of the practical applications of using surface EMG during dynamic exercise. Most importantly, by improving the precision of surface EMG, research in clinical studies can be performed with confidence, sensitivity and with less invasiveness. Sensitivity in EMG is often required for diagnosis and therefore most clinical studies have used invasive techniques to measure muscle activity (specifically muscle fiber conduction velocity and median frequency). Thus by ensuring the precision in recording true biological variation using surface EMG, credible research in the clinical population is possible.

The following chapters aim to review the most appropriate methods for normalisation during exercise to exhaustion in two different populations:

i) A healthy athletic population, during a standardised exercise trial to volitional exhaustion during both cycling and running exercise, and;

ii) A population of patients with Peripheral Vascular Disease (PVD), during exercise trials to volitional exhaustion during walking.

PVD has been selected as the clinical condition because this condition can be rapidly reversed through intervention and therefore provides a good research model. PVD is defined as atherosclerotic occlusion of the blood vessels of the lower extremities. Narrowing of the blood vessels reduces blood flow to the lower limb and results in claudicant type pain (intermittent claudication). This pain is induced during physical activity, specifically walking and is relieved by rest [57]. Therefore the pain significantly reduces functional capacity and exercise tolerance in these patients. The intervention used in some patients to alleviate the symptoms of the disease is angioplasty (unblocking of the affected artery), resulting in “reversing” occlusive vascular component of the disease.

By using a method of normalisation that is sensitive to changes in muscle activity, a possible alteration in muscle activity could resolve the underlying contributions to the decline in functional capacity and exercise tolerance experienced in these patients. It is unclear whether changes, if any, in the EMG signal may be attributed to the unblocking of the affected artery and blood flow to active muscles.

In both populations (athletes exercising to exhaustion and patients with PVD), measurements of surface EMG have the potential to provide insight to explain mechanisms of fatigue, as has been described in the literature [174]. A detailed discussion of the models and theories of fatigue, and the application of EMG activity as a means to evaluate these models, lies beyond the scope of this review. However, it is still an issue worthy of a brief discussion as this theme has application for both groups in exercise trials to exhaustion. The possibility exists that the use of EMG may provide insight to evaluate the theories of fatigue. Accordingly, the following sections will briefly discuss the physiological models of fatigue and the measurement of muscle activity at exhaustion.

4.2 MODELS OF FATIGUE

Fatigue is usually defined "*as a decrease in force production or an inability to regenerate the original force in the presence of an increase in perception of effort*" [173]. Various physiological models of fatigue attempt to explain muscle recruitment patterns, point of muscle failure as well as the onset of the symptoms of fatigue. The Peripheral Model [146], proposes that exercise performance is limited by chemical factors, often associated with metabolism, that acts on the exercising muscle, introducing what is termed "peripheral fatigue" [65].

In this model of fatigue, the physiological changes include metabolite accumulation or substrate depletion in the active muscles [65], and the inability of the cardio-respiratory structure to maintain oxygen delivery to the active muscles [14]. Furthermore, this model proposes that during maximal exercise or incremental exercise to exhaustion, the heart is unable to supply oxygen to the muscles at a sufficient rate to prevent the development of skeletal muscle anaerobiosis [144]. Oxygen independent glycolysis results in an increase production rate of lactic acid and other metabolites which accumulate and directly impair skeletal muscle contractile function. Thus, the impairment of muscle function ultimately leads to fatigue and the termination of exercise. In this model, the primary factor responsible for fatigue is the inability of the skeletal muscle to produce the required force.

According to this model the skeletal muscle is unable to produce the necessary force during fatigue, and a key logical prediction is that all skeletal muscle motor units involved in producing force must be active at the point where volitional fatigue occurs [146]. It is logical to assume this model predicts that at fatigue there is maximal recruitment of skeletal muscle to counteract the gradual decline in the ability of each motor unit to produce the required force output. However, this model does not consider or explain why exercise is terminated when muscle fibers are still available to be co-activated. This suggests that inactive muscles are impaired without ever being active.

An alternative model of fatigue, the Central Fatigue Model, is generally described in terms of a reduction in motor command or neural drive to active muscles which results in a reduced force output [69; 95; 185]. In the Central Fatigue Model, reductions of

power output during exercise or cessation of exercise are not caused by any limiting physiological changes in the muscle, or in any peripheral organ. Rather, these changes are caused by changes in, or altered efferent command from the brain structures which occur prior to the onset of maximal use of the peripheral structures or physiological systems [144]. This model has been studied extensively during static or isometric exercise. During these types of exercise, surface EMG has been widely used to evaluate the activation of skeletal muscle motor units. These studies are summarised below.

4.3 MAXIMAL RECRUITMENT OF MUSCLE FIBERS

By definition, a maximal contraction implies that all motor units are firing at their maximal capacity [197]. This was documented in 1954 by two studies Bigland & Lippold [16] and Merton[136]. Both studies used electrical stimulation of the ulnar nerve to compare the maximal force during stimulated tetanic contractions and the maximal voluntary force produced by the adductor pollicis muscle (without external stimulation). Both studies showed that the maximum force of voluntary isometric contractions matched the force output reached during the maximal tetanic stimulation of the ulnar nerve. Furthermore, Merton [136], demonstrated that this activation remained at maximum when the contraction was maintained for 3 minutes, since nerve stimulation failed to cause an increase in the declining force output (restoration of force output). If positioned properly, this muscle can be isolated and results in contraction of only the thumb adductor, which simplifies the measurement. However, when measuring muscle activity in larger muscle groups, isolation of one muscle becomes complicated and difficult due to co-activation of synergist/surrounding muscles. Therefore, when producing a movement, the resultant contraction could be due to not only the targeted muscle, but by the contribution of a number of synergistic muscles which may not all be contracting maximally and simultaneously during maximal effort [197].

In an extensive review of the literature, Gandevia [69] emphasised that it has not been shown that complete recruitment of all available motor units occurs in any form of voluntary exercise. Furthermore, muscle activation during submaximal exercise to exhaustion remains well below maximal levels at the point of maximal effort immediately preceding volitional fatigue. A study by Hunter et al. [90], which

measured muscle activity during 20 % MVC, found that exhaustion during submaximal contraction occurred at muscle activity levels of between 30 % - 50 % of MVC. These findings suggest that fewer than 50 % of available motor units are active at exhaustion during submaximal exercise [146].

This finding is in agreement with a recently developed model of fatigue, the central regulation model of exercise [145; 146], which is specifically relevant in whole body, dynamic exercise. This model proposes that the peripheral skeletal muscles are never recruited maximally during maximal voluntary activity. Rather, muscle recruitment is regulated either consciously or subconsciously from the onset of exercise, and is characterised by the presence of 'reserve' recruitment capacity [173]. It is proposed that a 'reserve' regulatory mechanism is designed to protect body organs from being damaged during exercise [145; 146; 173; 190].

The investigation of this model presents an opportunity to utilise surface EMG as a marker of the proposed regulation of skeletal muscle activation. As explained in Chapter 1, EMG is an indirect measure of the degree of skeletal muscle motor unit activation. During static or isometric exercise trials, techniques including fine-wire, intramuscular EMG and Transcranial magnetic stimulation have been used as methods to determine direct activation of motor units. However, in dynamic exercise, these techniques are not practical (reasons discussed in Chapter 1), therefore surface EMG is a more practical alternative for evaluating the models of fatigue during dynamic exercise.

During dynamic exercise trials, such as cycling or running, where the athlete is allowed to 'select' his/her own pace during a time-trial task for performance, it has been hypothesised that the central nervous system regulates exercise performance by integrating feed-forward control with afferent sensory feedback from multiple organs during exercise activity [173]. This integration results in a specific skeletal muscle recruitment pattern, leading to a pacing strategy, which is continuously modified on the basis of neural feedback during exercise [173; 184]. This specific muscle recruitment pattern was demonstrated in a study where 10 subjects cycled a 20 km time trial in hot (35 ºC) and cool (15ºC) conditions [183]. Power output and iEMG activity of the VL decreased early in the heat, while core temperatures, heart rate and ratings of perceived exertion (RPE) were similar between conditions. The

reduction in muscle activity was interpreted as being part of an anticipatory response where the brain adjusted muscle recruitment and power output to reduce heat production, thereby ensuring that thermal homeostasis was maintained during exercise in the heat.

Several other studies have provided evidence that fatigue during maximum dynamic exercise to exhaustion occurs when fewer than 100 % of the available muscle units in the active limbs are active. For example Kayser et al. [96] had subjects cycle at 75 % of VO_2 max until exhaustion, at sea level and high altitude. They found that lower limb iEMG did not increase during hypoxic conditions as was found at sea level. Furthermore, when supplemental oxygen was supplied at the point of exhaustion, after a few breaths, the iEMG activity increased, and the subjects were able to continue cycling for a longer period. This demonstrated that muscle activity could not have been 100 % at exhaustion. Earlier fatigue had thus occurred despite the presence of a motor unit reserve. After supplemental oxygen was provided the motor unit reserve was partly utilised. Therefore, this study suggests that a reduction in neural recruitment occurs to protect the tissues from hypoxia when available oxygen may be insufficient.

4.4 CENTRAL GOVERNOR MODEL IN CLINICAL POPULATION

Another practical application to test the models of fatigue is in special populations, including patients with specific diseases. For example, patients with peripheral vascular disease who have active occlusion of the vasculature of the lower limb, terminate exercise due to intermittent claudication (pain in the leg). It is of interest that exercise terminates at blood lactate concentrations lower than those found in healthy age-matched controls and in patients with other chronic diseases [152]. It has been presumed that the poor exercise intolerance of these patients' is determined by the excessive lactate accumulation in their ischemic muscles [177]. This theory is consistent with the peripheral model of fatigue. The alternative central regulation model suggests that exercise would be limited before profound skeletal muscle ischemia develops. The use of surface EMG to measure the degree of skeletal muscle activation in patients with PVD presents an opportunity to use EMG to evaluate models of fatigue and exercise intolerance in a clinical population.

A key conceptual challenge with the use of surface EMG during dynamic exercise is that EMG activity is usually normalised by comparison with the EMG activity measured during a static MVC, as was evaluated in Chapters 2 and 3 of the present thesis. This challenge further highlights the necessity for a more appropriate method of normalisation of EMG activity, a need which the present thesis aims to address. In Chapters Two and Three, the most appropriate methods for normalisation in terms of reliability, repeatability and sensitivity were identified. The next challenge is to evaluate the validity of these methods for use in evaluating the central regulation model of fatigue during dynamic exercise.

4.4 MEASUREMENTS OF MAXIMAL MOTOR UNIT RECRUITMENT

Validity of the central governor model has been debated by many exercise physiologists. Weir et al. [191] specifically questions the ability of the central governor model to explain muscle activity reserve capacity since the studies supporting the central governor model have all used surface EMG as a measurement of muscle activity and as an interpretation of central drive contributions to fatigue. Weir et al. [191] advised that researchers should use techniques, which artificially induce maximal recruitment, including magnetic supraspinal stimulation or twitch interpolation through electrical stimulation of the muscle or nerve.

4.4.1 Twitch interpolation

In theory, twitch interpolation shows an increase in force output above the voluntary level, and denotes electrical activation of the motor units that were not recruited under voluntary conditions, suggesting the presence of a reserve. An example of this reserve is shown in Figure 4.1. However, evidence for using twitch interpolation as a technique for measuring central activation failure is also unconvincing. Even though studies using small muscle groups (adductor pollicis) have revealed a linear relationship between voluntary force and superimposed twitches [117], in larger muscle groups (elbow flexors), the relationship between voluntary force and superimposed twitches is shown to be non-linear at higher efforts [3].

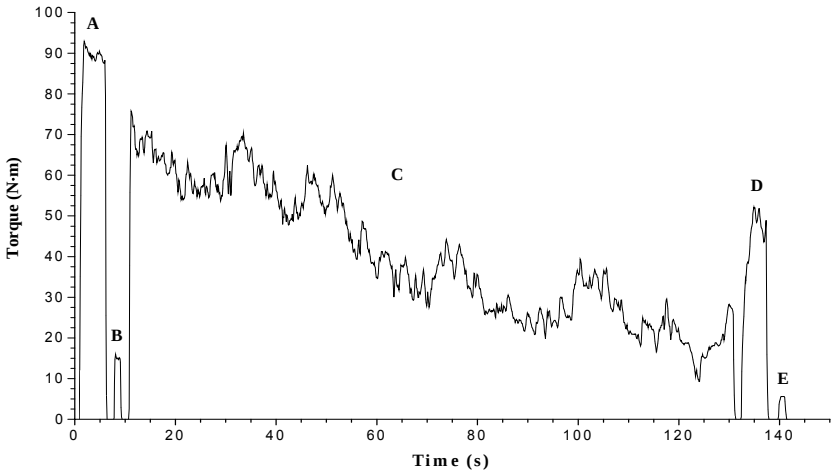


Fig 4.1: Representative torque data from a single subject from unpublished data. *A = 5sec MVC 1, B = Twitch Stimulation 1, C = 2min MVC, D = 5sec MVC 2, E = Twitch Stimulation 2*

4.4.2 Transcranial magnetic stimulation

Transcranial magnetic stimulation is a method used to determine the contribution of the cerebral cortex and central nervous system to muscle activation, through the stimulation of different areas of the brain and recording the responses from the skeletal muscle. A study by Todd et al. [180] attempted to compare voluntary activation using twitch interpolation by motor nerve stimulation and transcranial magnetic stimulation. The study showed that transcranial magnetic stimulation elicited a superimposed force in 92 % of all maximal efforts. In contrast, motor nerve stimulation elicited superimposed forces in only 68 % of maximal efforts suggesting that twitch interpolation techniques using percutaneous muscle stimulation are not able to measure central activation failure accurately for maximal and near maximal efforts. This finding confirms that fatigue occurs in the presence of a motor unit reserve, because the administration of motor nerve and then motor cortex stimulation produces graded increases in force output.

4.4.3 M-wave measurement

Weir et al. [191] also questions the interpretation of EMG amplitudes that have been used in studies showing submaximal recruitment of motor units, and because authors normalised surface EMG to a static, isometric MVC performed before the trial as a measurement of maximal muscle activity, rather than techniques that stimulate the muscle such as twitch interpolation. Weir et al. [191] recommend normalising EMG amplitude to changes in the size of M-waves. The supramaximal stimulation is seen as the equivalent of recruiting all motor units [26] and provides

information on the stability of neuromuscular propagation [50]. This method has only been shown to be repeatable for Biceps brachii [26], Tibialis anterior muscle [129] and Vastus medialis [130] muscles. However, Merletti et al. [129] found the M-wave shape appeared to be consistent over days and within day experiments (a number experiments conducted on the same day), but the amplitude and duration varied. In addition, repeatability of this method has only been found for relatively static exercise, unlike exercise which is used to evaluate physiology and fatigue during tasks like cycling and running.

However, the use of this method could possibly have adverse effects on the subjects, especially when both the major nerve supplies to the upper and lower limb are stimulated before a trial, as might occur during studies involving cycling and running. The common M-wave protocol involves stimulating the motor nerve in 4 Volt increments until a maximal M-wave is achieved, after which additional supramaximal stimulation is given to confirm no further enlargement in peak-to-peak M-wave amplitude [26]. This type of measurement is viewed as uncomfortable and possibly painful, and utilising this technique on the same person performing repeated trials would be impractical.

4.5 CONCLUSION

It is acknowledged that the measurement and interpretation of surface EMG during dynamic exercises is a complex and technical method and is influenced by intrinsic and extrinsic factors as discussed in Chapter 1. However, with few reasonable alternatives, this method has been shown to be repeatable, reliable and has the ability to detect changes in muscle activity with increasing exercise intensity (for cycling and running exercises). By using the most appropriate method of normalisation established in Chapters 2 and 3, the next studies described in Chapters 5 and 6 aims to investigate muscle activity at exhaustion in healthy athletic populations. The hypothesis tested in these studies is that muscle activity is submaximal at exhaustion. The objective of each study is to measure voluntary activation of muscle activation at exhaustion. In addition, the study in Chapter 7 examines a diseased population using an appropriate method of normalisation to measure possible changes in muscle activity after an intervention and the effect on functional capacity and exercise tolerance.

CHAPTER 5

MUSCLE ACTIVITY AT EXHAUSTION DURING CYCLING

5.1 INTRODUCTION

In previous chapters, various methods for normalising the EMG signal measured during dynamic exercise were evaluated. As explained in these chapters the purpose of identifying the most reliable and repeatable method for normalisation is to ensure that appropriate conclusions can be drawn from studies where surface EMG measurements are made. One such application for using surface EMG measurements is during exercise to volitional exhaustion, particularly to investigate various models which describe the aetiology of fatigue.

In the present chapter, the use of surface EMG as a research technique for exercise to exhaustion is considered during cycling trials to exhaustion to evaluate the merits of the peripheral model and the central governor model described briefly in Chapter 4.

As discussed, the peripheral model suggests that voluntary exercise is terminated due to a failure of the contractile properties of skeletal muscle. This model predicts that all the motor units will be active at exhaustion. In contrast, the main principle of the complex, regulatory central governor model is that the active muscle is never maximally recruited even during maximal voluntary exercise. It is hypothesised that the submaximal recruitment of muscle is due to the conscious or subconscious inhibition of muscle activation [173]. Several studies have shown that muscle activity is indeed not maximal at exhaustion during maximal exercise performances [95; 96; 182]. However, one of the main limitations of these studies is that the authors normalise surface EMG to a static, isometric MVC performed before the trial as a measurement of maximal muscle activity, rather than techniques that stimulate the muscle such as twitch interpolation.

The use of surface EMG in determining maximal voluntary activation of skeletal muscle during dynamic exercise can also be contentious due to the intrinsic and extrinsic factors (e.g. electrode shift, amplitude cancellation), which can affect the EMG signal. These factors are discussed in Chapter 1. Chapter 2 of the present thesis showed that the measurement of muscle activity normalised to dynamic cycling exercise was the most repeatable, reliable and able to detect changes in muscle activity corresponding to changes in cycling exercise intensity.

In determining a particular method for obtaining a reference value for normalisation, one must consider several issues (as discussed in Chapters 1, 2 and 3). The important issues are 1) repeatability and reliability of the reference EMG value, 2) the sensitivity of the method in detecting changes in exercise intensity, 3) the similarity between the muscle activity in the method of normalisation and the activity of interest and 4) the relevance of the normalising contraction to the specific question being addressed. Accordingly, the present chapter aims to apply the previously determined appropriate method of normalisation for cycling trials to measure maximal muscle activity during progressive cycling exercise to exhaustion. This is done with the aim of evaluating whether the degree of muscle activation at fatigue is maximal, compared to various pre-exercise 'maximum' contractions, and whether muscle activity at the point of exhaustion is repeatable.

In selecting the most appropriate method for EMG normalisation, it is recognised that the 70 % PPO method was established to be most reliable and appropriate for use during cycling trials, particularly where repeated trials would be performed (Chapter 2). However, considering that the present study aims to investigate if muscle activation is maximal at volitional exhaustion, the present chapter alternatively utilises the MVC and Sprint methods as normalisation techniques. These techniques measure maximal muscle activity during static isometric contraction and dynamic sprint cycling respectively.

5.2 METHODS

5.2.1 Subject selection

Thirteen well-trained cyclists were recruited from local cycling clubs to participate in this study. Subjects were included if they were currently between the ages of 18-35 years old and if they were able to complete the Cape Argus cycle race (109 km) in under 3 h 30 min. The study was approved by the Research and Ethics Committee of the Faculty of Health Sciences within the University of Cape Town. The study was performed in accordance with the principles of the declaration of Helsinki (October, 2000), ICH Good Clinical Practice (GCP) and the laws of South Africa. Subjects performed the same experimental protocol on each of the three testing days. Each testing day was separated by 5-7 days of normal training.

5.2.2 Experimental trial

These methods have been described in Chapter 2 Section 2.2.2. A summary of the experimental design is shown in Figure 5.1.

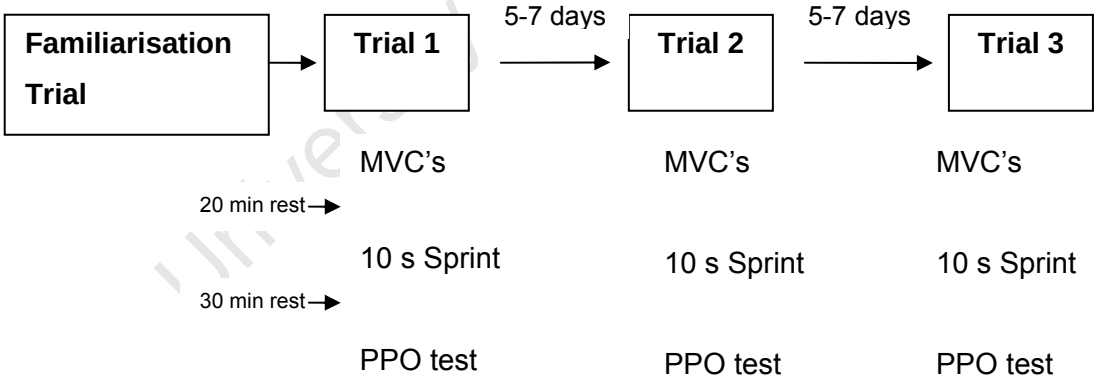


Figure 5.1: Experimental design

5.2.3 Anthropometry

The same protocol was used as described in Chapter 2 (Section 2.2.3)

5.2.4 Maximal voluntary contractions

These methods have been described in Chapter 2 (Section 2.2.4)

5.2.5 Peak power output test

These methods have been described in Chapter 2 (Section 2.2.5)

5.2.6 EMG data collection

These methods have been described in Chapter 2 (Section 2.2.6)

5.2.7 EMG analysis

These methods have been described in Chapter 2 (Section 2.2.7)

5.2.8 Methods of normalisation

5.2.8.1 MVC Method

These methods have been described in Chapter 2 (Section 2.2.8.1)

5.2.8.2 Sprint Method

These methods have been described in Chapter 2 (Section 2.2.8.2)

In selecting an appropriate method of normalisation which meets most of the requirements as described in Chapter 1 and evaluated in Chapter 2, the following table is used to present the advantage and disadvantage for using each method during cycling;

Table 5.1: Advantages and disadvantages for using the MVC and Sprint method of normalisation for muscle activity during cycling to exhaustion

Norm Method	Advantages	Disadvantages
MVC	• Higher repeatability for VL and BF • Sensitivity to tracking EMG over increasing exercise intensities, only usable for RF and BF muscles	• Intra-subject CV is high, thus reliability is weaker • Static movement thus not representative of muscle activity during cycling (does not account for electrode shift and tissue conductivity) • Constant activity, compared to 'on-off' bursts of activity during cycling • Lower repeatability for VM, RF and MG • Low sensitivity to tracking EMG over increasing exercise intensities, for VM,VL, MG and LG
Sprint	• Higher reliability, Intra-subject variability is lower • High repeatability for VM, RF and MG • Dynamic movement thus better representation of muscle activity during cycling trial (by accounting for electrode shift and tissue conductivity) • Higher sensitivity in tracking EMG over increasing exercise intensities for VM, VL, RF and BF	• Lower repeatability for VL and BF • Low sensitivity to tracking EMG over increasing exercise intensities, MG and LG

Considering the advantages and disadvantages of each method as outlined above, the Sprint method was deemed more appropriate for normalisation of muscle activity during cycling to exhaustion for the purpose of this study. The sprint method was considered to be the most reliable and repeatable method. It is also representative of the muscle movement used in cycling.

5.2.9 Statistical analysis

All results were analysed using a commercially available software programme (Statistica 7, StatSoft, Tulsa, OK, USA. Inc.©). Results were expressed as means ± standard deviation (SD). The degree of repeatability of muscle activity at exhaustion was investigated using the Intraclass Correlation Coefficient (ICC). When ICC ranged between R = 0.80 and 1.00 the reproducibility was defined as "good", between R = 0.60 and 0.79 as "fair" reproducibility and less than R = 0.60 as "poor" [170]. Reliability of muscle activity at exhaustion over 3 trials for each subject (Intra-subject reliability) was assessed using coefficient of variation (CV) analysis. Intra-subject variability was calculated by dividing the standard deviation by the mean over 3 trials. If CV values were less than 12 %, the data were considered to have good variability.

5.3 RESULTS

5.3.1 Descriptive characteristics

The descriptive characteristics of the cyclists are reported in Chapter 2 Table 2.2.

5.3.2 Muscle activity at exhaustion

Figure 5.2 shows the average muscle activity at exhaustion for the thirteen subjects, each muscle was expressed as a percentage of muscle activity during a maximal sprint. Muscle activity at exhaustion was similar over three trials for all muscles. It is important to note that all muscles were less active at exhaustion than when performing a maximal sprint. The mean muscle activity at exhaustion over 3 trials (expressed as a range of the percentage of sprint value) was; VM (59 % - 65%), VL (54 % - 60%), RF (44 % - 51%), BF (42 % - 45%), MG (40 % - 46%) and LG (36 % - 44%). The summed average EMG of all the muscles was also shown to be lower than muscle activity during a maximal sprint.

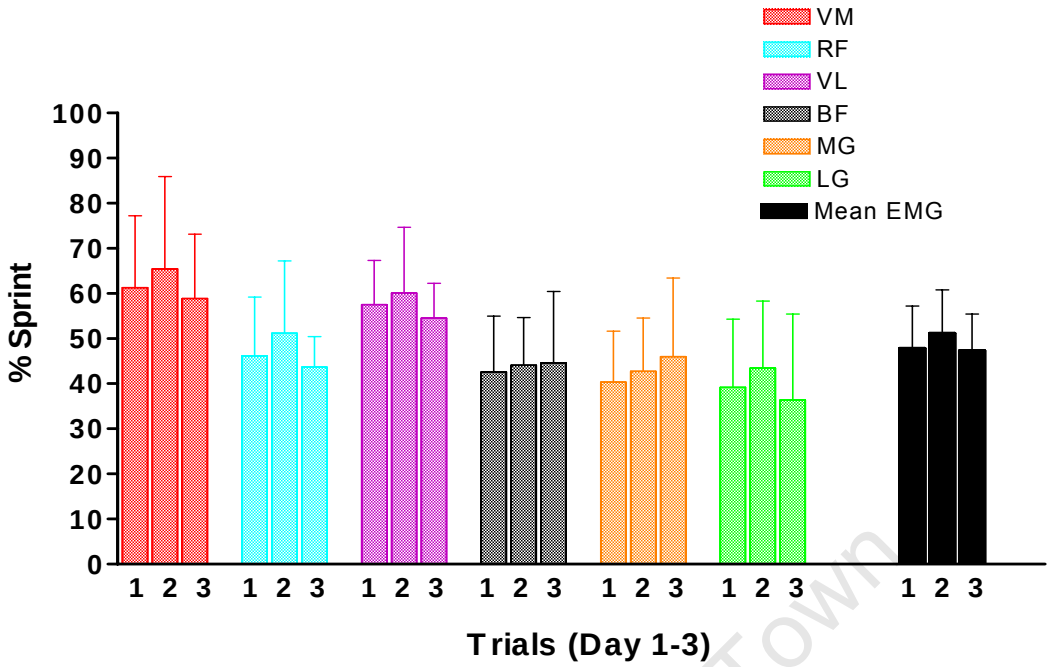


Fig 5.2: Muscle activity of Vastus medialis (VM), Rectus femoris (RF), Vastus lateralis (VL), Biceps femoris (BF), Medial and Lateral gastrocnemius (MG and LG respectively) at exhaustion for 3 PPO trials*. The graph also shows the mean EMG activity of all muscles summed during each trial.*

5.3.3 Intra-subject reliability of muscle activity at exhaustion

To further highlight the variation in muscle activity at exhaustion, the data for each subject at exhaustion (with accompanying ICC values), over 3 separate days of testing, are presented in Figure 5.3 and 5.4. The muscle activities of the quadriceps muscles at exhaustion have the greatest variability for each subject and lowest ICC. The VM, VL and RF showed “poor” repeatability.

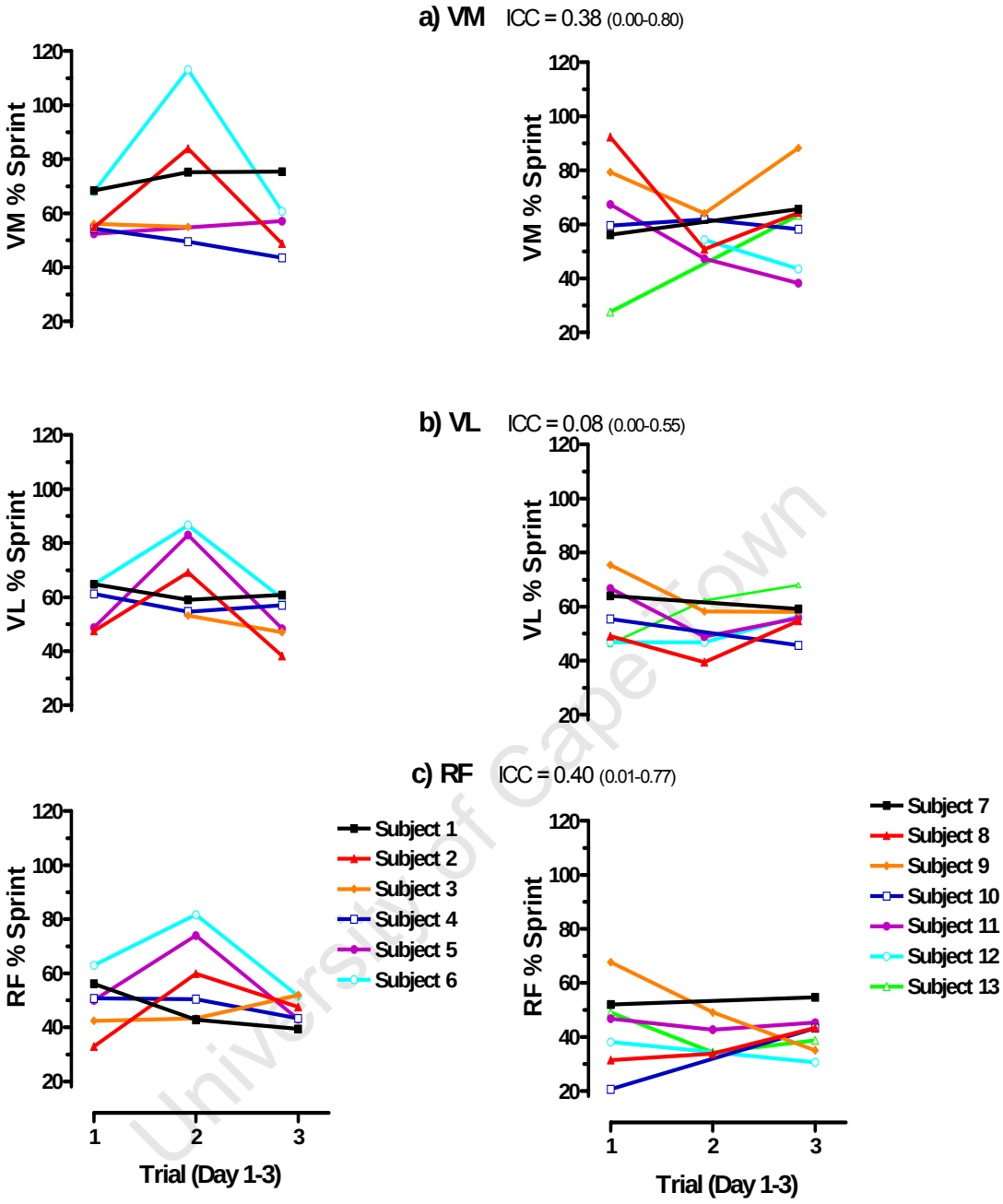


Figure 5.3: Variability of each subjects muscle activity at exhaustion over 3 trials with corresponding ICC values (95% confidence intervals). *Representing muscle activity of* ***a)*** *Vastus medialis (VM),* ***b)*** *Vastus lateralis (VL) and* ***c)*** *Rectus femoris (RF)*

The gastrocnemius muscles and BF showed less variability in muscle activity at exhaustion, and “fair” to “good” ICC values.

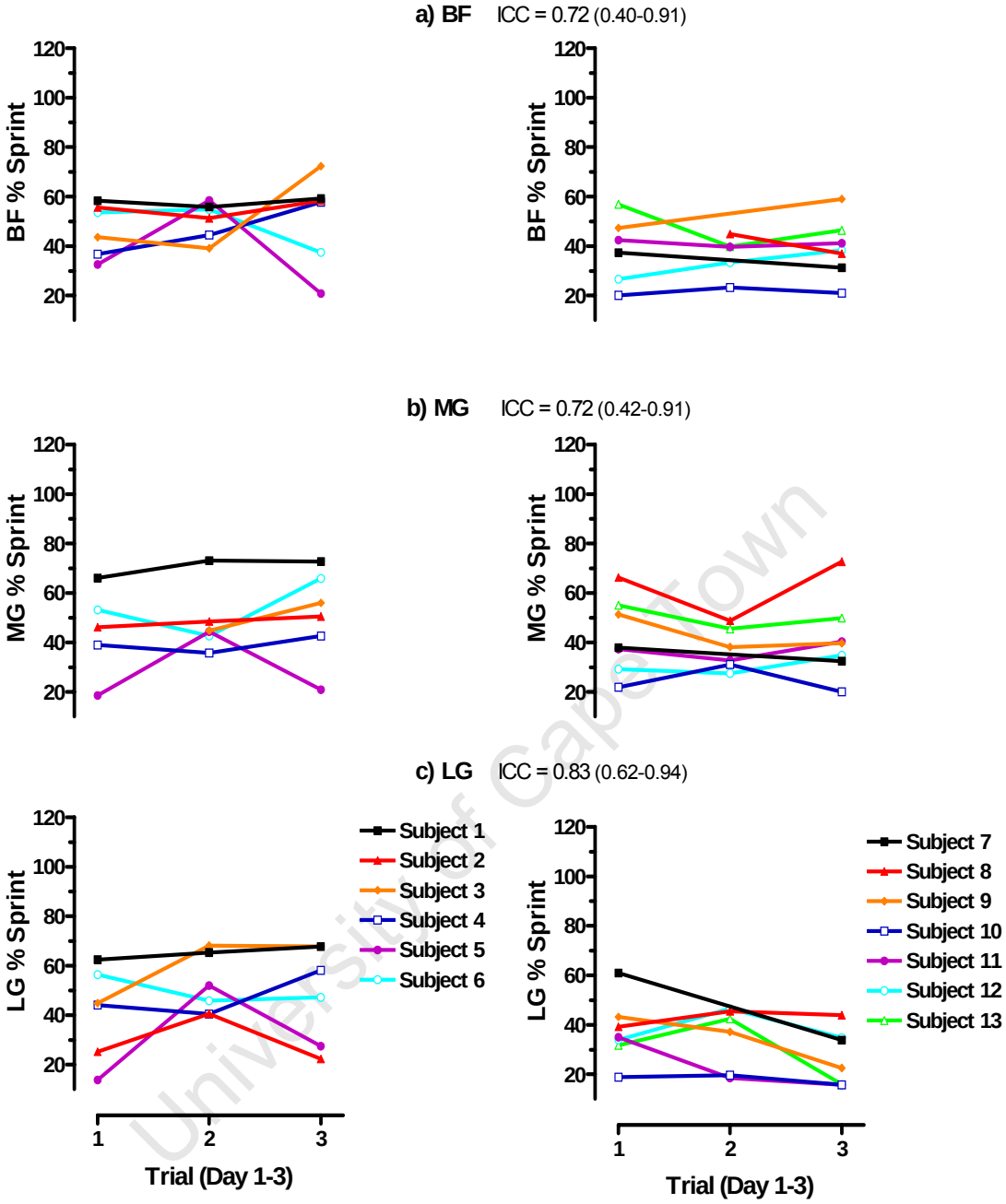


***Figure 5.4: Variability of each subjects muscle activity at exhaustion over 3 trials with corresponding ICC values (95 % confidence intervals)**. Representing muscle activity of **d)** Biceps femoris (BF), **e)** Medial gastrocnemius (MG) and **f)** Lateral gastrocnemius (LG).*

The intra-subject CV of muscle activity over the 3 trials gave an indication of the reliability of muscle activity at exhaustion (Table 5.2). Only six out of the thirteen subjects showed CV values less than 12 % for at least 3 muscles. Most subjects (5

out of 13) showed low CV values for VM. The average muscle activity of all muscles of each subject was highly variable at exhaustion.

Table 5.2: Intra-subject coefficient of variation of EMG activity at exhaustion for six muscles

Subject	VM	VL	RF	BF	MG	LG	Average
1	**5**	**5**	19	**3**	**4**	**4**	**7 ± 7**
2	30	31	29	**6**	**8**	33	23 ± 12
3	**1**	**9**	**11**	35	42	22	20 ± 16
4	**11**	**6**	**9**	23	18	20	14 ± 7
5	**6**	33	29	52	36	62	36 ± 19
6	35	20	23	20	30	**12**	23 ± 8
7	**11**	**6**	**4**	12	29	40	17 ± 14
8	31	16	18	14	32	**7**	20 ± 10
9	16	16	32	15	**12**	31	20 ± 10
10	**3**	14	50	**8**	31	**11**	19 ± 18
11	29	16	**5**	**3**	**10**	45	18 ± 16
12	16	**11**	15	18	27	18	18 ± 5
13	56	19	18	18	**6**	44	27 ± 20

Intra-subject CV of muscle activity of Vastus medialis (VM), Rectus femoris (RF), Vastus lateralis (VL), Biceps femoris (BF), Medial and Lateral gastrocnemius (MG and LG respectively) at exhaustion for 3 PPO trials. The values highlighted in bold represent "good" CV values ≤ 12 %.

5.4 DISCUSSION

This study examined EMG activity at volitional exhaustion during maximal cycling trials. By tabulating the advantages and disadvantages of the methods of normalisation evaluated in Chapter 2, the most appropriate method of normalisation for the present study was determined. Although the 70 % PPO method was the most repeatable and reliable (Chapter 2) this method was not used in this study because comparison of EMG during a maximal cycling bout to a 70 % PPO bout would reveal little insight into the degree of maximal muscle activation. Instead, the chosen methods for normalisation were the MVC method (a maximal static, isometric contraction), and the Sprint method (maximal dynamic movement).

In view of the advantages and disadvantages of these two methods, the Sprint method was selected as the most appropriate method of normalisation for use in the present study. In particular, this method had greater reliability, repeatability (predominantly for VM, RF and MG) and sensitivity (predominantly for VM, VL, RF and BF) than the MVC method. Most importantly the muscles used in the Sprint method involves the same dynamic muscle contractions as the exercise bout being investigated, thus better representing muscle activity during cycling. In contrast, the static, isometric MVC method has often limited conclusions based on the comparison of a dynamic bout with a static maximal muscle contraction of short duration. Thus the Sprint method for normalisation of EMG during cycling to exhaustion was used.

The main finding of this study was that muscle activity at exhaustion in all the major muscles during cycling was submaximal (Figure 5.2) compared to EMG activity during maximal sprint cycling. All six muscles measured in the study achieved peak activation of between 44 % - 65 % of that achieved during a 10 second all-out sprint. This finding suggests that peripheral skeletal muscles are never maximally recruited, even during maximal volitional effort during incremental exercise. It has been suggested that mechanisms of fatigue regulation may also incorporate neural mechanisms, which regulate the number of motor units that are recruited or derecruited during exercise in order to prevent any peripheral system reaching maximal capacity [145; 173].

These findings support the findings of other studies that show muscle activity during cycling to be less than maximal at the end of maximal cycling performances. A recently published study by Tucker et al. [182] investigated muscle activity during self-paced 20 km time- trials performed in normoxic and hyperoxic conditions. The study found that muscle activity at the end of the 20 km time-trial in normoxia was only 26 % - 46% of MVC. Furthermore, Kay et al. [95] studied muscle activity in cyclists during a 60 min time-trial with six 1 min maximal sprints at 10 min intervals, in warm humid conditions. The study showed that muscle activity (normalised to the first sprint) decreased in sprints 2-5 where iEMG values were 71 %, 71 %, 73 % and 77 % of the initial sprint. However, in the final sprint, muscle activity increased to 90 % of the initial sprint value, with associated increase in measured power output. These subjects were able to increase neural drive to the peripheral muscles resulting in an increase in power output at the end of the bout of exercise (endspurt) [95; 182].

The peripheral model of fatigue fails to explain how power output in the final sprint of the trial can increase to levels similar to that of the initial sprint. This occurs in the presence of metabolite accumulation which according to this model impairs muscle function. Kay et al. [95] suggest that there is a neuromuscular reserve present during self-paced exercise which maintains the ability to activate and deactivate muscles so as to maintain work output and therefore optimal exercise performance. In a similar study by St Clair Gibson et al. [175], muscle activity was also submaximal during a 100 km time-trial, interspersed with high intensity bouts. During the trials, power output decreased incrementally during the high intensity cycling bouts, despite verbal encouragement of the subjects. Similarly EMG amplitude decreased in parallel to the decrease in power output. These changes occurred despite only activating approximately 20 % of muscle activity during a MVC. It thus appears that the central nervous system down regulated power output by decreasing motor command to the skeletal muscles, despite the attempts of the subjects to maintain power output. Ansley et al. [6] has also shown muscle activity to be approximately 25 % of MVC at the point of peak power output during three consecutive 4 km cycling time-trials (separated by 17 min). The authors interpreted their findings as being due to centrally regulated pacing strategies.

The key consideration in the above mentioned studies is that EMG activity during cycling was normalised according to EMG measured during a sustained isometric

contraction. However, this has two potential limitations; firstly muscle activity during the MVC is not representative of muscle activity during cycling, secondly the analysis compares EMG during 3 or 5 seconds of sustained activity during the MVC to the same time period of cycling activity. Because cycling consists of 'on-off' bursts of muscle activation (intermittent activity, with muscle being inactive for part of the pedal stroke) in this time period, the MVC method therefore compares the intermittent muscle activity during cycling to the sustained muscle contraction of the MVC. Thus, by using the Sprint method to normalise muscle activity during cycling, the potential problems outlined above are reduced. Moreover the use of Sprint method (dynamic) of normalisation would possibly also reduce the contributions of extrinsic factors which affect the EMG amplitude (Chapter 2).

In addition, the present study has highlighted the variability of intra-subject muscle activity at exhaustion, where very few muscles showed CV values less than 12 %. Such variations are an indication that muscle activity at exhaustion varies from one day to the next. Whilst part of the variation could however be due to electrode placement and other influential factors, precautionary steps were taken to minimise these, as described in the Methods (Chapter 2). In addition, the intra-subject variation using the Sprint method is not as low as using the 70 % PPO method, but this variation was deemed less significant for the present study, given that the primary objective was the application of EMG to evaluate muscle activity at fatigue. However, given the variability of the Sprint method of normalisation, the variability in EMG activity at fatigue was greater than can be explained through these previously described extrinsic factors which would contribute to the variation in the EMG signal.

A possible limitation of the study could have been not additionally using twitch interpolation at exhaustion to verify a decrease in central drive.

In conclusion, by using the most appropriate method of EMG normalisation, the findings of this study indicate that muscle activity is sub-maximal at exhaustion during graded cycling exercise to volitional fatigue. Furthermore, the findings confirm that only a certain percentage of the limb muscle mass is active during maximal exercises. These findings are not compatible with the peripheral model of fatigue, as this model assumes that the total muscle mass is active at exhaustion.

CHAPTER 6

MEASUREMENT OF MUSCLE ACTIVITY AT EXHAUSTION IN RUNNING

6.1 INTRODUCTION

This chapter continues to address the application of the most appropriate method of normalisation during dynamic exercise, specifically in running. As discussed in Chapters 4 and 5, using surface EMG in determining maximal voluntary activation during dynamic exercise is seen as questionable due to the intrinsic and extrinsic factors (e.g. electrode shift, amplitude cancellation) affecting the EMG signal (as discussed in Chapter 1). No other research has been published on the repeatability and reliability of the EMG amplitude with increasing running speed. Thus the findings of Chapter 3 of the present thesis are indeed novel, as it has shown that the measurement of muscle activity during dynamic running exercise to be repeatable, reliable and able to detect changes in muscle activity corresponding to increasing running speed.

Accordingly, this chapter aims to apply the most appropriate method of normalisation, to measure maximal muscle activity during progressive running exercise to exhaustion and to investigate if muscle activity is indeed submaximal at exhaustion. In selecting the most appropriate method for EMG normalisation, it must be recognised that the 70 % PRS method does not provide information on the absolute level of muscle activation and is therefore not suitable for normalising the data in this context. The MVC and Sprint methods are more appropriate methods of normalisation as explained in Chapter 5.

6.2 METHODOLOGY

6.2.1 Subject Selection

Twelve well-trained runners were recruited from local running clubs to participate in this study. The same subject criteria were applied as in Chapter 3 Section 3.2.1. Once each subject signed the informed consent, they were required to visit the laboratory on four separate occasions. During the first visit to the laboratory, subjects underwent an anthropometrical assessment and answered the PAR-Q as described in Chapter 2 (Section 2.2.1 and 2.2.3). Each subject was then familiarised with the equipment used and the testing protocol. The study was approved by the Research and Ethics Committee of the Faculty of Health Sciences within the University of Cape Town. The study was performed in accordance with the principles of the declaration of Helsinki (October, 2000), ICH Good Clinical Practice (GCP) and the laws of South Africa. Subjects performed the same experimental protocol on each of the three testing days. Each testing day was separated by 5-7 days of normal training.

6.2.2 Experimental trial

The same methods have been described in Chapter 3 (Section 3.2.2). A summary of the experimental design is shown in Figure 6.1.

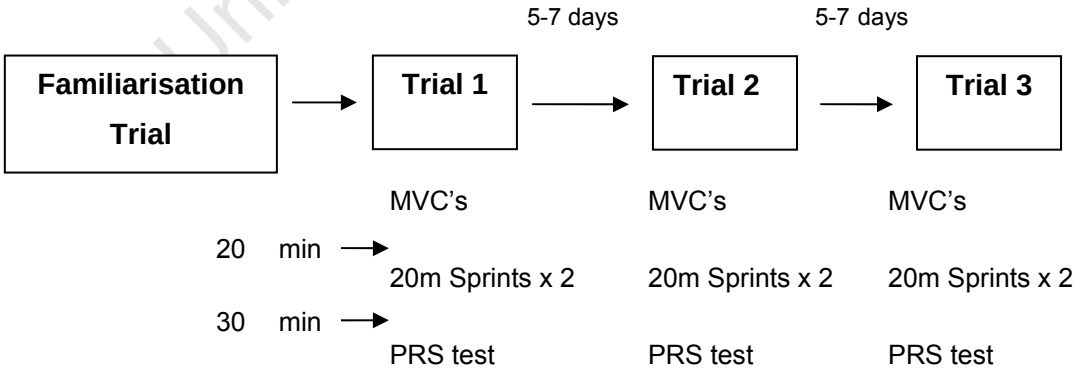


Figure 6.1: Experimental design

6.2.3 Familiarisation trial

The same protocol has been described in Chapter 3 (Section 3.2.2.1)

6.2.4 Maximal voluntary contractions

The same protocol was used as described in Chapter 2 (Section 2.2.4)

6.2.5 20 m sprints

These methods have been described in Chapter 3 (Section 3.2.4)

6.2.6 Peak running speed trial (PRS)

These methods have been described in Chapter 3 (Section 3.2.5)

6.2.7 EMG collection and analysis

These methods have been described in Chapter 3 (Section 3.2.6 and 3.2.7)

6.2.8 Methods of normalisation

These methods have been described in Chapter 3 (Section 3.2.8). From Chapter 3 it was concluded that normalising EMG to the Sprint and MVC methods resulted in the most repeatable EMG amplitudes. This study aims to investigate the maximal muscle activity at exhaustion, thus the use of the 70 % PRS method is not feasible.

6.2.8.1 MVC Method

These methods have been described in Chapter 3 Section 3.2.8.1

6.2.8.2 Sprint Method

These methods have been described in Chapter 3 Section 3.2.8.2.

In selecting an appropriate method of normalisation which meets most of the requirements as described in Chapter 1 and evaluated in Chapter 3, the following table is used to present the advantages and disadvantages for using each method during running;

Table 6.1: Advantages and disadvantages for using the MVC and Sprint methods of normalisation for muscle activity during running to exhaustion.

Norm Method	Advantages	Disadvantages
MVC	• Higher repeatability for VM, BF and MG. • Sensitivity to tracking EMG over increasing exercise intensities is similar to Sprint method but slightly better in detecting changes in VL and LG.	• Intra-subject CV is high, thus reliability is weaker. • Static movement, thus not representative of muscle activity during running (does not account for electrode shift and tissue conductivity). • Lower repeatability for VL, RF and LG.
Sprint	• Higher reliability–Intra subject variability is lower. • High repeatability for VL, RF and LG. • Dynamic movement thus better representation of muscle activity during running (by accounting for electrode shift and tissue conductivity). • Sensitivity to tracking EMG over increasing exercise intensities is similar to MVC method but slightly better in detecting changes in RF.	• Lower repeatability for VM, BF and MG

Considering the advantages and disadvantages of each method as outlined above, the Sprint method was deemed the more appropriate method to normalise muscle activity during running to exhaustion for the purpose of this study. The sprint method was the most reliable and repeatable method. It is also representative of the muscle movement used during running.

6.2.9 Statistical analysis

All results were analysed using a commercially available software programme (Statistica 7, StatSoft, Tulsa, OK, USA. Inc©.). Results were expressed as means ± standard deviation (SD). The degree of repeatability of muscle activity at exhaustion was investigated using the Intraclass Correlation Coefficient (ICC). When ICC ranged between R = 0.80 and 1.00 the reproducibility was defined as "good", between R = 0.60 and 0.79 as "fair" reproducibility and less than R = 0.60 as "poor" [170]. Reliability of muscle activity at exhaustion over 3 trials for each subject (Intra-subject reliability) was assessed using coefficient of variation (CV) analysis. Intra-subject variability was calculated by dividing the standard deviation by the mean over 3 trials. If CV values were less than 12 % a good variability was considered.

6.3 RESULTS

6.3.1 Descriptive characteristics

The descriptive characteristics of the runners are shown in Chapter 3 (Table 3.1).

6.3.2 Muscle activity at exhaustion

Due to EMG lead failure, the muscle activity of certain subjects was not captured. Figure 6.2 shows the average muscle activity at exhaustion for twelve subjects, expressed as a percentage of muscle activity during a maximal sprint for each muscle. Muscle activity at exhaustion was similar over three trials for all muscles. Important to note is that all muscles were less active at exhaustion than when performing a maximal sprint. The mean muscle activity at exhaustion over 3 trials (expressed as a range of the percentage of sprint value) was; VM (53 % - 58 %), RF (55 % - 64 %), VL (42 % - 51%), BF (58 % - 59 %), MG (60 % - 66 %) and LG (61 % - 63 %). The summed mean EMG of all the muscles was less than 100 % sprint. As seen from the above the muscle activity ranges, the Medial and Lateral gastrocnemius muscles are the most activated during running followed by

the bilateral RF and BF. The summed EMG of all the muscles was lower than muscle activity during a maximal sprint.

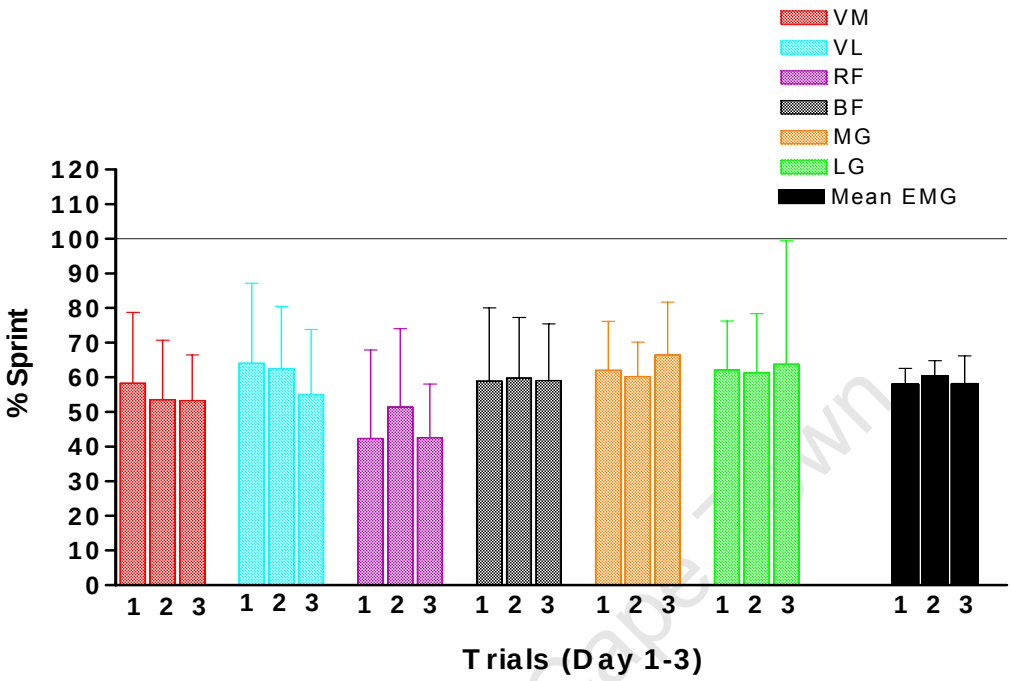


Figure 6.2: Muscle activity of Vastus medialis (VM), Rectus femoris (RF), Vastus lateralis (VL), Biceps femoris (BF), Medial and Lateral gastrocnemius (MG and LG respectively) at exhaustion for 3 PRS trials. *The graph also shows the mean EMG activity of all muscles summed during each trial.*

Trial to trial reliability, using EMG data at exhaustion of each subject, was applied to further highlight the variation in muscle activity at exhaustion. The muscle activity of each subject at exhaustion, over 3 separate days of testing, are presented in Figures 6.3 and 6.4 with accompanying ICC values. The quadriceps muscles and BF are seen to have the least variability and "good" ICC values at exhaustion (Figure 6.3). The MG and LG muscles had "poor" repeatability at exhaustion (Figure 6.4).

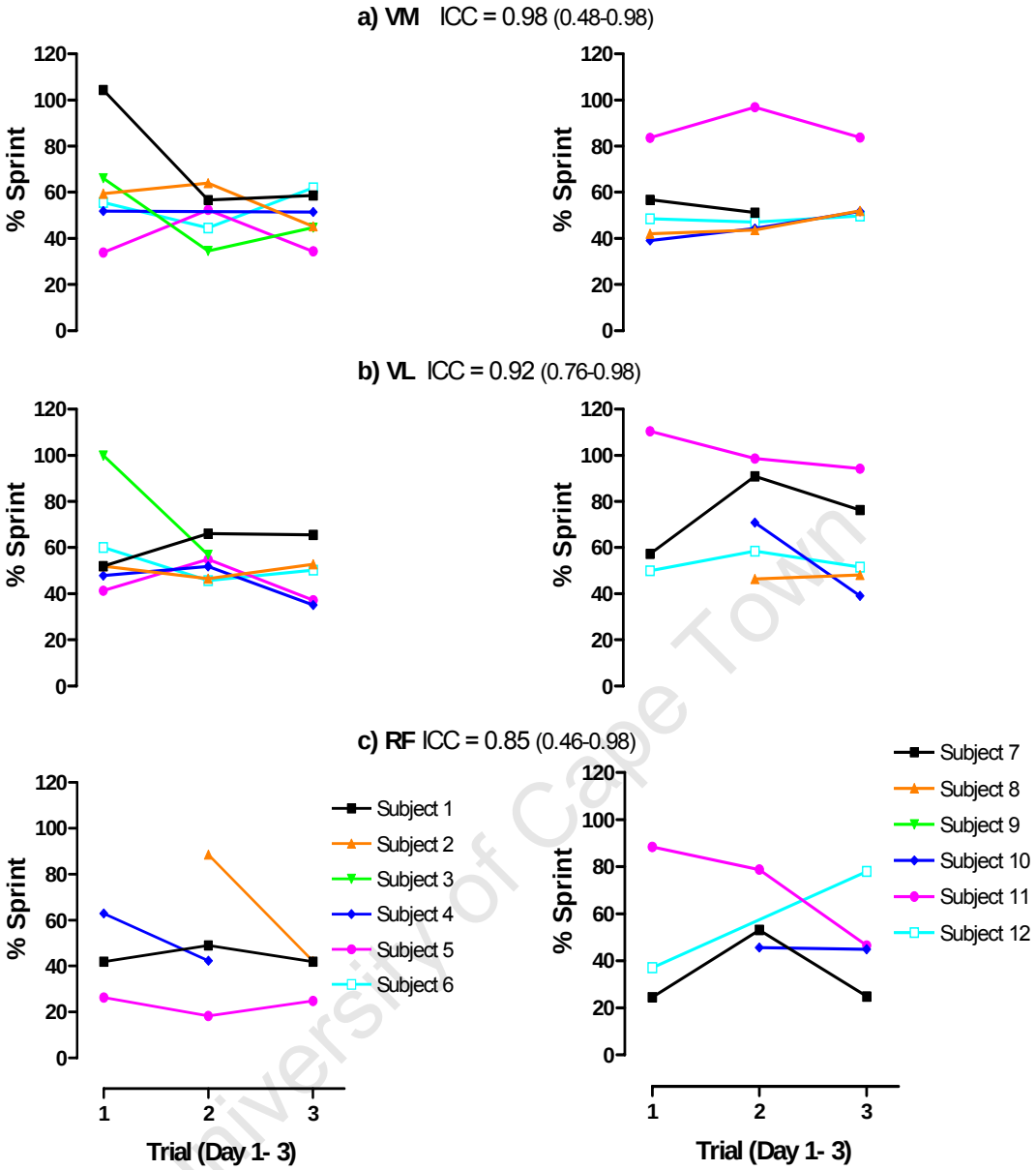


Figure 6.3: Variability of each subjects muscle activity at exhaustion over 3 trials with corresponding ICC values (95% confidence intervals). *Representing muscle activity of* ***a)*** *Vastus medialis (VM),* ***b)*** *Vastus lateralis (VL) and* ***c)*** *Rectus femoris (RF). Note only one data point was available for Subject 9's VM, RF and VL. For RF, only one data point was available for subjects 3, 6 and 8.*

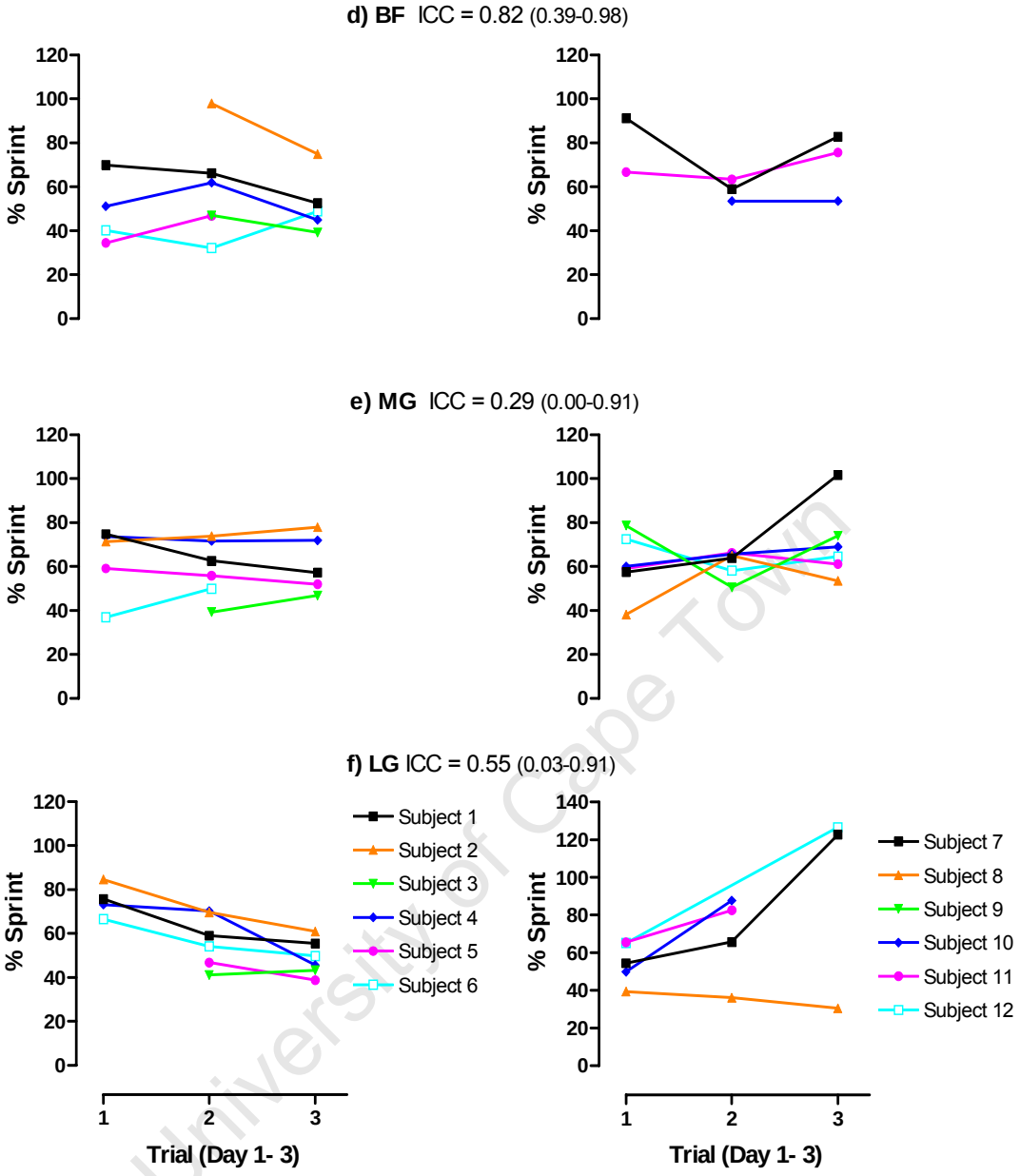


Figure 6.4: Variability of each subjects muscle activity at exhaustion over 3 trials with corresponding ICC values (95% confidence intervals). *Representing muscle activity of* ***d)*** *Biceps femoris (BF),* ***e)*** *Medial gastrocnemius (MG) and* ***f)*** *Lateral gastrocnemius (LG). Note only one data point was available for Subject 9's BF and LG. For BF, only one data point was available for subject 8 and 12.*

6.3.3 Intra-subject reliability of muscle activity at exhaustion

The intra-subject CVs of muscle activity over the 3 trials at exhaustion (with accompanying ICC values) are shown in Table 6.2. Only two out of the eleven subjects showed CV values less than 12 % for at least 4 muscles and only four subjects showed CV values of less than 12 % for 2 muscles. Most subjects (6 out of 11) showed low CV values for MG. however, overall muscle activity variation was seen to be high at exhaustion.

Table 6.2: Intra-subject coefficient of variation of EMG activity at exhaustion for six muscles

Subject	VM	VL	RF	BF	MG	LG	Average
1	37	13	**9**	14	14	17	**17 ± 10**
2	18	**7**	50	19	**4**	17	19 ± 16
3	33	39		13	12	**3**	20 ± 15
4	**0**	19	28	16	**1**	24	15 ± 11
5	26	21	18	21	**6**	13	18 ± 7
6	16	14		21	21	15	17 ± 3
7	**7**	22	48		32	45	31 ± 17
8	**11**	**3**		22	26	13	15 ± 9
9					22		22
10	14	41	**1**		**7**	39	20 ± 18
11	**9**	**8**	31	**0**	**6**	16	12 ± 11
12	**2**	**8**	50	**9**	**11**	45	21 ± 21

Intra-subject CV of muscle activity of Vastus medialis (VM), Rectus femoris (RF), Vastus lateralis (VL), Biceps femoris (BF), Medial and Lateral gastrocnemius (MG and LG respectively) at exhaustion for 3 PRS trials. The values highlighted in bold represent "good" CV values ≤ 12 %.

6.4 DISCUSSION

The aim of this study was similar to Chapter 5 i.e. to use the most appropriate method of normalisation to measure maximal muscle activity during progressive exercise to exhaustion and to investigate whether muscle activity is indeed submaximal at exhaustion, specifically in running. The results of Chapter 3 provided novel data on the ability of static and dynamic methods of normalisation to show repeatability, reliability and sensitivity. The most appropriate method of normalisation in this study was decided upon by tabulating the advantages and disadvantages of the MVC and Sprint methods (Table 6.1). In view of the advantages and disadvantages, the Sprint method was selected as the most appropriate method of normalisation to answer the question of whether muscle activity is indeed submaximal at exhaustion during running exercise. In particular, this method had greater reliability, repeatability (predominantly for VL, RF and LG) and sensitivity to changes in exercise intensity.

More importantly this method involves the same dynamic movement as the trial being investigated (i.e. running). Whereas using the MVC method, the analysis compares EMG during 3 s of sustained activity (during the MVC) to 'on-off' bursts of muscle activity (running trial). This could create further variability in the data, since the characteristic of each burst or active period can vary concurrently with changes in stride length and frequency [78].

As discussed in Chapter 3, running requires a complex sequence and combination of muscle activation which includes an interplay of muscular forces and external forces (inertial, gravitational and reaction forces) [63]. By using a running method as an EMG reference, would better represent the muscle activity of trial being investigated.

The main finding of this study showed that muscle activity in all muscles during running at exhaustion was submaximal (Figure 6.2) (as compared to EMG activity during 20 m sprint). This finding is in agreement with results of Chapter 5. All six muscles measured in the study achieved an average maximal activity range of between 42 % - 66 % of that achieved during a 20 m maximal sprint. Indeed, these

findings suggest that peripheral skeletal muscles are never maximally recruited, even during maximal volitional effort during sustained exercise.

These findings are in agreement with other studies that have shown muscle activity to be less than maximal in running. Sloniger et al. [171] investigated muscle activity during horizontal and uphill running to exhaustion (at 115 % peak oxygen uptake). The authors used magnetic resonance imaging to measure muscle activity. The active muscle volume was divided by the post-exercise muscle volume to obtain the percentage of muscle volume that was active. The study found that lower leg muscles were not maximally activated for both running trials. The percentage of volume of muscle activated varied from 41 % - 90 % during horizontal and 44 % - 88 % during uphill running. It is important to note that this method of measurement has not been fully investigated for its repeatability in measuring muscle activity volumes.

In addition, the present study has found that the agonist and antagonist muscles are 'highly' activated (RF (55 % - 64 %) and BF (58 % - 59 %) respectively) to possibly assist in creating high joint stiffness, to prevent unnecessary yielding of the ankle, knee and hip joints [111]. The hip and knee extensors as well as the plantar flexors need higher muscle activity to endure the higher impact loads experienced with increasing running speed [111]. The high muscle activity of MG (60 % - 66 %) and LG (61% - 63 %), could be due to the contribution of increased preactivation in gastrocnemius activity with increasing running speed, which has been demonstrated by Komi et al. [107]. Kyröläinen et al. [111] interprets preactivity to be a preparatory requirement for the enhancement of EMG activity during the braking phase, and for the timing of muscular actions with respect to the ground contact with increased running speeds.

Furthermore, the present study has highlighted the variability of intra-subject muscle activity at exhaustion, where very few muscles showed CV values to be less than 12 %. This is an indication that muscle activity is never totally fixed at exhaustion. In addition the variation in each muscle over the different trials could be indicative of compensatory motor unit firing. However, this study has found lower intra-subject variability than in cycling (Chapter 5). This could be due to either the loss in captured data of certain subjects (due to EMG lead failure); or it could be due to the fact that muscle activity during running profile is seen to be 'programmed' within an

individual especially during faster running speeds [75]. A study by Guidetti et al. [75] investigated the intra and inter-subject variability of EMG patterns during running. They found little variation within individuals EMG profiles but differences between subjects were significant.

In conclusion, by using the most appropriate method of EMG normalisation, this study found that muscle activity was sub-maximal at exhaustion during incremental running. However, certain individuals have shown muscle activity to be greater than muscle activity during maximal sprint at exhaustion, but they were never the same maximal activity values over the three trials. Furthermore, the findings confirm that only a certain percentage of the limb muscle mass is active during maximal exercises, which is in agreement with the findings in Chapter 5. These findings are not compatible with the peripheral model of fatigue, as this model assumes that the total muscle mass is active at exhaustion.

CHAPTER 7

METHODS OF NORMALISATION OF EMG IN CLINICAL SETTINGS: CASE STUDIES OF MUSCLE ACTIVITY IN PATIENTS WITH PERIPHERAL VASCULAR DISEASE BEFORE AND AFTER ANGIOPLASTY

7.1 INTRODUCTION

In Chapters 2 and 3 of this thesis, the most appropriate method for normalisation of EMG activity during dynamic exercise was evaluated. Based on the findings of these studies, the application of EMG measurements to specific exercise conditions was examined in Chapters 5 and 6, in which EMG activity was measured during maximal volitional exercise.

A further application of EMG measurement in dynamic exercise is to assess muscle activity and effort tolerance in clinical populations. This method of evaluation is important to assist in the understanding of how interventions or exercise training might affect effort tolerance and functional capacity. One such condition is Peripheral Vascular Disease (PVD).

The predominant pathology in PVD is atherosclerotic occlusion of the blood vessels of the lower extremities. The occlusion reduces blood flow to the lower limb at rest and during exercise [192] and results in claudicant type pain (intermittent claudication). Typically this pain is induced by walking and is relieved by rest [57]. This pain limits walking activity and it is not uncommon for patients to walk only short distances at a time.

It was traditionally believed that the exercise intolerance experienced by patients with PVD was due to ischemia of the lower limbs resulting in accumulation of lactate in the skeletal muscles and blood [118; 177]. This theory is consistent with a peripheral model of fatigue. Yet findings from studies concluded in our laboratory, suggest that

patients with PVD terminate exercise at lower blood lactate concentrations compared to healthy age matched controls or patients with other chronic disease states [152].

The central regulation model of fatigue suggests that exercise in patients with PVD terminates before profound skeletal muscle ischemia develops, thus preventing possible damage to muscle from either a result of ischemia or metabolite accumulation/depletion [146]. Termination of exercise would therefore occur as the result of reduced motor drive to the lower limb (measured as a decrease in muscle activity). Thus, altered skeletal muscle activity might be responsible for the exercise intolerance experienced by these patients. Furthermore, when patients with PVD undergo angioplasty to remove the restriction of blood flow, the resultant improvement in exercise tolerance could be measured as an increase in surface EMG activity that might translate to an increased capacity to tolerate higher exercise workloads. Therefore a primary aim of the study was to investigate muscle activity during exercise in patients with PVD before and after angioplasty.

EMG methods of normalisation in clinical populations have been an issue of contention. The use of the standard method of normalisation, the MVC method, seems to be somewhat limited, as it often causes pain in patients during the maximal contraction. Studies have evaluated the repeatability of surface EMG in clinical population and rehabilitation of injuries [21; 33; 34; 114; 142]. The researchers concluded that even though the MVC method had higher repeatability the application of this method in the symptomatic population might not be accurate, as these patients might not elicit a true MVC due to pain and/or muscle inhibition.

Using the MVC method of normalisation in studies of gait profiles has also been questioned [44; 198], as muscle activity from an MVC (isometric contraction) is used to normalise muscle activity during gait cycles (which are non-isometric contractions). Yet, it is of interest that the MVC method of normalisation of the EMG signal continues to be advocated for use in clinical practice [172].

An alternative method for normalisation of the EMG signal in the clinical population is the Mean Dynamic method. This method was developed to use EMG as a diagnostic tool in gait analysis [198]. The limitation of the Mean Dynamic method is

that it serves only as a measurement of muscle activity throughout the gait cycle in relation to the average EMG recorded during gait, thus not providing information on the degree of muscle activation, as would a maximal contraction [25].

Therefore in order to analyse the EMG activity in the clinical population group accurately, the appropriate methods of normalisation need to be applied. Due to the findings of the previous chapters and the above mentioned research, both the MVC and Mean Dynamic methods of normalisation were selected. Thus, a further aim of this chapter is to apply an appropriate method of normalisation to measure and evaluate muscle activity in patients with PVD. This chapter describes the detailed findings in a case series of patients with PVD and functional incapacity, who underwent angioplasty.

7.2 METHODS

7.2.1 Patient recruitment

Patients with Peripheral Vascular Disease were recruited from the Department of Vascular Surgery at Kingsbury Hospital (Cape Town, South Africa) to participate in this pilot study. To protect the identities of these patients they are referred to by initials only.

In order to be included into the trial, patients with PVD and intermittent claudication had to show significant evidence for their disease as documented by the vascular surgeon using Color Flow Doppler and angiography. Patients were not recruited if they had exclusion criteria including; absence of claudication; pain at rest or tissue loss, dyspnea, exercise limiting arthritis, active cancer, liver disease or renal disease. Patients were also excluded based on the contraindications specified by the ACSM guidelines [12].

Two patients, Case number 1 (AA) a habitual walker and Case number 2 (AB) a Western Province League squash player were diagnosed with peripheral vascular disease and were scheduled to have percutaneous transluminal angioplasty (PTA).

The patients agreed to be examined and performed the exercise tests three days before and again three days after the angioplasty procedure.

On the first visit to the laboratory, each patient was examined by a sports physician at the clinical practice of the Sports Science Institute of South Africa before they performed the required exercise trials. The physician recorded each subject's medical history and examined the patient to ensure that there were no contraindications to exercise. Thereafter the patients signed consent to participate in the study. The study was approved by the Research and Ethics Committee of the Faculty of Health Sciences within the University of Cape Town. The study was performed in accordance with the principles of the Declaration of Helsinki (October, 2000), ICH Good Clinical Practice (GCP) and the laws of South Africa. The methods used to assess body composition are described in Chapter 2 (Section 2.2.3).

7.2.2 Experimental design

After the evaluation by the sports physician, each patient was familiarised with the equipment. Patients first performed MVC's on the Biodex Dynamometer and then were familiarised with walking on the treadmill (methods described in Section 7.2.4 and 7.2.5 respectively). Patients were also familiarised with the ratings of perceived exertion and pain scores as described in Section 7.2.7 and 7.2.8.

On the second visit to the laboratory (Pre-Angioplasty trial), subjects were prepared for the placement of the electrodes on four muscles of both the right and left legs namely, Vastus medialis (VM), Vastus lateralis (VL), Medial gastrocnemius (MG) and Lateral gastgrocnemius (LG). Subjects then performed maximal voluntary contractions on the Biodex Dynamometer. Following this, subjects were instructed to walk on the treadmill until exhaustion using the Graded Treadmill Exercise Test (GTET) protocol as explained in Section 7.2.6. Exhaustion was defined as the moment the patients could not walk any further due to pain or fatigue. Heart rate and brachial blood pressure were monitored every two minutes during exercise. Patients were asked to report their perceived pain using perception of pain scale and rate their perceived exertion, by indicating a value on the Borg scale. During exercise, total inspiratory ventilation, oxygen uptake and respiratory exchange ratio were

measured using the Oxycon Alpha Analyzer (Oxycon, Viasis, Hoechberg, Germany).

Prior to the initiation of exercise, a resting venous blood sample (two millilitres) was drawn from a subcutaneous forearm vein in patient AB. An additional two millilitre blood sample was collected at three, four and five minutes after exercise. The blood was then analysed to determine blood lactate concentrations.

Three days after the Pre-Angioplasty trial, patient AA underwent angioplasty of the popliteal artery of the left leg and patient AB underwent angioplasty of the femoral artery of the right leg. Before and after angioplasty the same radiographer measured the blood flow velocity across the lesion using a duplex Doppler, to measure change in blood velocity. Following a three day recovery period from the angioplasty, subjects returned to the laboratory where they repeated the exercise trial (Post-Angioplasty trial).

7.2.3 Ankle and brachial pressure measurements

After subjects remained supine in a quiet environment for a period of fifteen minutes the resting brachial blood pressure (Korotoff Phase I and IV) was measured and recorded by means of audible sphygmomanometry using a calibrated mercury column sphygmomanometer with an appropriately sized cuff. Left and right brachial pressures were measured with a Doppler (©Huntleigh Technology PLC 1997 Dopplex ® Advanced Pocket Dopplers United Kingdom) and blood pressure cuffs were affixed just above the ankles. The Doppler probe was placed over the posterior tibial or dorsalis pedis artery. The cuff was then inflated until the sound disappeared; the cuff was then slowly deflated until the sound returned. The pressure at which the sound returned was recorded as the ankle systolic pressure. Ankle brachial index (ABI) was calculated for each patient by dividing the ankle pressure reading by the brachial pressure reading. An additional brachial and ankle systolic blood pressure was taken immediately post-exercise.

7.2.4 Maximal voluntary contractions

The same procedure was used as described in the previous chapters. Only two MVC attempts of the quadriceps and gastrocnemius muscles of both the diseased and healthy legs were performed prior to the exercise trials.

7.2.5 Graded treadmill exercise test (GTET) to maximal claudication

On the first visit to the laboratory, subjects were familiarised with the treadmill. During this visit, they were given the opportunity to walk on the treadmill for three bouts of two minutes at a comfortable speed.

On the second visit to the laboratory, patients performed the GTET. During the GTET protocol the treadmill speed was initially set at 3.2 $km.h^{-1}$ with a 2 % gradient [156]. The gradient was then increased by 2 % every two minutes. Pain free walking distance was recorded from the start of the test until the moment when the patient first noticed calf pain. Maximum walking distance was defined as the distance covered on the treadmill before the patient chose to discontinue exercise because of severe claudication pain.

7.2.6 Heart rate and ECG

Heart rate during the GTET was recorded using an electrocardiogram monitor (Cardio Perfect® 3.3, Rijswijk, Netherlands) with self-adhering electrodes placed on the patient in a modified 10-electrode (Maser-Likar) configuration [1].

7.2.7 Ratings of perceived exertion scale (RPE scale)

Ratings of subjective perceived exertion were quantified using the Borg 6-20 Scale [22]. During their first laboratory visit patients were provided with printed instructions to familiarise themselves with the scale prior to testing. Instructions were given for the RPE scale as suggested by Pandolf [150]. The Borg scale was used to quantify overall exertion experienced during the trials. Once instructions were given and the patients were familiarised with the scale, no further assistance was provided by the

investigator in translating sensations to the numerical ratings on the RPE scales during the GTET.

7.2.8 Pain scores

The pain scale ranges from 0 to 10, where 0 is no pain felt and 10 is the most pain ever felt [112]. Subjects were familiarised with this scale on their first laboratory visit and once again before the testing trials.

7.2.9 Blood sample collection and analysis

Prior to the start of the GTET, a resting venous blood sample was drawn from the subcutaneous forearm vein of patient AB. Two millilitres of blood was drawn directly into a sodium oxalate lined glass test tube and immediately placed on ice for later analysis of plasma lactate concentrations. Additional two millilitres blood samples drawn at minute 3, 4 and 5 post-exercise. The blood samples were then centrifuged using a Sigam 302-K centrifuge (Munich, Germany) at 3000 rpm for 10 minutes at 4°C. Blood lactate concentrations was measured in plasma by means of an enzymatic kit technique (bioMerieux SA, lactate PAP 69280, Marcy I Etoille, France).

7.2.10 EMG data collection

The identical procedure for EMG data collection was used as in all previous chapters. However, in this trial only muscle activity of Vastus medialis (VM), Vastus lateralis (VL), Medial and Lateral gastrocnemius (MG and LG respectively) from both the symptomatic (Disease) and asymptomatic (Healthy) leg of each patient was examined.

7.2.11 EMG analysis

EMG data were analysed as described in Chapter 2 Section 2.2.7. EMG was recorded for 10 s at the end of each minute. Three strides with similar peak amplitudes in each 10 s EMG recording were averaged and the resultant mean amplitudes were normalised.

7.2.12 Methods of normalisation

Due to the nature of the clinical patient population being investigated, it is important to carefully choose the appropriate testing parameters for these patients due to risk factors of possible illness or injury as outlined in the ACSM guidelines [12]. For example, in the previous chapters subjects were able to perform maximal static and dynamic activities. However, using a maximal dynamic protocol (in this instance a sprint on the treadmill) would be an inappropriate method for this clinical population. In addition, due to the stringent exclusion criteria in this study, insufficient numbers of patients were recruited to test the repeatability of methods of normalisation. Therefore, the most appropriate methods of normalisation are based on previously published literature.

7.2.12.1 Mean Dynamic Method of normalisation

EMG was normalised as described by Burden et al. [24] and Yang & Winter [198]. The method analyses each stride of the gait cycle to the mean value recorded during that gait cycle. However, this method has been shown to be repeatable on level ground only whereas the protocol for this study measured EMG activity on an increasing gradient. Averaging the EMG amplitude of the entire trial was therefore not possible due to the increasing gradient. Therefore, a 10 s recording of walking on the treadmill at 0 degree gradient (level surface) at a speed of 3.2 $km.h^{-1}$ was measured. The mean amplitude from this recording (as described in Chapter 3 Section 3.2.8.3) was then used as the normalisation value for muscle activity at each increase in gradient. This method will only provide information about the level muscle activation throughout the GTET in relation to the average activity recorded whilst walking at 0 % gradient at speed of 3.2 $km.h^{-1}$. Nevertheless, this method allows detection of a change in muscle activity when comparing EMG measurements taken before and after angioplasty.

7.2.12.2 MVC Method of normalisation

This method of normalisation is described in Chapter 3 section 3.2.8.1.

Table 7.1 tabulates the advantages and disadvantages of both methods of normalisation which were derived from the findings in Chapter 3 and the published literature.

Table 7.1: Advantages and disadvantages in using MVC and Mean Dynamic Method for normalisation during gait

Normalisation Method	Advantages	Disadvantages
MVC	• Provides a degree of muscle activation in relation to maximal static activation capacity. • Still debatable if best to use in gait studies, however still recommended by Soderberg & Knutson [172].	• Maximal effort required, in patient population this could cause pain and discomfort. • Static movement is not representative of muscle activity during walking (does not account for electrode shift and tissue conductivity). • Shown not to be repeatable and shown to have large variability when used to normalised gait studies [44; 198]. • Self limiting performance, unable to control their performance output.
Mean Dynamic	• High reliability–Intra subject variability is low [25; 198]. • High repeatability • Dynamic movement, thus better representation of muscle activity. • Walking pace is controlled for by walking at 0 % gradient at 3.2 $km.h^{-1}$.	• Only provides us with muscle activation in relation to a submaximal walk

Both methods of normalisation were chosen to determine which method was more sensitive to change in workload before and after the intervention.

7.3 CASE STUDIES

7.3.1 Case 1

7.3.1.1 Case history

AA, a 65 year old male who participated in daily recreational walking, walking an average of 6 - 8 km per day, reported to the vascular surgeons with increasing claudicant type discomfort of the lower leg. AA achieved smoking cessation in 1978. He was diagnosed with coronary artery disease and underwent subsequent CABG in 1992. Thereafter, he developed occlusion in a vein graft to the circumflex artery which was successfully stented. He had an abnormal ECG at rest and on effort; however this pattern has not changed over the past 14 years and he did not have active angina pectoris. His risk factors included hypertension and elevated blood cholesterol concentration. His medication included Atorvastatin (10 $mg.day^{-1}$), Moducren (10 $mg.day^{-1}$) and Ecotrin (125 $mg.day^{-1}$). AA had prior history of injury to the Achilles tendon of his right leg.

Color flow Doppler and subsequent angiography revealed Peripheral Vascular Disease with a 70 % stenosis in the popliteal artery above the level of the knee of his left leg. Due to the fact that this was a single localised lesion, vascular surgeons advised management of the occlusion with balloon angioplasty.

Blood velocity at the area of stenosis was measured by flow duplex Doppler evaluation at 206 $cm.s^{-1}$ before angioplasty and reduced to 97 $cm.s^{-1}$ after the procedure. Figure 7.1 demonstrates the area of stenosis in the lower leg.

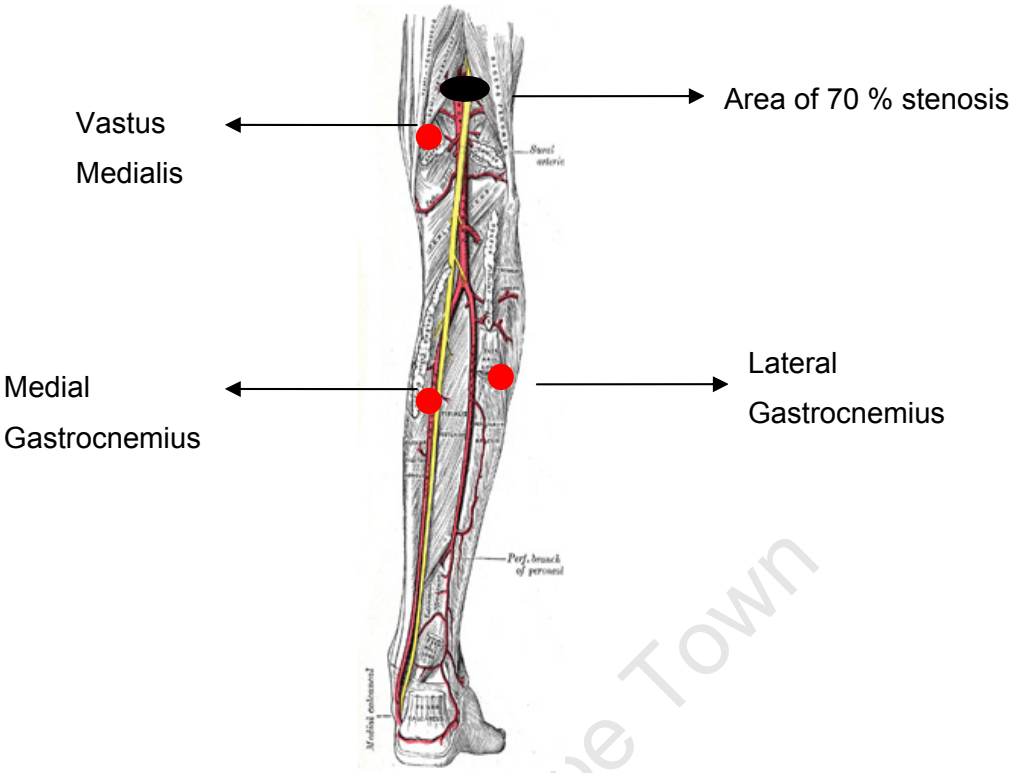


***Figure 7.1*: Lower limb indicating area of stenosis, arteries of the lower leg and affected muscles** *(modified from the public domain: http://www.bartleby.com/107/151.html).*

7.3.1.2 Clinical evaluation and special investigations

AA weighed 82 kg, was 1.76 m tall, and had a body mass index (BMI) of 26.5. Table 7.2 shows measurements of ABI at rest and after exercise before and after angioplasty. Prior to angioplasty, ABI at rest was measured as 0.89 and reduced after exercise to 0.67. However ABI after angioplasty was 1.07 at rest and was 0.99 after exercise.

Table 7.2: Ankle Brachial Index measurements Pre and Post Angioplasty

Trial	ABI at rest	ABI after exercise
Pre-A	0.89	0.67
Post-A	1.07	0.99

Ratio of ankle pressure to brachial pressure in Pre-Angioplasty (Pre – A) and Post-Angioplasty (Post – A) trials.

AA developed pain in his left leg (Diseased leg) in the Pre - A trial at 4 min 23 s resulting in a pain free walking distance of 230 m. AA walked a total distance of 467 m at a final gradient of 8 % at minute 08:30 as the trial was discontinued by the supervising physician due to ST-segment depression of more than 2 mm was noticed on the ECG.

Following angioplasty, AA's pain free walking distance increased to 384 m. However, in the Post - A trial, AA once again experienced significant ST-segment changes and the test was terminated by the attending physician at minute 08:30.

7.3.1.3 Measurement of physiological variables during exercise

Both oxygen consumption (Figure 7.2 a) and heart rate (Figure 7.2 b) were lower throughout the exercise trial after angioplasty.

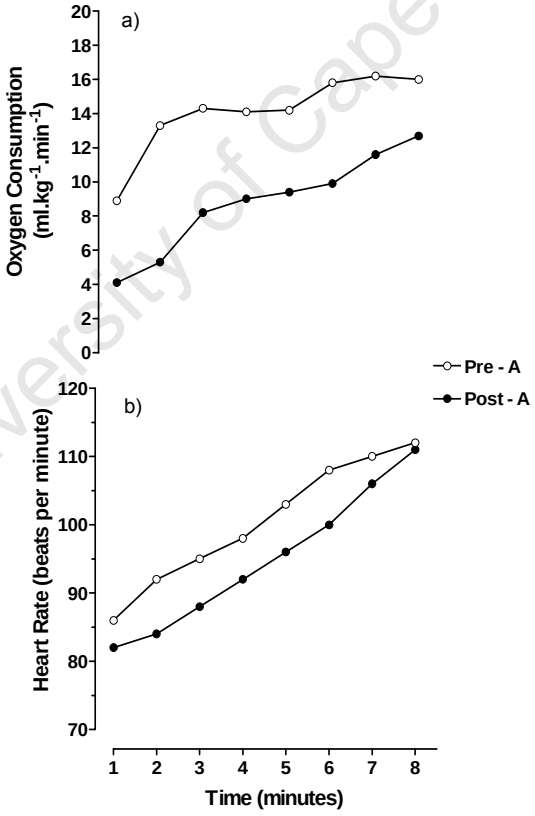


Figure 7.2: Changes in (a) Oxygen consumption and (b) Heart rate measured in Pre-Angioplasty (Pre – A) and Post Angioplasty (Post – A) trials.

Blood lactate concentration was not measured because the patient reported history of a profound vasovagal response to needles, and declined consent for this part of the clinical trial.

Figure 7.3 shows the changes in pain and RPE scores during exercise, before and after angioplasty. Prior to angioplasty, pain was reported at minute 4 during exercise but only presented at minute 7 of exercise after angioplasty. The peak pain score was also lower following angioplasty (Figure 7.3 a). Furthermore the RPE scale indicated that AA's perception of effort also decreased from minute 4 after angioplasty compared to the Pre- A trial.

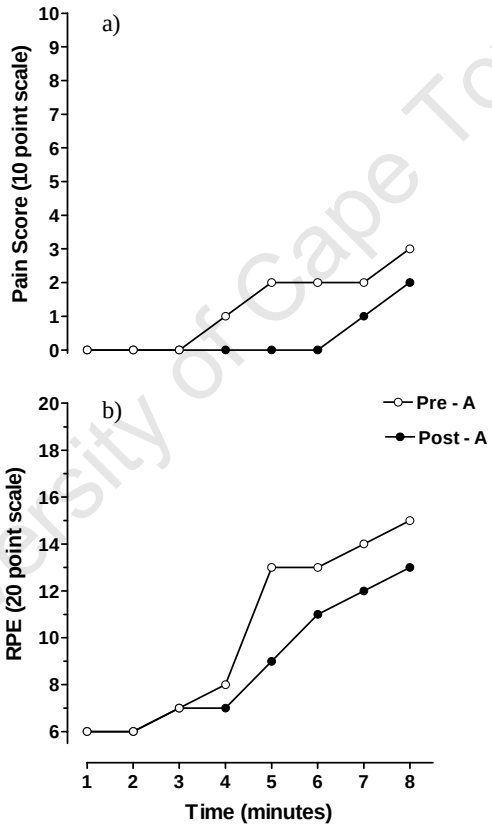


Figure 7.3: Changes in (a) Pain Scores and (b) RPE in Pre-Angioplasty (Pre – A) and Post Angioplasty (Post – A) trials.

7.3.1.4 Force output from MVC

Table 7.3 shows the changes in maximal and average force output during the MVC of the quadriceps muscles before and after angioplasty. Peak force output of the diseased leg increased by 19 Nm after angioplasty. Peak force also increased in his healthy right leg after angioplasty by 18 Nm. Furthermore, average force was also increased after angioplasty in both the disease and healthy leg.

Table 7.3: Force output measurements during maximal voluntary contractions of the quadriceps muscles of the right (Healthy) and left (Diseased) leg in the Pre and Post Angioplasty trial.

Trial	Right Leg (Healthy) Force output (Nm)		Left Leg (Diseased) Force output (Nm)	
	Peak	Average	Peak	Average
Pre - A	213	201	185	179
Post - A	225	221	204	191

Healthy and Diseased leg force output in the Pre-Angioplasty (Pre-A) and Post-Angioplasty (Post-A) trials. Average force of 2 maximal contractions. Force output in Newton meters (Nm).

Table 7.4 shows the changes in maximal and average force output during the MVC of the calf muscles before and after angioplasty. The calf muscles of the diseased leg produced a greater peak force after angioplasty (with an increase of 20 Nm) and the average force produced by the calf muscles over 2 MVC's increased by 60 Nm. Interestingly, the force output increased slightly in the healthy leg after angioplasty.

Table 7.4: Force output measurements from maximal voluntary contractions of the calf muscle of the right (Healthy) and left (Diseased) leg, Pre and Post Angioplasty trial.

Trial	Right Leg (Healthy) Force output (Nm)		Left Leg (Diseased) Force output (Nm)	
	Peak	Average	Peak	Average
Pre - A	89	85	132	87
Post - A	99	96	152	147

Healthy and Diseased leg force output in the Pre-Angioplasty (Pre-A) and Post-Angioplasty (Post-A) trials. Average force of 2 maximal contractions. Average force resultant of 2 maximal contractions. Force output in Newton meters (Nm).

7.3.1.5 Methods of normalisation to measure muscle activity pre and post - angioplasty

EMG activity of the leg muscles before and after angioplasty using the two methods of normalisation are shown in Figures 7.4 – 7.7.

Figure 7.4 shows the differences in muscle activity between the healthy and diseased leg before angioplasty, using the MVC and Mean Dynamic methods of normalisation. Muscle activity normalised using the MVC method displayed similar trends where muscle activity of all 4 muscles of the healthy leg was greater than in the diseased leg (Figure 7.4 a, c, e and g). This pattern was similar for VM and LG when EMG was normalised using the Mean Dynamic method. However VL and MG activity normalised to the Mean Dynamic method in the healthy leg was not considerably greater than in the diseased leg.

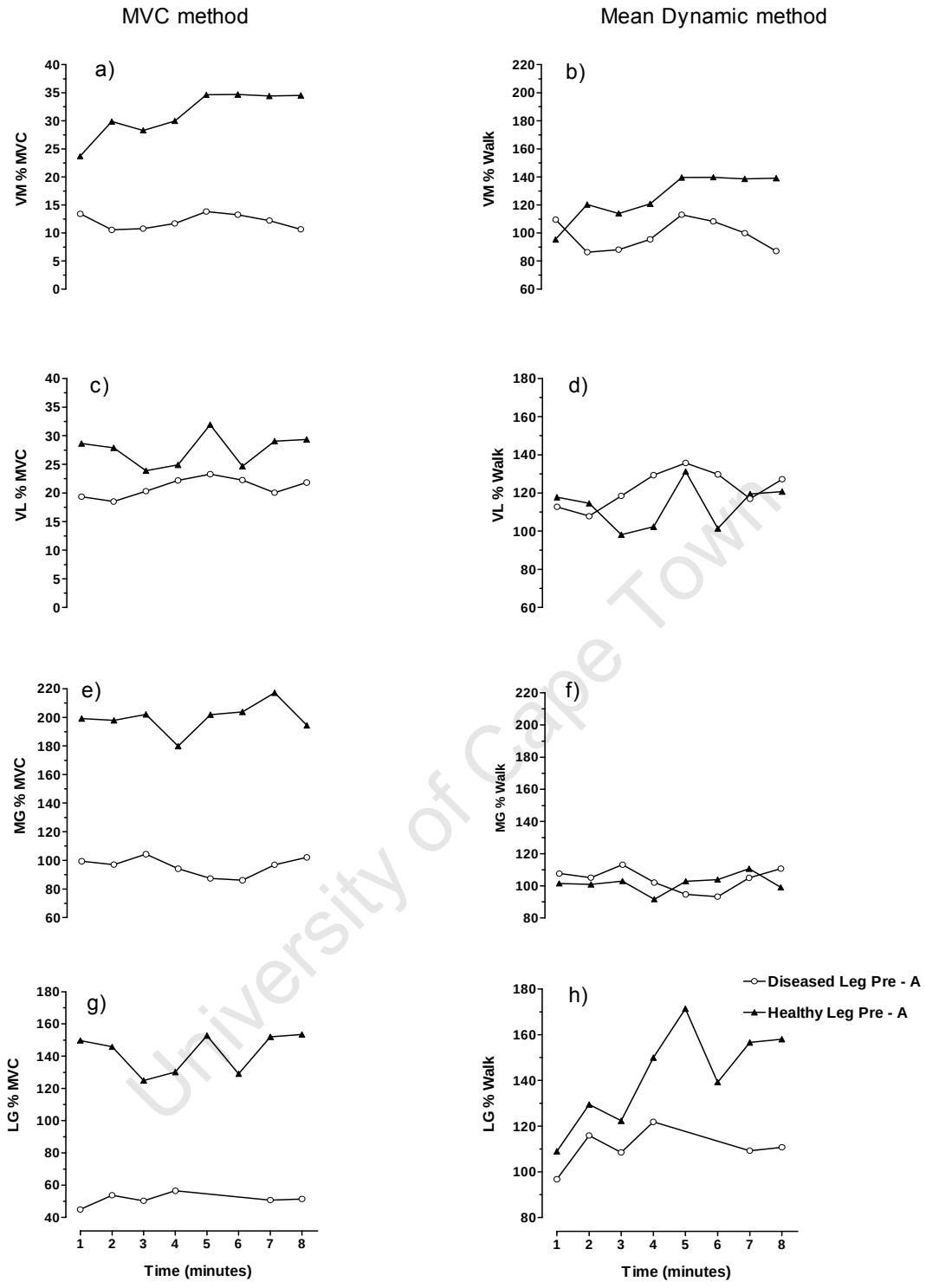


Figure 7.4: Muscle activity normalised to the MVC Method (left column) and the Mean Dynamic Method (right column) of the Healthy and Diseased leg during the Pre-Angioplasty trial (Pre- A) ***(a) & (b)*** *Vastus medialis (VM);* ***(c) & (d)*** *Vastus lateralis (VL)* ***(e) & (f)*** *Medial gastrocnemius and* ***(g) & (h)*** *Lateral gastrocnemius*

Figure 7.5 displays EMG activity during exercise, normalised to both methods, in the diseased leg before and after angioplasty. The quadriceps muscle activity displays different muscle activity patterns when normalised using the two methods. VM and VL activity normalised using the MVC method (Figures 7.5 a and c), shows no difference in activity levels in the diseased leg before and after angioplasty. Whereas, EMG activity normalised using the Mean Dynamic method (Figures 7.5 b and d) showed muscle activity to be greater after angioplasty. The Mean Dynamic method also appears more sensitive in showing increasing muscle activity in the diseased leg with increasing exercise intensity over time for the quadriceps muscles, unlike the MVC method. MG activity normalised using the MVC method, showed a decrease in the diseased leg activity after angioplasty (Figure 7.5 e), whereas when normalised using the Mean Dynamic method, MG activity was similar in both trials, but demonstrated a sensitivity to increasing workload after angioplasty (Figure 7.5 f). LG activity was the same when normalised to both methods, showing greater muscle activity after angioplasty (Figures g and h).

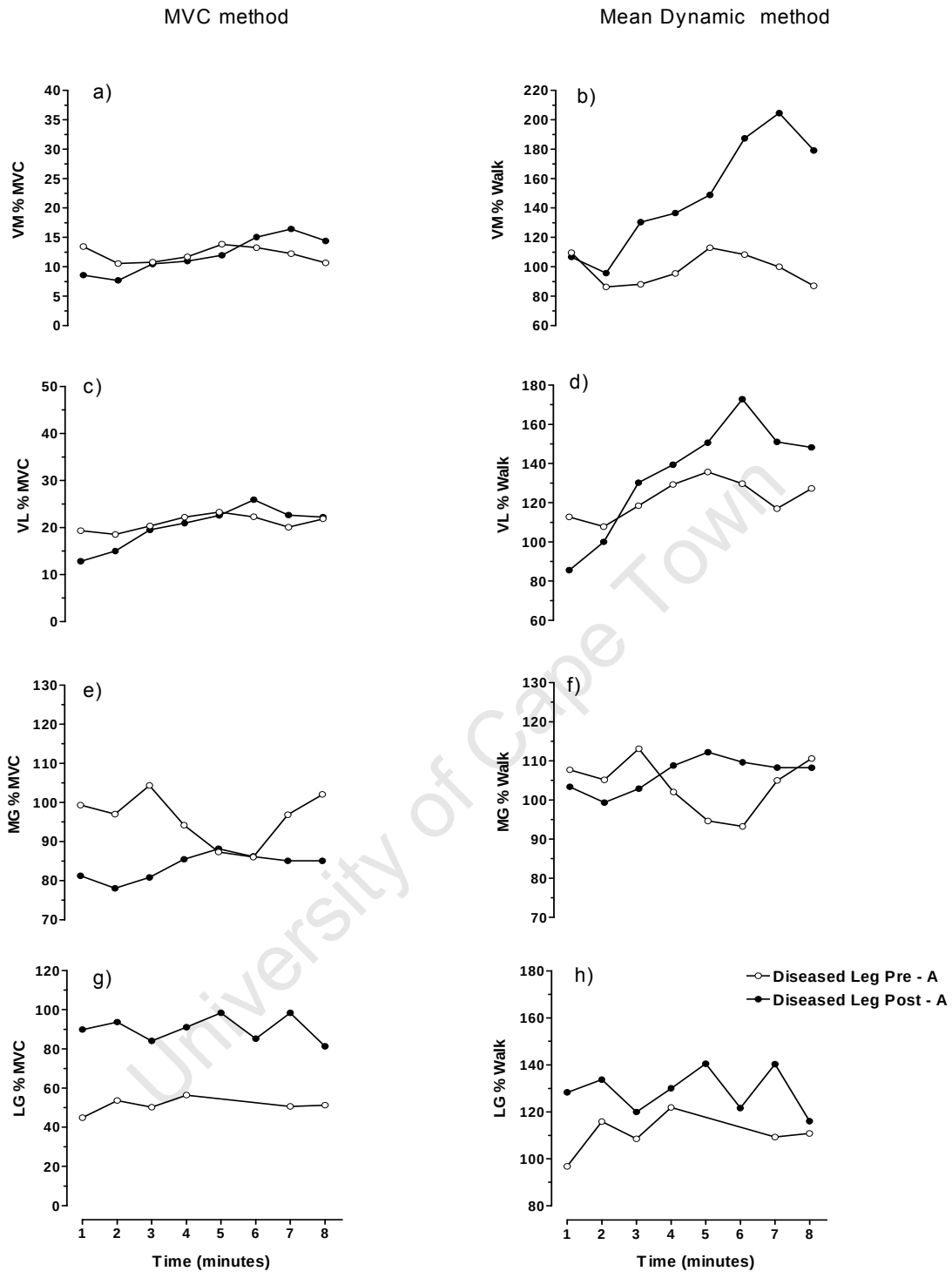


Figure 7.5: Muscle activity normalised to the MVC Method (left column) and the Mean Dynamic Method (right column) of the Diseased leg during the Pre-Angioplasty (Pre- A) and Post-Angioplasty (Post-A) trial, *(a) & (b)* Vastus medialis (VM); *(c) & (d)* Vastus lateralis (VL) *(e)& (f)* Medial gastrocnemius and *(g) & (h)* Lateral gastrocnemius

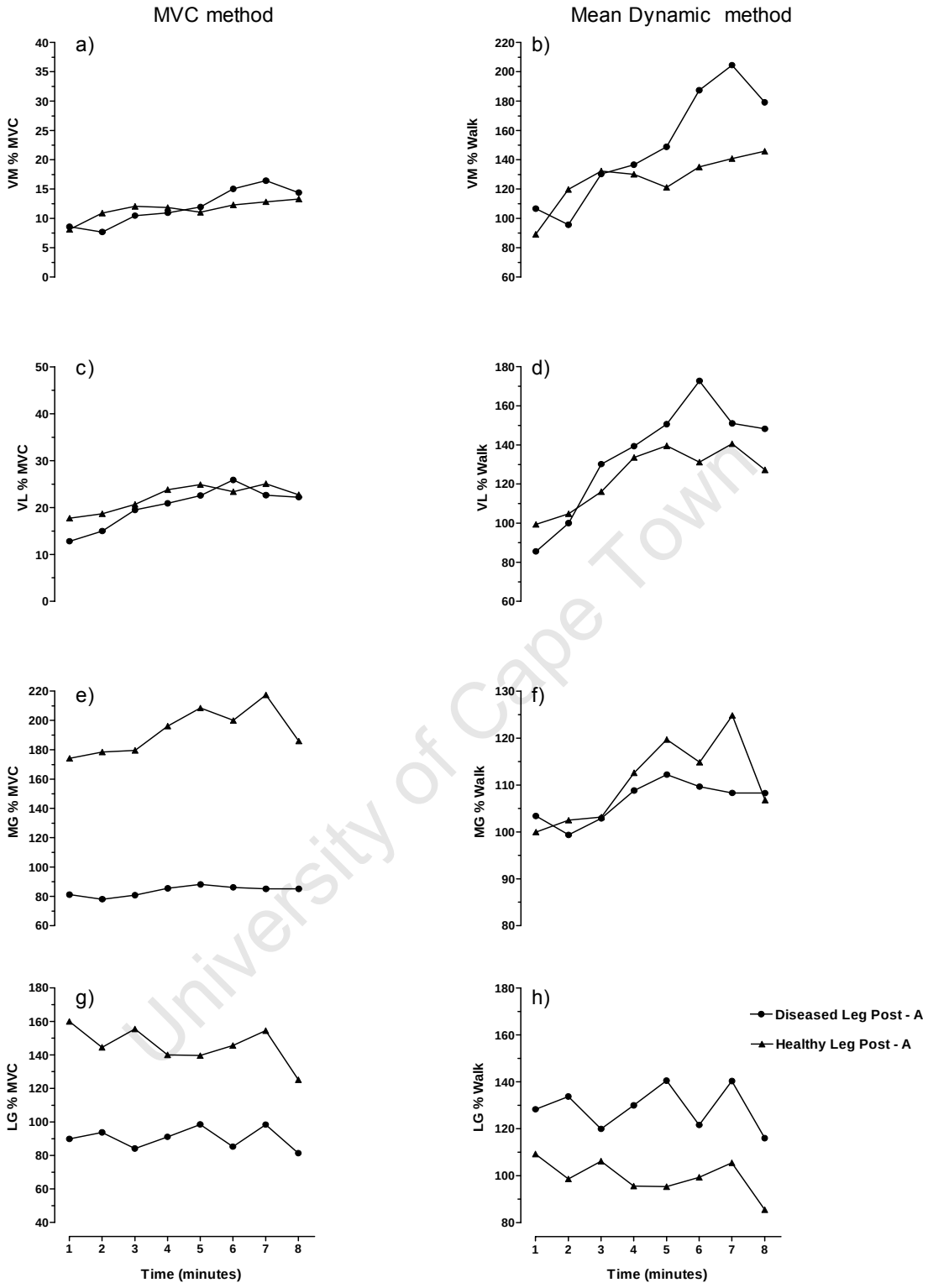


Figure 7.6 Muscle activity normalised to the MVC Method (left column) and the Mean Dynamic Method (right column) of the Healthy and Diseased leg during the Post-Angioplasty trial (Post - A), ***(a) & (b)*** *Vastus medialis (VM);* ***(c) & (d)*** *Vastus lateralis (VL)* ***(e)& (f)*** *Medial gastrocnemius and* ***(g) & (h)*** *Lateral gastrocnemius.*

Muscle activity of the healthy and diseased leg after angioplasty, normalised using both methods during exercise, is shown in Figure 7.6. The quadriceps activity in the healthy and diseased leg was similar after angioplasty, when normalised using the MVC method. However, normalised using the Mean Dynamic method, quadriceps activity in the diseased leg increased to levels greater than the healthy leg activity after angioplasty (Figure 7.6 b and d). Furthermore, the quadriceps activity was sensitive to increasing exercise intensity, which was not found by normalising using the MVC method.

Normalising gastrocnemius muscle activity using the MVC method, demonstrated a notably greater muscle activity in the healthy leg than the diseased leg after angioplasty (Figure 7.6 e and g). MG activity normalised using the Mean dynamic method also showed a greater activity level in the healthy leg. However, when normalising LG activity using the Mean Dynamic method, the activity in the diseased leg was greater than in the healthy leg after angioplasty (Figure 7.6 h).

7.3.2 Case 2

7.3.2.1 Case history

AB, a 58 year old male elite squash player, presented to his doctor with leg pain during vigorous exercise. This discomfort increased to an extent where participation in squash was no longer possible, walking however was unaffected. The discomfort started after approximately 30 minutes of squash and would disappear at rest. His risk factors included hypertension, hypercholesterolaemia and cigarette smoking (20 per day). Medications at the time of testing included Enalapril (10 $mg.day^{-1}$) and Atorvastatin (10 $mg.day^{-1}$). AB had no prior history of injury to the limb or lower back.

He was referred to Vascular Surgeons at Kingsbury Hospital where he was diagnosed with Peripheral Vascular Disease. Color flow Doppler evaluation and subsequent angiography revealed PVD with a greater than 80 % stenosis in the proximal femoral artery of his right leg. Due to the fact that this was a single

localised lesion, the vascular surgeons advised management of the occlusion by balloon angioplasty.

Blood velocity at the area of stenosis was measured by flow duplex Doppler evaluation at 328 $cm.s^{-1}$ before angioplasty and reduced to 159 $cm.s^{-1}$ after the procedure. Figure 7.7 demonstrates the area of stenosis in AB's proximal femoral artery.

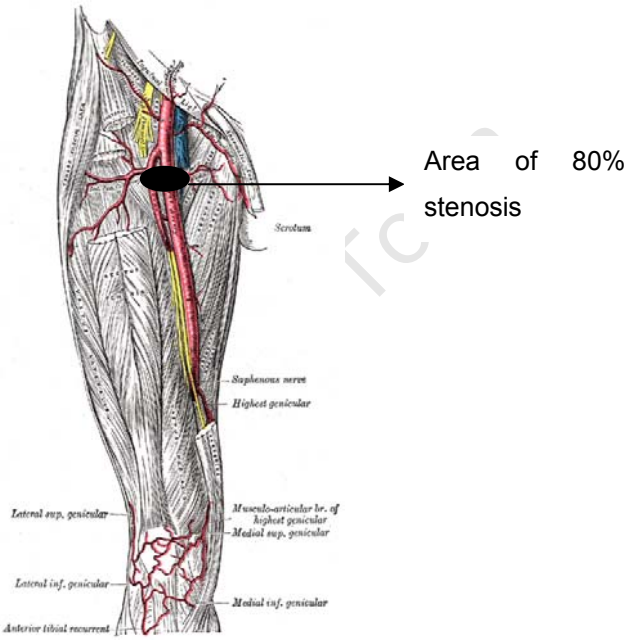


Figure 7.7: Lower limb indicating area of stenosis and arteries of the lower leg. *(modified from the public domain: http://www.bartleby.com/107/157.html)*

7.3.2.2 Clinical evaluation and special investigations

AB weighed 89.3 kg, was 1.78 m tall, and had a BMI of 28. Table 7.5 shows ABI measurements at rest and after exercise in both before and after angioplasty trials. Prior to angioplasty, ABI at rest was measured as 0.80 and increased after exercise to 0.87. However ABI after angioplasty was 1.09 at rest and decreased to 0.98 after exercise.

Table 7.5: Ankle Brachial Index measurements Pre and Post Angioplasty

Trial	ABI at rest	ABI post exercise
Pre - A	0.80	0.87
Post - A	1.09	0.98

Ratio of ankle pressure to brachial pressure in Pre-Angioplasty (Pre – A) and Post-Angioplasty (Post – A) trials.

In the Pre - A Trial, AB was able to walk up to the maximal treadmill gradient of 15 %, which was reached after 16 minutes of walking. At 15 minutes into the trial, he developed pain in his right leg (Diseased leg), equivalent to 849 m. In the Post - A Trial, AB experienced no pain in his diseased leg throughout the trial.

7.3.2.3 Measurement of physiological variables during exercise

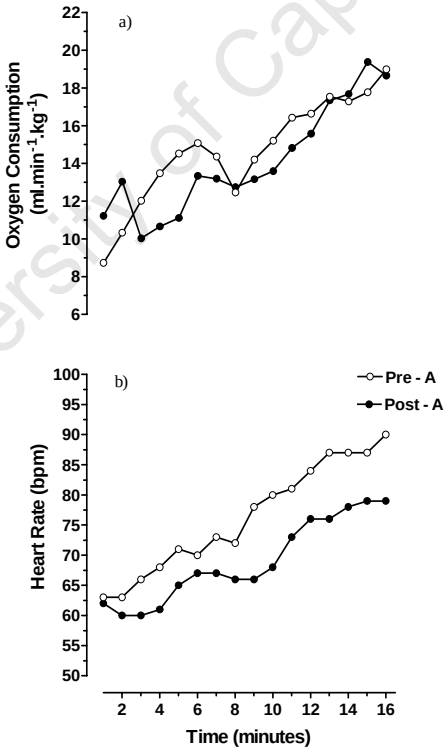


Figure 7.8: Changes in (a) Oxygen consumption and (b) Heart rate measured in Pre-Angioplasty (Pre – A) and Post Angioplasty (Post – A) trials.

Oxygen consumption was similar during exercise in both the pre and post - angioplasty trials (Figure 7.8.a). Heart rate started at similar values in both trials but

increased more rapidly in the Pre - A trial. Heart rate during exercise was substantially lower after angioplasty. For example heart rate was 90 bpm at minute 15 of exercise trial prior to angioplasty but reached 79 bpm at the same time after angioplasty.

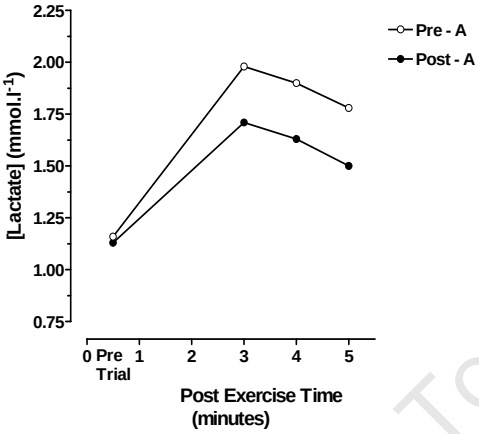


Figure 7.9: Changes in blood lactate concentration in Pre-Angioplasty (Pre – A) and Post Angioplasty (Post – A) trials.

Figure 7.9 shows the changes in blood lactate concentrations before and after angioplasty. Peak blood lactate concentrations were similar before the start of both trials. However, 3 minutes after the trials, peak blood lactate concentration was lower in the Post-A trial, and remained lower at the 4th and 5th minute post exercise. Blood lactate concentrations from the 3rd to 5th minute post exercise ranged between 2.00 - 1.75 mmol.l^{-1} in the Pre - A trial and decreased to the range of 1.75 - 1.50 mmol.l^{-1} in the Post - A trial. The pain and RPE score are shown in Figure 7.10.

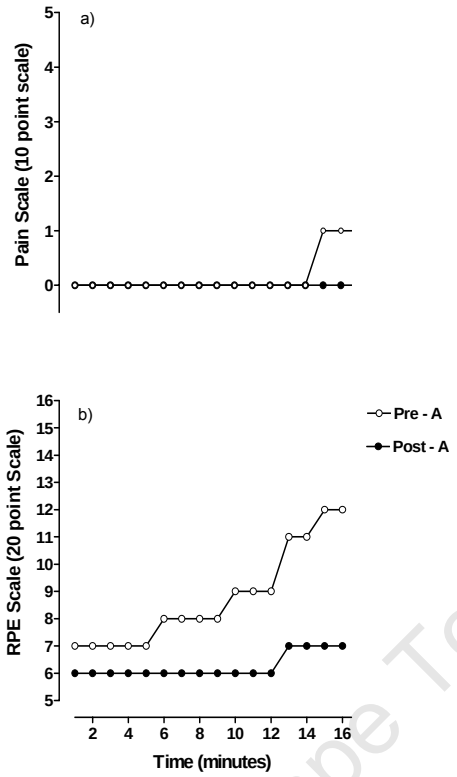


Figure 7.10: Changes in (a) Pain Scores and (b) RPE in Pre-Angioplasty (Pre –A) and Post Angioplasty (Post – A) trials.

Despite 80 % occlusion of the femoral artery, AB displayed few symptoms related to exercise intolerance during the GTET test (Pre - A). AB only experienced pain in his diseased leg at the 15th minute of the Pre - A trial whilst no pain was felt at any time during the Post - A trial. RPE was seen to increase in stages in the Pre - A trial and reached a maximum rating of 12. In the Post - A trial, AB's rating of perceived exertion remained at 6 until the 12th minute of exercise after which the rating only increased to 7 for the remainder of trial.

7.3.2.4 Force output from MVC

Table 7.6 shows the changes in peak and average force output in the diseased and healthy leg before and after angioplasty. Peak force increased by 24 Nm after angioplasty in the diseased leg. Interestingly, the peak force decreased by 20 Nm in his healthy leg after angioplasty.

Table 7.6: Force output measurements during maximal voluntary contractions of the quadriceps muscles of the right and left leg, Pre and Post-angioplasty.

Trial	Right Leg (Diseased) Force output (Nm)		Left Leg (Healthy) Force output (Nm)	
	Peak	Average	Peak	Average
Pre - A	142	142	262	251
Post - A	166	159	242	220

Healthy and Diseased leg force output in the Pre-Angioplasty (Pre-A) and Post-Angioplasty (Post-A) trials. Average force of 2 maximal contractions. Force output in Newton meters (Nm)

Table 7.7 shows the changes in peak and average force output of the calf muscles. The diseased leg produced a greater peak force in the Post-A trial (increased by 16 Nm). The average force of his calf muscles (2 MVC's), increased by 19 Nm in the diseased leg. There was a slight decrease in force production in the healthy leg after angioplasty.

Table 7.7: Force output measurements during maximal voluntary contractions of the calf muscle of the right and left leg, Pre and Post angioplasty.

Trial	Right Leg (Diseased) Force output (Nm)		Left Leg (Healthy) Force output (Nm)	
	Peak	Average	Peak	Average
Pre - A	86	83	121	118
Post - A	102	102	117	117

Healthy and Diseased leg force output in the Pre-Angioplasty (Pre-A) and Post-Angioplasty (Post-A) trials. Average force of 2 maximal contractions. Force output in Newton meters (Nm)

7.3.2.5 Methods of normalisation to measure muscle activity pre and post angioplasty

EMG activity from the VM was not measured in the Pre - A trial due to technical problems with the EMG leads. Thus only results of VL, MG and LG are displayed in the following graphs.

The differences in EMG activity between the healthy and diseased leg before angioplasty are shown in Figure 7.11. VL, MG and LG normalised using the MVC method (Figure 7.11 a, c and e) of the diseased leg had greater muscle activity than the healthy leg prior to angioplasty, MG and LG specifically demonstrates a considerable difference between healthy and diseased leg. However, when normalising VL and MG using the Mean Dynamic method, the VL and MG activity in the healthy leg was slightly greater than in the diseased leg. Furthermore, LG activity in the healthy was considerably greater than the diseased leg (Figure 7.11 f).

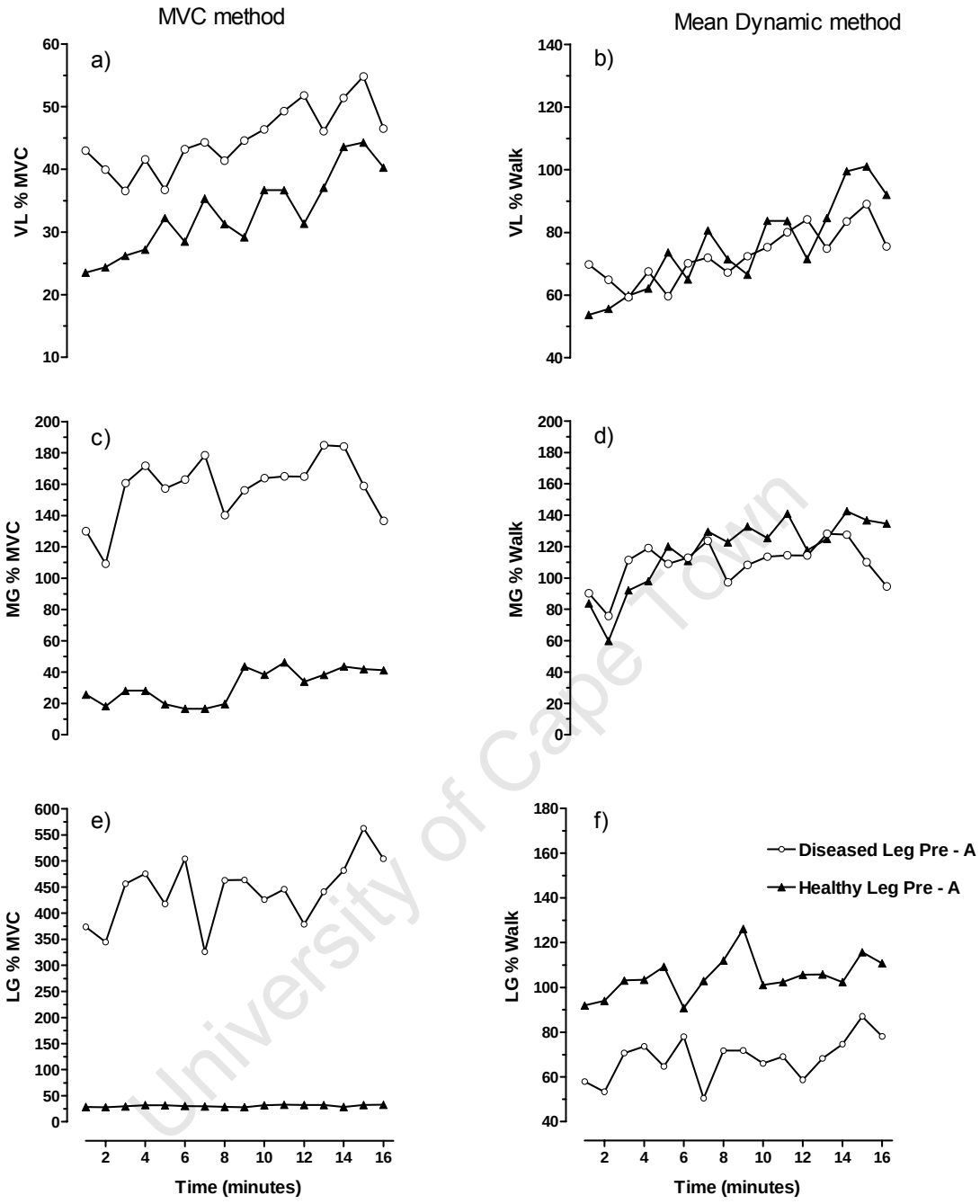


Figure 7.11: Muscle activity normalised to the MVC Method (left column) and the Mean Dynamic Method (right column) of the Healthy and Diseased leg during the Pre-Angioplasty trial (Pre- A) ***(a) & (b)*** *Vastus lateralis (VL)* ***(c) & (d)*** *Medial gastrocnemius and* ***(e) & (f)*** *Lateral gastrocnemius*

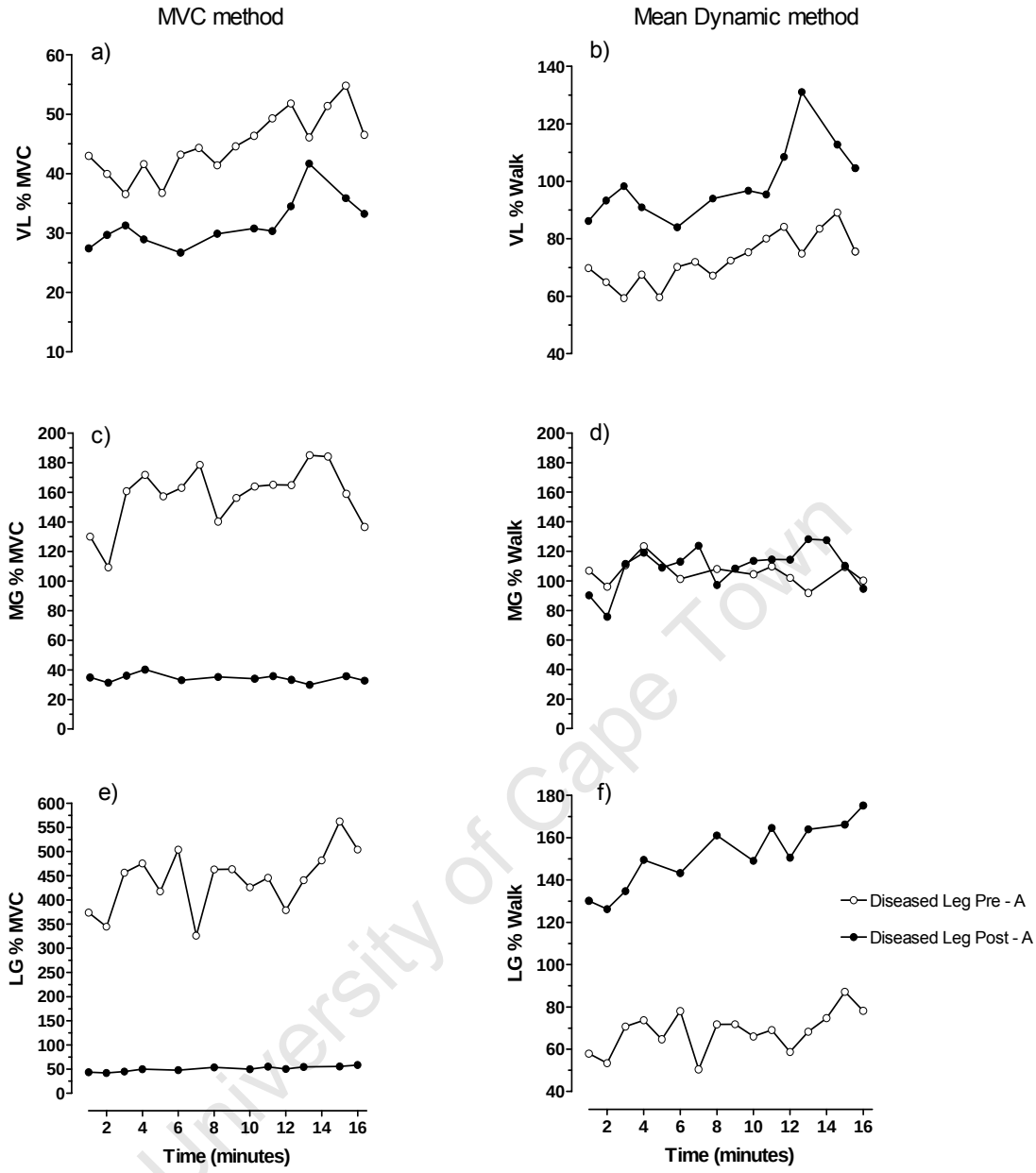


Figure 7.12: Muscle activity normalised to the MVC Method (left column) and the Mean Dynamic Method (right column) of the Diseased leg during the Pre-Angioplasty (Pre- A) and Post-Angioplasty (Post-A) trial, ***(a) & (b)*** *Vastus lateralis (VL)* ***(c) & (d)*** *Medial gastrocnemius and* ***(e) & (f)*** *Lateral gastrocnemius*

Figure 7.12 shows changes in muscle activity in the diseased leg before and after angioplasty when normalised using the MVC and Mean Dynamic methods. VL. MG and LG, normalised using the MVC method, showed a greater muscle activity before angioplasty. Whereas when normalised using the Mean Dynamic method, their

activity levels were only greater after angioplasty, except for MG which remained similar over the trials.

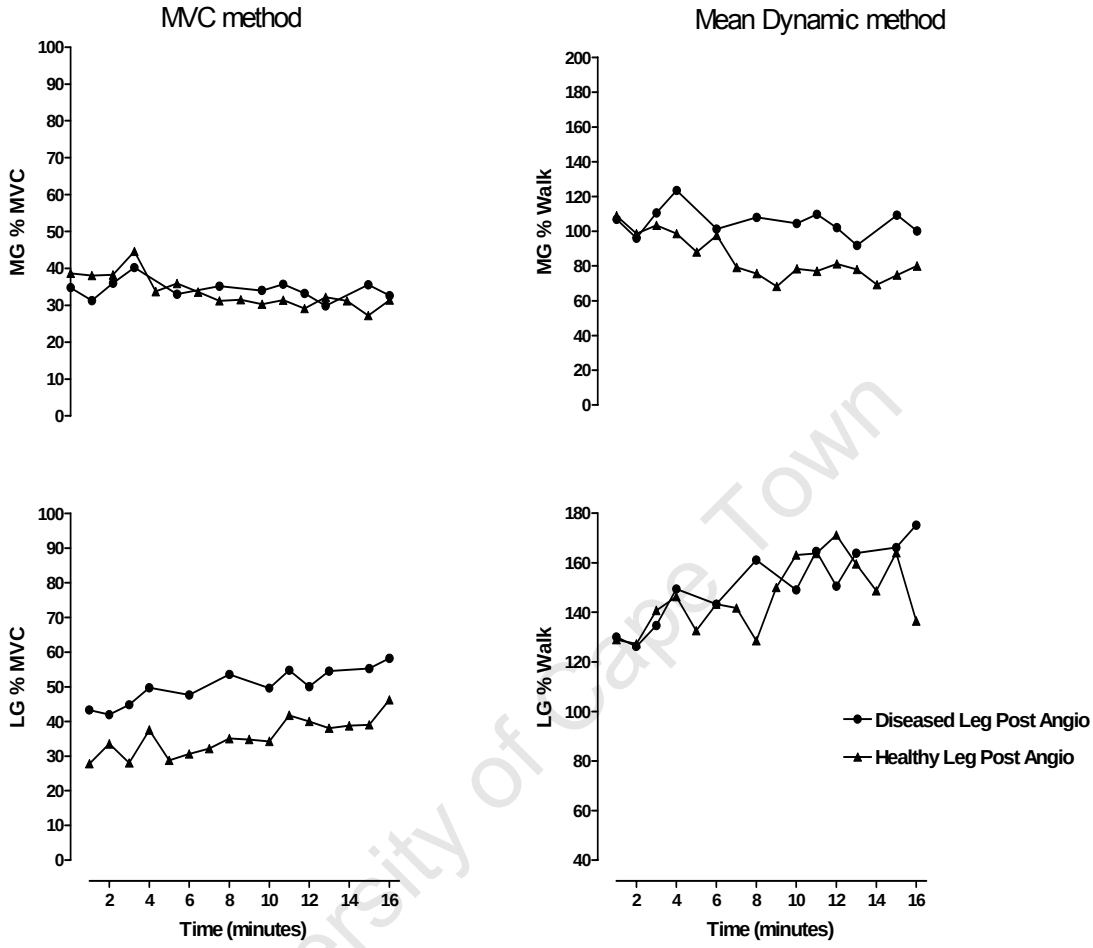


Figure 7.13: Lower limb muscles of the Healthy and Diseased leg in the Post - Angioplasty trial (Post - A) normalised to MVC Method (left column) and Mean Dynamic Method (right column) ***(a) & (b)*** *Medial gastrocnemius and* ***(c) & (d)*** *Lateral gastrocnemius*

The differences between muscle activity in the healthy and diseased leg after angioplasty is shown in Figure 7.13. VL activity in the healthy leg is not shown due to technical problems with the EMG leads. The MG activity was similar between the healthy and diseased leg after angioplasty, when normalised using the MVC method. Whereas using the Mean Dynamic method of normalisation, MG activity in the diseased leg was greater than the healthy leg after angioplasty. LG normalised using the MVC method, showed LG activity in the diseased leg to be greater than

the healthy leg after angioplasty. However, LG activity was similar in both legs when normalised using the Mean Dynamic method.

7.4 DISCUSSION

Angioplasty is an invasive procedure that is used commonly in the management of PVD where there is a predominant single lesion causing obstruction of blood flow. This procedure was successful in both of the patients described. After angioplasty there was decreased blood flow velocity at the site of the lesion (indicating improved, less turbulent flow), decreased pain during walking and increased functional capacity in both patients. Improved functional capacity was always thought to be related to improved blood flow to the ischemic leg. To my knowledge, this is the first study to measure muscle activity before and then within days after angioplasty. This was performed to determine the role of muscle activity in exercise intolerance in patients with PVD.

Surface EMG is considered an appropriate non-invasive method to investigate the underlying mechanism of exercise intolerance in patients with PVD. This study has also evaluated the two recommended methods of EMG normalisation described in the clinical population (MVC method and Mean Dynamic method) to analyse muscle activity in these patients before and after angioplasty.

7.4.1 Methods of normalisation

One of the main findings of this study was that muscle activity of the lower leg changed after restoration of blood flow through angioplasty. Altered muscle activity patterns are seen using both methods of normalisation. The MVC method of normalisation is the standard method of normalisation. The MVC method is still recommended for the measurement of EMG in the clinical population [172]. In the present study, muscle activity normalised using the MVC method, decreased in the diseased leg after angioplasty for most lower leg muscles, in both patients (Figures 7.5 e and 7.12). However, when EMG activity was normalised using the Mean Dynamic method, muscle activity in the diseased leg, increased after angioplasty in

both patients. Furthermore, the results of this study show that the MVC method of normalisation is less sensitive than the Mean Dynamic method in tracking changes in muscle activity with increasing exercise intensity.

It is reasonable to expect muscle force output and EMG amplitude to have a linear relationship, as both variables are dependant on the number of motor units recruited [60]. For this reason, EMG amplitude has been shown to increase with increasing power output/workload [18; 51; 52; 119]. A possible reason for the muscle activity of the diseased leg being greater before angioplasty (normalised to the MVC method) could be explained by the application of the normalisation equation.

Muscle activity during the MVC contraction (before angioplasty) produced lower force output with a resultant lower EMG amplitude compared to the trial after angioplasty (Tables 7.3, 7.4, 7.6 and 7.7). This resulted in a lower value of the denominator of the normalisation equation, which in turn equates to a higher % of MVC value (refer to equation in Chapter 1 Section 1.3.1). After angioplasty, force output increased, resulting in a possible increase in EMG amplitude, and thus a higher denominator value and lower % MVC. For example, in Figure 7.12 e, patient AB demonstrated muscle activity of the LG before angioplasty to be approximately 600 % MVC. This high percentage could be explained by abnormally low EMG activity, leading to an apparent activation of 600 % of MVC. This implies that muscle activity was greater during walking on a treadmill at 3.2 $km.h^{-1}$ than during a maximal contraction (before angioplasty). However, this changes after angioplasty, as muscle activity of LG decreases to approximately 50 % of MVC. This demonstrates that it is possible to misinterpret the EMG data when normalising using the MVC method especially in a clinical population. In addition, the repeatability of force output during MVCs is a requirement for the method's reproducibility. Highly reliable force output measurements are a basic requirement for reliable EMG measurements [105].

The discrepancy between the two methods of normalisation is further highlighted when muscle activity is compared in the healthy and the diseased leg before and after angioplasty. Using the MVC method for normalisation of EMG resulted in the muscle activity in the healthy leg of AA to be greater than in his diseased leg, before angioplasty. However, for AB, muscle activity in the healthy leg was considerably

lower than his diseased leg, before angioplasty. In contrast, in both patients, muscle activity was greater in their healthy legs (for most muscles) when normalised using the Mean Dynamic method before angioplasty. Thus the use of the MVC method displayed conflicting results between patients, whereas using the Mean Dynamic method showed more consistency in the results as both patients displayed the same outcome (increase in muscle activity after angioplasty).

The MVC method of normalisation involves a maximal static contraction. The nature of this contraction does not represent the same physiological mechanisms, including lengthening and shortening muscle actions and speed of muscle contraction, which are evident in walking. Furthermore, maximal contractions in the patient population could cause pain and inhibition of muscle activity [21]. Thus, conscious or subconscious inhibition could occur through patients anxiety related to their diseased state.

Bolgla & Uhl [21] highlighted that previous studies investigating EMG amplitude using the MVC method used healthy populations and inferred the similar findings to symptomatic subject populations. Bolgla & Uhl [21] have therefore also recognised the difficulty in using the MVC method of normalisation and proposed the alternative Mean Dynamic method of normalisation since it was able to identify differences in EMG amplitude between exercises but with less risk of injury or discomfort.

Thus utilisation of the Mean Dynamic method in the clinical population is favoured for the following reasons. Firstly, the Mean Dynamic method is repeatable with preferable inter and intra-subject variability [24; 168; 169; 198]. Secondly, this method of normalisation uses the same muscle action which is used during walking. This could possibly reduce the contribution of extrinsic factors associated with errors in measuring EMG during dynamic activity as outlined in Chapter 1. Thus, by using this method, changes in muscle activity are influenced less by these extrinsic factors and influenced more by biological variation. Importantly, during the application of this method work output of the patients is controlled, as they walked at the same speed and gradient both before and after angioplasty. It must be acknowledged that the muscle activity during the normalisation (walking at 3.2 $km.h^{-1}$) could also have altered after angioplasty, affecting the denominator in the normalisation equation. Yet, muscle activity using this method of normalisation, tracks changes in force

output and exercise tolerance. Thus, the results of this study favour the use of the Mean Dynamic method of normalisation in this patient population.

Further discussion is therefore based on the data generated by using the Mean Dynamic method of normalisation for EMG analysis.

7.4.2 Muscle activity before and after angioplasty

Muscle activity in the diseased leg increased in both patients after only three days recovery from the angioplasty procedure. By comparing the healthy leg to the disease leg in the Pre-A trial, AA and AB showed different muscle activity patterns. AA was diagnosed with unilateral popliteal artery occlusion of the lower leg. VL was assumed to be the least affected by the limited blood flow as it obtains collateral blood flow from the femoral profunda artery. VM, MG and LG do not receive collateral flow.

Indeed, VL activity appeared to be slightly greater in the diseased leg than the healthy leg before angioplasty. This could be due to a compensation of VL activity for the muscles that were directly affected by the stenosis in the diseased leg. VM, MG and LG activity decreased in the diseased leg compared to the healthy leg, after angioplasty. After angioplasty, VL activity remained greater in the diseased leg than in the healthy leg. But, VM, MG and LG muscle activity improved in the diseased leg after angioplasty.

AB was diagnosed with a unilateral femoral artery occlusion of greater than 80 %. The muscle activity in all the muscles measured increased after angioplasty, in the diseased leg. However, before angioplasty, all the measured muscles in the diseased leg were found to be less active than the healthy leg. Furthermore, after angioplasty, all the measured muscles EMG amplitudes increased more than the EMG amplitudes in the healthy leg. It is possible that the inhibition of skeletal muscle activity in his diseased leg might occur due to the inhibition of central drive to the skeletal muscle [(146)]. It is possible that these changes could reflect the readjustment of the central drive to the lower limbs musculature, resulting in a possible regulatory mechanism to protect the body from being harmed, especially in this diseased state.

To my knowledge, these findings have not been described in any literature thus far. It is interesting to note that a study conducted by Kayser et al. [96] investigated the effects of hypoxia on muscle activity. The study found that lower limb iEMG did not increase as much during cycling in hypoxic conditions as it did during normoxic conditions (in which a higher maximum power output was achieved). However, when oxygen was supplied, iEMG activity and power output increased. The same analogy could thus be applied to this patient population. It is possible that the restriction in blood flow to the active muscles results in lower oxygen saturation with a decrease in muscle activity. Once the restriction is removed, oxygen saturation increases and muscle activity increases accordingly. Therefore, in the study by Kayser et al. [96] as in the present study, lack of oxygen to the active muscle might cause central reduction in neural recruitment to protect the tissues from hypoxia.

The only published studies investigating EMG in patients with PVD, have studied changes in median frequency (MDF) and nerve conduction velocity of peroneal and tibial nerves in patients with PVD [8; 125; 151; 155]. McDermott et al. [125] measured peroneal nerve conduction velocity in patients with PVD and control subjects. Patients with PVD had slower nerve conduction velocity and a lower muscle power output compared to controls. This study suggested a decrease in muscle fiber stimulation from the central nervous system. A further study conducted by Pedrinelli et al. [155] examined conduction velocity and MDF of Tibialis anterior muscle during tetanic electrical stimulations in patients with PVD and controls. Conduction velocity ranges did not differ significantly between patients and healthy controls. However, MDF of both the ischemic and non-ischemic legs were found to be significantly lower than the controls, as the healthy limb also showed a decrease in MDF. This finding suggests that chronic ischemia was not the cause of lower MDF in patients with PVD. It is of interest to note that this study used a 3 week walking program intervention, which resulted in improved exercise tolerance in patients with PVD. These findings suggest that central drive increases following a supervised walking program and that factors other than ischemia and physical inactivity underlie the abnormal EMG signal in patients with PVD.

It is possible that a change in fiber type could also account for the changes in MDF [109; 125; 126; 155]. In addition, fiber type composition rather than oxygen availability could limit the endurance capacity of skeletal muscle in patients with PVD [166]. Thus

the altered neural drive and fiber type composition in patients with PVD could both be contributing factors to the lower functional capacity experienced by these patients.

7.4.3 Changes in other physiological variables

Blood flow velocity in the affected arteries decreased in both patients substantially after angioplasty. AA's blood velocity decreased from 206 to 97 $cm.s^{-1}$ (normal blood velocity range of the popliteal artery is 69 ± 14 $cm.s^{-1}$) and AB's blood velocity decreased from 382 to 159 $cm.s^{-1}$ (normal blood velocity range of the femoral artery is 114 ± 25 $cm.s^{-1}$) [176]. Although these values were not within the normal range, the decrease in blood velocity is indicative of the improved blood flow after angioplasty in both patients.

Peak and average force outputs from the quadriceps and calf muscles improved in both patients after angioplasty (Tables 7.3, 7.4, 7.6 and 7.7). In addition, both patients showed improvements in the physiological variables measured during graded maximal exercise. Heart rate and oxygen consumption decreased after angioplasty (Figures 7.2 and 7.8.). Research suggests that peak oxygen consumption in patients with PVD is approximately 18 $ml.kg^{-1}.min^{-1}$ [83]. Both patients showed similar peak values, where AA's peak oxygen consumption was 16.2 $ml.kg^{-1}.min^{-1}$ and AB's was 19.7 $ml.kg^{-1}.min^{-1}$ before angioplasty. Therefore their peak oxygen consumptions can be regarded as normal for their age group.

It is of interest to note that exercise intolerance in patients with PVD is assumed to be due to blood flow to the lower limbs and/or accumulation of lactate in the skeletal muscles and blood as a result of ischemia [101; 118; 177]. However, patients with PVD terminate exercise at lower peak blood lactate concentrations than do patients with other chronic diseases [152]. A study conducted in this laboratory by Parr [152] found that PVD patients who performed a GTET, showed peak blood lactate concentrations of 3.3 ± 1.4 $mmol.l^{-1}$, whereas patients with chronic heart failure have peak lactate concentrations of 4.6 ± 0.5 $mmol.l^{-1}$ [41] and patients with chronic obstructive pulmonary disease have peak lactate concentrations of 5.1 ± 2.7 $mmol.l^{-1}$ [32]. Healthy controls performing similar exercise to exhaustion had peak lactate

concentrations of 9.7 ± 2.7 mmol.l^{-1}. Furthermore, only post-exercise blood lactate concentrations ≥ 8 mmol.l^{-1} are supposedly a marker of oxygen dependant glycolysis [15; 87]. The patient, described in this study, had a peak lactate concentration of 2 mmol.l^{-1} after 3 minutes of performing the GTET. Peak lactate concentration decreased to 1.8 mmol.l^{-1} in the Post-A trial (Figure 7.9). It is therefore unlikely that the blood lactate concentration of this patient is related to low blood oxygen dependant glycolysis and pain, as the blood lactate concentrations were very low. However, it must be noted that lactate concentrations could have been high in the skeletal muscles, and unable to diffuse out due to low blood flow.

Ratings of perceived exertion seemed to have increased prior to pain becoming evident. It is possible that different physiological factors affect the perception of effort and pain. The pain and RPE scores of both patients were lower after angioplasty (Figures 7.3 and 7.10). Since the rate of increase in RPE predicts time to exhaustion [55], this indicates the central processes generating the RPE "knew" that angioplasty had produced cardiovascular and metabolic changes which would allow exercise to continue for longer. Note this effect was present within 6 minutes of start of exercise in the Pre – A trial, and only presented at minute 12 after angioplasty (Fig 7.10 b).

There are certain limitations to this study, whilst the aim was to recruit up to 15 patients with PVD who had single lesion amenable by angioplasty only two patients were recruited for this study. More subjects would have allowed an appropriate investigation of methods of normalisation for this patient population. The many challenges experienced in recruiting these patients involved the fact that 1) most patients diagnosed at the hospital suffered from severe diabetes or had to undergo emergency angioplasty; 2) due to peripheral neuropathy, diabetic candidates were not ideal for a study investigating EMG and muscle activity; 3) most of the patients diagnosed with Peripheral Vascular Disease (PVD) have multiple areas of atherosclerotic plaque development and not just one lesion amenable to angioplasty as required by this study. Thus the stenosis is not isolated to one area. This inclusion criterion was necessary for this study, which examined the effects of rapid reversal of a single occlusion. Future studies should include investigation of time course changes following angioplasty and consider the measurement of muscle

fiber conduction velocity. Furthermore, evaluation of muscle biopsy for lactate analysis and fiber typing should be considered.

In conclusion, this case series found clinical changes in EMG and functional capacity in two patients with PVD, following angioplasty. Both patients described in this series demonstrated improvement in functional capacity and changes in muscle activity during incremental exercise until exhaustion. These findings suggest that a reduction in muscle activity is seen as a physiological attempt to protect the body from serious harm in this diseased state. In addition, both patients showed improvements in other physiological variables, including a decrease in heart rate, perception of effort, pain and peak blood lactate concentrations.

CHAPTER 8

SUMMARY AND CONCLUSIONS: PRACTICAL GUIDELINES FOR NORMALISATION OF EMG IN THE LABORATORY

8.1 OVERVIEW

The objective of the thesis was to critically analyse the various methods of normalising EMG during dynamic exercise with the goal of minimising the contribution of extrinsic factors by using the most appropriate method of normalisation. Furthermore, the thesis aimed to illustrate that decisions regarding the most appropriate method of normalisation should be based on the study design, research question (absolute muscle activity or changes in muscle pattern) and the muscles being investigated.

8.1.1 Chapter 2

Few studies have used alternative methods of normalisation for EMG during cycling. Therefore the aim of this study was to critically examine alternative methods of EMG normalisation during cycling, where each method's ability to show repeatability, reliability and sensitivity was examined. Two maximal static methods (MVC and AP MVC methods) and two dynamic methods (Sprint and 70 % PPO) were analysed and compared.

In summary, the data showed that for the normalisation of EMG during cycling:

(i) The most appropriate method was the submaximal dynamic method of 70 % PPO, showing the highest repeatability, lowest intra-subject variation and highest sensitivity to changes in exercise intensity.

(ii) The Sprint method of normalisation provided a better representation of muscle activity than the MVC method when investigating the amount of muscle activated during cycling. The Sprint method showed greater

repeatability, lower intra-subject variability and better sensitivity than the MVC method.

(iii) The MVC method showed 'fair' repeatability for most muscles examined. However, this method was unable to adequately detect changes in EMG amplitude with corresponding increases in power output.

In conclusion, these data suggest that normalising EMG to dynamic methods (70 % PPO and the Sprint methods) are more suitable for examining muscle activity during cycling over different testing days and for once-off measurements.

8.1.2 Chapter 3

The aim of this study was to critically examine alternative methods of EMG normalisation during running. The ability of each method of normalisation in demonstrating repeatability, reliability and sensitivity was examined. The maximal static method (MVC method) and two dynamic methods (Sprint and 70 % PRS) were investigated. In summary, the data showed that for the normalisation of EMG during running:

(i) The most appropriate method of normalisation was the maximal dynamic Sprint method, as this method showed the highest repeatability, low intra-subject variation and high sensitivity to changes in exercise intensity.

(ii) The submaximal dynamic method had 'poor' repeatability for most muscles; however this method also had the lowest intra-subject CV and greatest ability to detect changes with increasing running speed.

In conclusion, the data suggests that normalising EMG during running to dynamic methods is more suitable than normalising to the static MVC method. The Sprint method of normalisation is recommended for researchers who are investigating maximal muscle activity over different days and for once-off measurements. Moreover, the 70 % PRS method is also recommended for measurement over days

since it has the lowest intra-subject variability and the greatest ability to track changes in muscle activity with increase in exercise intensity.

8.1.3 Chapter 5

By applying the findings from Chapter 2, this chapter attempted to answer a physiological question regarding the muscle activity at exhaustion during cycling. The aim of this chapter was to use an appropriate method of normalisation to measure maximal muscle activity at exhaustion and to investigate if muscle activity is indeed submaximal during maximal exercise to exhaustion. The main findings of this study were;

(i) The Sprint method was the most appropriate method of normalisation to answer the research question, because this method had a greater reliability, greater repeatability (predominantly for VM, RF and MG) and greater sensitivity (predominantly for VM, VL, RF and BF) than the MVC method. This method also uses the same dynamic movement which occurs in the activity being investigated and is thus a better representation of muscle activity during cycling.

(ii) Muscle activity was submaximal at exhaustion ranging between 36 % - 65 % of muscle activity during a maximal cycle sprint. This indicates that muscle activity does not reach 'maximal' capacity at exhaustion.

(iii) Muscle activity at exhaustion varied on different days for each subject.

In conclusion, this study showed that muscle activity was submaximal during an incremental cycle test to exhaustion (PPO). The finding provides evidence of central regulation of exercise and confirms that only a certain percentage of the limb muscle mass is active during maximal exercises. These findings are not compatible with the peripheral model of fatigue, as the model assumes that the total muscle mass must be active at exhaustion.

8.1.4 Chapter 6

This study was analogous to Chapter 5, in applying the most appropriate method of normalisation to measure maximal muscle activity at exhaustion during running (from Chapter Three) and to investigate if muscle activity is indeed submaximal during maximal running exercise to exhaustion. The main findings of this study were:

(i) The Sprint method of normalisation was the most appropriate method of normalisation to answer the research question. The method had greater repeatability (predominantly for VL, RF and LG), reliability and sensitivity; than the MVC method.

(ii) Muscle activity was submaximal at exhaustion ranging between 42 % - 66 % of a 20 m sprint, indicating muscle activity does not reach 'maximal' capacity at exhaustion.

(iii) The agonist and antagonist muscles were the most activated, RF (55 % - 64 %) and BF (58 % - 59 %) respectively, possibly to assist with increasing the joint stiffness, to prevent unnecessary yielding of the ankle, knee and hip joints.

(iv) At exhaustion, quadriceps muscle activities were the most repeatable over 3 days, whereas the gastrocnemius muscles showed 'poor' repeatability at exhaustion.

In conclusion muscle activity during running is indeed submaximal at exhaustion and supports the findings of Chapter Five. These findings also support the model of central regulation of exercise and confirm that only a certain percentage of muscle is active during maximal exercises.

8.1.5 Chapter 7

The aims of the chapter were to firstly apply an appropriate method of normalisation for measurement and evaluation of muscle activity in patients with PVD (before and after angioplasty). The chapter used two recommended methods of normalisation from the literature (MVC method and Mean Dynamic method), since the low subject number impeded the measurement of repeatability, reliability and sensitivity. Secondly, the study aimed to investigate the effect of angioplasty on skeletal muscle activity and physiological variables in patients with PVD. The main findings of the chapter were;

i) EMG data analysis using the MVC method of normalisation resulted in conflicting results when the data was normalised using the Mean Dynamic method. The MVC method demonstrated that muscle activity in the diseased leg decreased after angioplasty, whereas the Mean Dynamic method showed the muscle activity in the diseased leg increased after angioplasty.

ii) The Mean Dynamic method was chosen as the more appropriate method, as the MVC method could have produced abnormally low EMG activity during the MVC (due to possible muscle inhibition). In addition, the MVC method of normalisation is considered less sensitive than the Mean Dynamic method in tracking changes in muscle activity with increasing exercise intensity.

iii) By using the Mean Dynamic method, the study found muscle activity to be reduced in the state of peripheral vascular disease and muscle activity in the diseased leg was able to increase to muscle activity percentages greater than the asymptomatic (healthy) leg, after angioplasty.

iv) Peak blood lactate concentration (which has been assumed to be the reason for claudication pain) was less than that of healthy controls and in patients with other chronic diseases.

(v) Both patients showed improvements in the other physiological variables measured.

In conclusion, the MVC method of normalisation should not be used in the clinical population. The subjects are unable to perform a valid MVC possibly due to pain and/or muscle inhibition. Therefore a submaximal method is the most appropriate method to normalise EMG in the clinical population as it is able to identify differences in EMG amplitude between trials and with increasing workload but with less risk of injury or discomfort. Furthermore this study demonstrated that exercise intolerance in these patients is affected by muscle activity. These findings advocate the central regulation of exercise, where the reduction in muscle activity is viewed as a physiological attempt to protect the body from serious harm so as to maintain homeostasis, in the diseased state.

It is important to note that the thesis was unable to provide these guidelines for the clinical population (patients with PVD). The study was unable to investigate the repeatability, reliability and sensitivity of the MVC and Mean dynamic methods of normalisation used in the clinical case studies, due to the low subject number. Therefore no valid recommendations are made for the appropriate method of normalisation to be used in clinical studies; however, the thesis has questioned the credibility of the MVC method, since it is not as sensitive in detecting changes in workload.

8.2 INTERPRETATION AND GUIDELINES

The overall theme of the thesis is that researchers should use a method of normalisation that is appropriate for their study design, research question and skeletal muscles being investigated. This increases the chance of detecting true biological variation rather than incurring experimental error which will subsequently reduce the chance of detecting biological variation.

The thesis has also highlighted the importance of using the ideal method of normalisation for the analysis of EMG during cycling and running (Chapter 2 and 3 respectively). The data from the various studies in this thesis provides possible guidelines to researchers who are planning studies involving EMG measurements.

The following organograms (Figure 8.1 for cycling and 8.2 for running) offer evidence-based guidance on the selection of the most appropriate method of normalisation for different research designs.

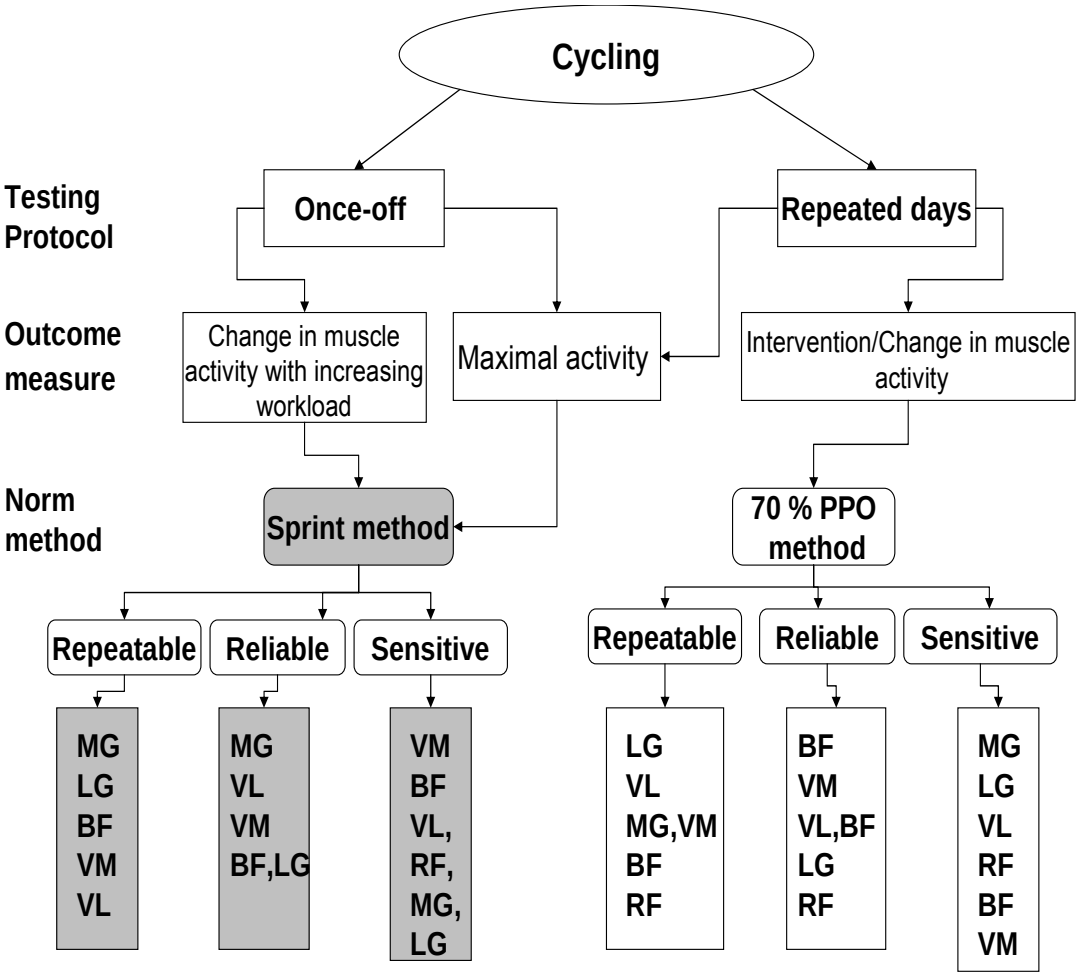


Figure 8.1: Schematic guideline for normalising EMG during cycling that is suitable for the study design, research question and skeletal muscles being investigated. *The muscles are displayed from highest to lowest ranking order. The muscles corresponding to the Sprint method is highlighted in grey.*

Figure 8.1 presents a schematic guideline for normalising EMG during cycling studies. If a researcher is investigating muscle activity on a once-off cycling trial, and intends to measure maximal muscle activity or changes in muscle activity, the Sprint method of normalisation is recommended. This method has demonstrated 'fair' to 'good' repeatability for most of the muscles measured, where the MG and LG have shown to have the highest repeatability. The MG and VL are the recommended muscles to use for low intra-subjection variation. The VM and BF are the most

sensitive muscles in tracking changes in workload, followed by the remaining four muscles (VL, RF, MG and LG). Important to note is that RF is not recommended as a muscle to measure when investigating maximal muscle activity, since RF has 'poor' repeatability and intra-subject reliability. However, RF activity does track changes in workload to the same extent as does VL, MG and LG.

Furthermore if a researcher is investigating muscle activity over repeated days, and intends on measuring maximal muscle activity, the Sprint method of normalisation is also recommended. The Sprint method demonstrates the same guidelines as mentioned above. However, if a researcher intends on measuring a change in muscle activity (through an intervention), the 70 % PPO method is recommended. All the measured muscles had 'good' repeatability, where LG and VL demonstrated the highest ICC values. The lowest intra-subject variability was demonstrated with BF and VM muscles. The gastrocnemius muscles were the most sensitive in tracking changes in workload.

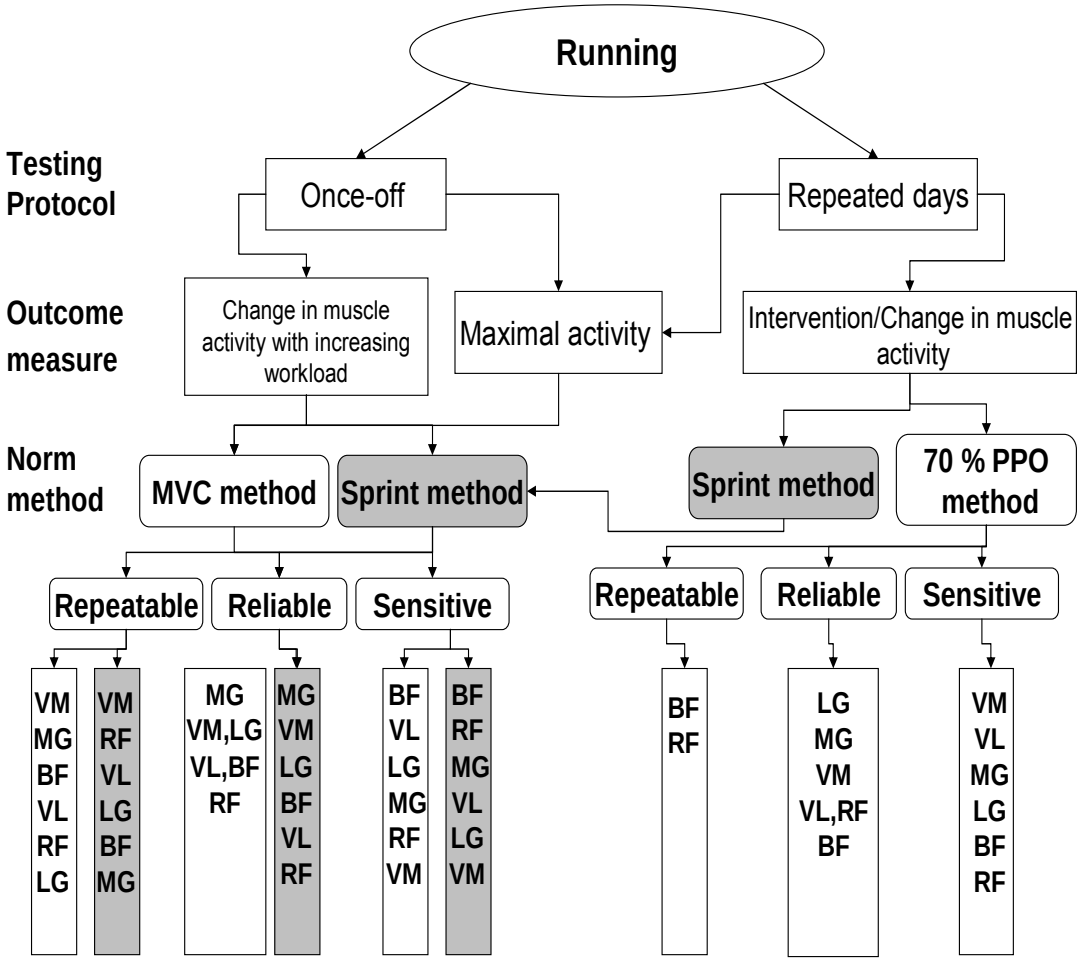


Figure 8.2: Schematic guideline for normalising EMG during running that is suitable for the study design, research question and skeletal muscles being investigated. *The muscles are displayed from highest to lowest ranking order. The muscles corresponding to the Sprint method is highlighted in grey.*

Figure 8.2 presents a schematic guideline for normalising EMG during running studies. If a researcher is investigating muscle activity in a once-off running trial, and intends to measure maximal muscle activity or changes in muscle activity, the Sprint and MVC methods of normalisation are recommended. These methods have demonstrated 'fair' to 'good' repeatability for most of the muscles measured, where the VM had the highest repeatability for both methods. The MG and VM are the recommended muscles to use for low intra-subject variation. The BF is the most sensitive muscle in tracking changes in workload.

Furthermore if a researcher is investigating running muscle activity over repeated days, and intends on measuring maximal muscle activity or changes in muscle

activity, the Sprint method of normalisation is recommended. The Sprint method demonstrates the same guidelines as mentioned above. In addition, the 70 % PRS method is also recommended. Although this method has 'poor' repeatability for most muscles (except BF and RF which had 'fair' repeatability), this method has the lowest intra-subject variability, particularly in the gastrocnemius muscles. The 70 % PRS method of normalisation had the most sensitivity to changes in workload. VM and VL muscles showed the greatest ability to track EMG changes to increasing workloads.

REFERENCES

1. *ACSM Guidelines for exercise testing and prescription.* Lea & Febinger, 1991.

2. **Aaras A, Veierod MB, Larsen S, Ortengren R and Ro O.** Reproducibility and stability of normalised EMG measurements on musculus trapezius. *Ergonomics* 39: 171-185, 1996.

3. **Allen GM, McKenzie DK and Gandevia SC.** Twitch interpolation of the elbow flexor muscles at high forces. *Muscle Nerve* 21: 318-328, 1998.

4. **Andreassen S and Rosenfalck A.** Recording from a single motor unit during strong effort. *IEEE Trans Biomed Eng* 25: 501-508, 1978.

5. **Andrian ED and Bronk DW.** The Discharge of impulses in motor nerve fbersII: The frequency of discharge in reflex and voluntary Contractions. *J Physiol* 67: 119-151, 1929.

6. **Ansley L, Schabort E, St Clair GA, Lambert MI and Noakes TD.** Regulation of pacing strategies during successive 4-km time trials. *Med Sci Sports Exerc* 36: 1819-1825, 2004.

7. **Arendt-Nielsen L, Mills KR and Forster A.** Changes in muscle fiber conduction velocity, mean power frequency, and mean EMG voltage during prolonged submaximal contractions. *Muscle Nerve* 12: 493-497, 1989.

8. **Argyriou AA, Tsolakis I, Papadoulas S, Polychronopoulos P, Gourzis P and Chroni E.** Dynamic F wave study in patients suffering from peripheral arterial occlusive disease. *Acta Neurol Scand* 115: 84-89, 2007.

9. **Arsenault AB, Winter DA and Marteniuk RG.** Bilateralism of EMG profiles in human locomotion. *Am J Phys Med* 65: 1-16, 1986.

10. **Arsenault AB, Winter DA, Marteniuk RG and Hayes KC.** How many strides are required for the analysis of electromyographic data in gait? *Scand J Rehabil Med* 18: 133-135, 1986.

11. **Avela J, Kyrolainen H and Komi PV.** Altered reflex sensitivity after repeated and prolonged passive muscle stretching. *J Appl Physiol* 86: 1283-1291, 1999.

12. **Balady GJ, Berra KA, Golding LA, Gordan NF, Mahler DA, Myers JN and Sheldahl LM.** *ACSM's guidelines for exercise testing and prescription*. Lippincott Williams & Wilkins, 2000.

13. **Basmajian JV and Deluca CJ.** *Muscles alive: Their function revealed by electromyography*. Baltimore: Williams and Wilkins, 1985.

14. **Bassett DR, Jr. and Howley ET.** Maximal oxygen uptake: "classical" versus "contemporary" viewpoints. *Med Sci Sports Exerc* 29: 591-603, 1997.

15. **Bassett DR, Jr. and Howley ET.** Limiting factors for maximum oxygen uptake and determinants of endurance performance. *Med Sci Sports Exerc* 32: 70-84, 2000.

16. **Bigland B and Lippold OC.** Motor unit activity in the voluntary contraction of human muscle. *J Physiol* 125: 322-335, 1954.

17. **Bigland-Ritchie B.** EMG/force relations and fatigue of human voluntary contractions. *Exerc Sport Sci Rev* 9: 75-117, 1981.

18. **Bigland-Ritchie B and Woods JJ.** Integrated EMG and oxygen uptake during dynamic contractions of human muscles. *J Appl Physiol* 36: 475-479, 1974.

19. **Bilodeau M, Arsenault AB, Gravel D and Bourbonnais D.** The influence of an increase in the level of force on the EMG power spectrum of elbow extensors. *Eur J Appl Physiol Occup Physiol* 61: 461-466, 1990.

20. **Bilodeau M, Goulet C, Nadeau S, Arsenault AB and Gravel D.** Comparison of the EMG power spectrum of the human soleus and gastrocnemius muscles. *Eur J Appl Physiol Occup Physiol* 68: 395-401, 1994.

21. **Bolgla LA and Uhl TL.** Reliability of electromyographic normalisation methods for evaluating the hip musculature. *J Electromyogr Kinesiol* 17: 102-111, 2007.

22. **Borg GA.** Psychophysical bases of perceived exertion. *Med Sci Sports Exerc* 14: 377-381, 1982.

23. **Brooks GA, Fahey TD and Baldwin KM.** *Exercise physiology: Human bioenergetics and its application*. New York: McGraw-Hill, 2004.

24. **Burden A and Bartlett R.** Normalisation of EMG amplitude: an evaluation and comparison of old and new methods. *Med Eng Phys* 21: 247-257, 1999.

25. **Burden A, Trew M and Baltzopoulos V.** Normalisation of gait EMGs: a re-examination. *J Electromyogr Kinesiol* 13: 519-532, 2003.

26. **Calder KM, Hall L, Lester SM, Inglis JG and Gabriel DA.** Reliability of the biceps brachii M-wave. *J Neuroengineering Rehabil* 2: 2005.

27. **Clarys JP.** Hydrodynamics and electromyography: ergonomics aspects in aquatics. *Appl Ergon* 16: 11-24, 1985.

28. **Clarys JP.** Electrology and localised electrisation revisited. *J Electromyogr Kinesiol* 4: 5-14, 1994.

29. **Clarys JP.** Electromyography in sports and occupational settings: an update of its limits and possibilities. *Ergonomics* 43: 1750-1762, 2000.

30. **Clarys JP and Alewaeters K.** Science and sports: a brief history of muscle, motion and ad hoc organisations. *J Sports Sci* 21: 669-677, 2003.

31. **Clarys JP, Massez C, Van Den Broeck M, Piette G and Robeaux R.** Total telemetric surface of the front crawl. In: Biomechanics VIII-B. international series on biomechanics, Champaign: Human Kinetics, 1983, p. 951-958.

32. **Coleman KL.** *Exercise tolerance and skeletal muscle structure and function in patients with chronic obstructive pulmonary disease* (Dissertation). University of Cape Town, 1998.

33. **Croisier JL, Malnati M, Reichard LB, Peretz C and Dvir Z.** Quadriceps and hamstring isokinetic strength and electromyographic activity measured at different ranges of motion: a reproducibility study. *J Electromyogr Kinesiol* 17: 484-492, 2007.

34. **Dankaerts W, O'Sullivan PB, Burnett AF, Straker LM and Danneels LA.** Reliability of EMG measurements for trunk muscles during maximal and sub-maximal voluntary isometric contractions in healthy controls and CLBP patients. *J Electromyogr Kinesiol* 14: 333-342, 2004.

35. **Davis RB, DeLuca PA and Õunpuu S.** A gait analysis data collection and reduction technique. *Hum Mov Sci* 10: 575-587, 1991.

36. **Day SJ and Hulliger M.** Experimental simulation of cat electromyogram: evidence for algebraic summation of motor-unit action-potential trains. *J Neurophysiol* 86: 2144-2158, 2001.

37. **De Luca CJ.** Myoelectrical manifestations of localised muscular fatigue in humans. *Crit Rev Biomed Eng* 11: 251-279, 1984.

38. **De Luca CJ.** Electromyography. In: Encyclopedia of medical devices and instrumentation, edited by Webster J. John Wiley & Sons, Inc, 2006, p. 98-109.

39. **De Luca CJ and Merletti R.** Surface myoelectric signal cross-talk among muscles of the leg. *Electroencephalogr Clin Neurophysiol* 69: 568-575, 1988.

40. **Deluca CJ.** The use of surface electromyography in biomechanics. *J Appl Biomech* 13: 135-163, 1997.

41. **Derman K.** *Exercise tolerance and skeletal muscle structure and function in patients with severe chronic heart failure* (Dissertation). University of Cape Town, 1995.

42. **DeVries HA.** "Efficiency of electrical activity" as a physiological measure of the functional state of muscle tissue. *Am J Phys Med* 47: 10-22, 1968.

43. **Dietz V, Schmidtbleicher D and Noth J.** Neuronal mechanisms of human locomotion. *J Neurophysiol* 42: 1212-1222, 1979.

44. **Dubo HI, Peat M, Winter DA, Quanbury AO, Hobson DA, Steinke T and Reimer G.** Electromyographic temporal analysis of gait: normal human locomotion. *Arch Phys Med Rehabil* 57: 415-420, 1976.

45. **Durnin JV and Womersley J.** Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. *Br J Nutr* 32: 77-97, 1974.

46. **Earl JE, Schmitz RJ and Arnold BL.** Activation of the VMO and VL during dynamic mini-squat exercises with and without isometric hip adduction. *J Electromyogr Kinesiol* 11: 381-386, 2001.

47. **Edwards RH.** Human muscle function and fatigue. In: Human muscle fatigue: Physiological mechanisms, edited by Porter R and Whelan J. London: Pitman Medical, 1981, p. 1-18.

48. **Eloranta V and Komi PV.** Function of the quadriceps femoris muscle under maximal concentric and eccentric contractions. *Electromyogr Clin Neurophysiol* 20: 159-54, 1980.

49. **Enoka RM.** Eccentric contractions require unique activation strategies by the nervous system. *J Appl Physiol* 81: 2339-2346, 1996.

50. **Enoka RM.** *Neuromechanics of Human Movement*. Champaign, IL: Human Kinetics, 2002.

51. **Ericson MO.** On the biomechanics of cycling: a study of joint and muscle load during exercise on the bicycle ergometer. *Scand J Rehabil Med* 16: 1-43, 1986.

52. **Ericson MO, Nisell R, Arborelius UP and Ekholm J.** Muscular activity during ergometer cycling. *Scand J Rehabil Med* 17: 53-61, 1985.

53. Ericson MO, Nisell R and Ekholm J. Quantified electromyography of lower-limb muscles during level walking. *Scand J Rehabil Med* 18: 159-163, 1986.

54. Esposito F, Orizio C and Veicsteinas A. Electromyogram and mechanomyogram changes in fresh and fatigued muscle during sustained contraction in men. *Eur J Appl Physiol Occup Physiol* 78: 494-501, 1998.

55. Eston R, Faulkner J, St Clair GA, Noakes T and Parfitt G. The effect of antecedent fatiguing activity on the relationship between perceived exertion and physiological activity during a constant load exercise task. *Psychophysiology* 44: 779-786, 2007.

56. Falck B, Stalberg E and Bischoff C. Influence of recording site within the muscle on motor unit potentials. *Muscle Nerve* 18: 1385-1389, 1995.

57. Falcone RA, Hirsch AT, Regensteiner JG, Treat-Jacobson D, Williams MA, Hiatt WR and Stewart KJ. Peripheral arterial disease rehabilitation: a review. *J Cardiopulm Rehabil* 23: 170-175, 2003.

58. Farina D. Interpretation of surface electromyography in dynamic contractions. *Exerc Sport Sci Rev* 34: 121-127, 2006.

59. Farina D, Macaluso A, Ferguson RA and De Vito G. Effect of power, pedal rate, and force on average muscle fiber conduction velocity during cycling. *J Appl Physiol* 97: 2035-2041, 2004.

60. Farina D, Merletti R and Enoka RM. The extraction of neural strategies from the surface EMG. *J Appl Physiol* 96: 1486-1495, 2004.

61. Farina D, Merletti R, Nazzaro M and Caruso I. Effect of joint angle on EMG variables in leg and thigh muscles. *IEEE Eng Med Biol Mag* 20: 62-71, 2001.

62. Farina D and Rainoldi A. Compensation of the effect of sub-cutaneous tissue layers on surface EMG: a simulation study. *Med Eng Phys* 21: 487-497, 1999.

63. Felici F. Applications in exercise physiology. In: Electromyography: physiology, engineering, and noninvasive applications, edited by Merletti R and Parker PA. New Jersey: John Wiley & Sons Inc, 2004, p. 365-379.

64. Felici F. Neuromuscular responses to exercise investigated through surface EMG. *J Electromyogr Kinesiol* 16: 578-585, 2006.

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

66. **Frigo C and Shiavi R.** Applications in movement and gait analysis. In: Electromyography: physiology, engineering, and noninvasive applications, edited by Merletti R and Parker PA. New Jersey: John Wiley & Sons, Inc, 2004, p. 381-401.

67. **Fuglevand AJ, Winter DA, Patla AE and Stashuk D.** Detection of motor unit action potentials with surface electrodes: influence of electrode size and spacing. *Biol Cybern* 67: 143-153, 1992.

68. **Gamet D, Duchene J and Goubel F.** Reproducibility of kinetics of electromyogram spectrum parameters during dynamic exercise. *Eur J Appl Physiol Occup Physiol* 74: 504-510, 1996.

69. **Gandevia SC.** Spinal and supraspinal factors in human muscle fatigue. *Physiol Rev* 81: 1725-1789, 2001.

70. **Gleeson NP and Mercer TH.** Reproducibility of isokinetic leg strength and endurance characteristics of adult men and women. *Eur J Appl Physiol Occup Physiol* 65: 221-228, 1992.

71. **Gollhofer A, Komi PV, Miyashita M and Aura O.** Fatigue during stretch-shortening cycle exercises: changes in mechanical performance of human skeletal muscle. *Int J Sports Med* 8: 71-78, 1987.

72. **Goto S, Toyoshima S and Hoshikawa T.** Study of the integrated EMG of leg muscles during pedalling at various loads, frequency, and equivalent power. In: Biomechanics V-1A, Baltimore: Univeristy Park Press, 1976, p. 246-252.

73. **Goubel F.** Muscle mechanics: fundamental concepts in SSC. *Med Sport Sci* 26: 24-35, 1987.

74. **Gregor RJ, Cavanagh PR and LaFortune M.** Knee flexor moments during propulsion in cycling--a creative solution to Lombard's Paradox. *J Biomech* 18: 307-316, 1985.

75. **Guidetti L, Rivellini G and Figura F.** EMG Patterns during Running: Intra- and Inter-individual Variability. *J Electromyogr Kinesiol* 6: 37-48, 1996.

76. **Hagberg M.** Muscular endurance and surface electromyogram in isometric and dynamic exercise. *J Appl Physiol* 51: 1-7, 1981.

77. **Hägg GM, Melin B and Kadefors R.** Applications in ergonomics. In: Electromyography: physiology, engineering, and noninvasive applications, edited by Merletti R and Parker PA. New Jersey: John Wiley & Sons Inc, 2004, p. 343-363.

78. **Hanon C, Thepaut-Mathieu C and Vandewalle H.** Determination of muscular fatigue in elite runners. *Eur J Appl Physiol* 94: 118-125, 2005.

79. **Hautier CA, Arsac LM, Deghdegh K, Souquet J, Belli A and Lacour JR.** Influence of fatigue on EMG/force ratio and cocontraction in cycling. *Med Sci Sports Exerc* 32: 839-843, 2000.

80. **Hawley JA and Noakes TD.** Peak power output predicts maximal oxygen uptake and performance time in trained cyclists. *Eur J Appl Physiol Occup Physiol* 65: 79-83, 1992.

81. **Heckathorne CW and Childress DS.** Relationships of the surface electromyogram to the force, length, velocity, and contraction rate of the cineplastic human biceps. *Am J Phys Med* 60: 1-19, 1981.

82. **Hermens HJ, Freriks B, Disselhorst-Klug C and Rau G.** Development of recommendations for SEMG sensors and sensor placement procedures. *J Electromyogr Kinesiol* 10: 361-374, 2000.

83. **Hiatt WR, Regensteiner JG and Nawaz D.** The evaluation of exercise performance in patients with peripheral arterial disease. *J Cardiopulm Rehabil* 12: 525-532, 1988.

84. **Hopkins WG.** Measures of reliability in sports medicine and science. *Sports Med* 30: 1-15, 2000.

85. **Hopkins, W. G.** A new view of statistics. 2007. Ref Type: Internet Communication. www.newstats.org.

86. **Howatson G and van Someren KA.** The reproducibility of peak isometric torque and electromyography activity in unfamiliarised subjects using isokinetic dynamometry on repeated days. *Isokinet Exerc Sci* 13: 103-109, 2005.

87. **Howley ET, Bassett DR, Jr. and Welch HG.** Criteria for maximal oxygen uptake: review and commentary. *Med Sci Sports Exerc* 27: 1292-1301, 1995.

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

89. **Hunter AM, St Clair GA, Lambert MI, Nobbs L and Noakes TD.** Effects of supramaximal exercise on the electromyographic signal. *Br J Sports Med* 37: 296-299, 2003.

90. Hunter SK and Enoka RM. Changes in muscle activation can prolong the endurance time of a submaximal isometric contraction in humans. *J Appl Physiol* 94: 108-118, 2002.

91. Johnson J and Siegel D. Reliability of an isokinetic movement of the knee extensors. *Res Q* 49: 88-90, 1978.

92. Kadefors R, Kaiser E and Petersen I. Dynamic spectrum analysis of myopotentials and with special reference to muscle fatigue. *Electromyography* 8: 39-74, 1968.

93. Kamen G and Caldwell GE. Physiology and interpretation of the electromyogram. *J Clin Neurophysiol* 13: 366-384, 1996.

94. Karamanidis K, Arampatzis A and Bruggemann GP. Reproducibility of electromyography and ground reaction force during various running techniques. *Gait Posture* 19: 115-123, 2004.

95. Kay D, Marino FE, Cannon J, St Clair GA, Lambert MI and Noakes TD. Evidence for neuromuscular fatigue during high-intensity cycling in warm, humid conditions. *Eur J Appl Physiol* 84: 115-121, 2001.

96. Kayser B, Narici M, Binzoni T, Grassi B and Cerretelli P. Fatigue and exhaustion in chronic hypobaric hypoxia: influence of exercising muscle mass. *J Appl Physiol* 76: 634-640, 1994.

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

98. Kellis E and Baltzopoulos V. The effects of normalisation method on antagonistic activity patterns during eccentric and concentric isokinetic knee extension and flexion. *J Electromyogr Kinesiol* 6: 235-245, 1996.

99. Keskula DR, Dowling JS, Davis VL, Finley PW and Dell'Omo DL. Interrater reliability of isokinetic measures of knee flexion and extension. *J Athl Training* 30: 167-170, 1995.

100. Kettenmann H. Alexander von Humboldt and the concept of animal electricity. *Trends Neurosci* 20: 239-242, 1997.

101. Klass M, Guissard N and Duchateau J. Limiting mechanisms of force production after repetitive dynamic contractions in human triceps surae. *J Appl Physiol* 96: 1516-1521, 2004.

102. Knowlton GC, Bennett RL and McClure R. Electromyography of fatigue. *Arch Phys Med Rehabil* 32: 648-652, 1951.

103. Knutson LM, Soderberg GL, Ballatyne BT and Clarke WR. A study of various normalisation procedures for within day electromyographic data. *J Electromyogr Kinesiol* 4: 47-59, 1994.

104. Knutsson E and Richards C. Different types of disturbed motor control in gait of hemiparetic patients. *Brain* 102: 405-430, 1979.

105. Kollmitzer J, Ebenbichler GR and Kopf A. Reliability of surface electromyographic measurements. *Clin Neurophysiol* 110: 725-734, 1999.

106. Komi PV and Buskirk ER. Reproducibility of electromyographic measurements with inserted wire electrodes and surface electrodes. *Electromyography* 4: 357-367, 1970.

107. Komi PV, Gollhofer A, Schmidtbleicher D and Frick U. Interaction between man and shoe in running: considerations for a more comprehensive measurement approach. *Int J Sports Med* 8: 196-202, 1987.

108. Komi PV and Tesch P. EMG frequency spectrum, muscle structure, and fatigue during dynamic contractions in man. *Eur J Appl Physiol Occup Physiol* 42: 41-50, 1979.

109. Kupa EJ, Roy SH, Kandarian SC and De Luca CJ. Effects of muscle fiber type and size on EMG median frequency and conduction velocity. *J Appl Physiol* 79: 23-32, 1995.

110. Kyröläinen H, Avela J and Komi PV. Changes in muscle activity with increasing running speed. *J Sports Sci* 23: 1101-1109, 2005.

111. Kyröläinen H, Belli A and Komi PV. Biomechanical factors affecting running economy. *Med Sci Sports Exerc* 33: 1330-1337, 2001.

112. Lambert MI, Marcus P, Burgess T and Noakes TD. Electro-membrane microcurrent therapy reduces signs and symptoms of muscle damage. *Med Sci Sports Exerc* 34: 602-607, 2002.

113. Laplaud D, Hug F and Grelot L. Reproducibility of eight lower limb muscles activity level in the course of an incremental pedalling exercise. *J Electromyogr Kinesiol* 16: 158-166, 2006.

114. Lariviere C, Arsenault AB, Gravel D, Gagnon D and Loisel P. Evaluation of measurement strategies to increase the reliability of EMG indices to assess back muscle fatigue and recovery. *J Electromyogr Kinesiol* 12: 91-102, 2002.

115. Larsson B, Mansson B, Karlberg C, Syvertsson P, Elert J and Gerdle B. Reproducibility of surface EMG variables and peak torque during three sets of ten dynamic contractions. *J Electromyogr Kinesiol* 9: 351-357, 1999.

116. Lloyd AJ. Surface electromyography during sustained isometric contractions. *J Appl Physiol* 30: 713-719, 1971.

117. Loring SH and Hershenson MB. Effects of series compliance on twitches superimposed on voluntary contractions. *J Appl Physiol* 73: 516-521, 1992.

118. Lundgren F, Dahllof AG, Schersten T and Bylund-Fellenius AC. Muscle enzyme adaptation in patients with peripheral arterial insufficiency: spontaneous adaptation, effect of different treatments and consequences on walking performance. *Clin Sci (Lond)* 77: 485-493, 1989.

119. MacIntosh BR, Neptune RR and Horton JF. Cadence, power, and muscle activation in cycle ergometry. *Med Sci Sports Exerc* 32: 1281-1287, 2000.

120. Marsh AP and Martin PE. The association between cycling experience and preferred and most economical cadences. *Med Sci Sports Exerc* 25: 1269-1274, 1993.

121. Marsh AP and Martin PE. The relationship between cadence and lower extremity EMG in cyclists and noncyclists. *Med Sci Sports Exerc* 27: 217-225, 1995.

122. Masuda K, Masuda T, Sadoyama T, Inaki M and Katsuta S. Changes in surface EMG parameters during static and dynamic fatiguing contractions. *J Electromyogr Kinesiol* 9: 39-46, 1999.

123. Mathiassen SE and Hägg G. Amplitude aspects and functional considerations on surface EMG electrode displacement with particular emphasis on the upper trapezius muscle. In: The state of the art on sensors and sensor placement procedures for SEMG, edited by Hermens HJ and Freriks B. The Netherlands: Roessingh Research and Development, 1997, p. 84-95.

124. Mathur S, Eng JJ and MacIntyre DL. Reliability of surface EMG during sustained contractions of the quadriceps. *J Electromyogr Kinesiol* 15: 102-110, 2005.

125. McDermott MM, Guralnik JM, Albay M, Bandinelli S, Miniati B and Ferrucci L. Impairments of muscles and nerves associated with peripheral arterial disease and their relationship with lower extremity functioning: the InCHIANTI Study. *J Am Geriatr Soc* 52: 405-410, 2004.

126. McGuigan MR, Bronks R, Newton RU, Sharman MJ, Graham JC, Cody DV and Kraemer WJ. Resistance training in patients with peripheral arterial disease: effects on myosin isoforms, fiber type distribution, and capillary supply to skeletal muscle. *J Gerontol A Biol Sci Med Sci* 56: B302-B310, 2001.

127. Menz HB, Latt MD, Tiedemann A, Mun SK and Lord SR. Reliability of the GAITRite walkway system for the quantification of temporo-spatial parameters of gait in young and older people. *Gait Posture* 20: 20-25, 2004.

128. Merletti R. Standards for reporting EMG data. *J Electromyogr Kinesiol* 9: III-IV, 1999.

129. Merletti R, Conte LRL and Sathyan D. Repeatability of electrically-evoked myoelectric signals in the human tibialis anterior muscle. *J Electromyogr Kinesiol* 5: 67-80, 1995.

130. Merletti R, Fiorito A, Lo Conte LR and Cisari C. Repeatability of electrically evoked EMG signals in the human vastus medialis muscle. *Muscle Nerve* 21: 184-193, 1998.

131. Merletti R and Hermens HJ. Detection and conditioning of the surface EMG signal. In: Electromyography: physiology, engineering, and noninvasive applications, edited by Merletti R and Parker PA. New Jersey: John Wiley & Sons Inc, 2004, p. 107-131.

132. Merletti R, Knaflitz M and DeLuca CJ. Electrically evoked myoelectric signals. *Crit Rev Biomed Eng* 19: 293-340, 1992.

133. Merletti R and Parker PA. *Electromyography: physiology, engineering, and noninvasive applications*. New Jersey: John Wiley & Sons Inc, 2004.

134. Mero A and Komi PV. Force-, EMG-, and elasticity-velocity relationships at submaximal, maximal and supramaximal running speeds in sprinters. *Eur J Appl Physiol Occup Physiol* 55: 553-561, 1986.

135. Mero A and Komi PV. Electromyographic activity in sprinting at speeds ranging from sub-maximal to supra-maximal. *Med Sci Sports Exerc* 19: 266-274, 1987.

136. Merton PA. Voluntary strength and fatigue. *J Physiol* 123: 553-564, 1954.

137. Mesin L, Joubert M, Hanekom T, Merletti R and Farina D. A finite element model for describing the effect of muscle shortening on surface EMG. *IEEE Trans Biomed Eng* 53: 593-600, 2006.

138. Morimoto S. Effect of length change in muscle fibers on conduction velocity in human motor units. *Jpn J Physiol* 36: 773-782, 1986.

139. Moritani T, Muramatsu S and Muro M. Activity of motor units during concentric and eccentric contractions. *Am J Phys Med* 66: 338-350, 1988.

140. Moritani T, Muro M and Kijima A. Electromechanical changes during electrically induced and maximal voluntary contractions: electrophysiologic responses of different muscle fiber types during stimulated contractions. *Exp Neurol* 88: 471-483, 1985.

141. Moritani T, Stegeman DF and Merletti R. Basic physiology and biophysics of EMG signal generation. In: Electromyography: physiology, engineering, and noninvasive applications, edited by Merletti R and Parker PA. New Jersey: John Wiley & Sons, Inc, 2004, p. 1-25.

142. Ng JK and Richardson CA. Reliability of electromyographic power spectral analysis of back muscle endurance in healthy subjects. *Arch Phys Med Rehabil* 77: 259-264, 1996.

143. Nilsson J, Thorstensson A and Halbertsma J. Changes in leg movements and muscle activity with speed of locomotion and mode of progression in humans. *Acta Physiol Scand* 123: 457-475, 1985.

144. Noakes TD. The central governor model of exercise regulation applied to the marathon. *Sports Med* 37: 374-377, 2007.

145. Noakes TD, Peltonen JE and Rusko HK. Evidence that a central governor regulates exercise performance during acute hypoxia and hyperoxia. *J Exp Biol* 204: 3225-3234, 2001.

146. Noakes TD and St Clair GA. Logical limitations to the "catastrophe" models of fatigue during exercise in humans. *Br J Sports Med* 38: 648-649, 2004.

147. Nordander C, Willner J, Hansson GA, Larsson B, Unge J, Granquist L and Skerfving S. Influence of the subcutaneous fat layer, as measured by ultrasound, skinfold calipers and BMI, on the EMG amplitude. *Eur J Appl Physiol* 89: 514-519, 2003.

148. Nummela A, Rusko H and Mero A. EMG activities and ground reaction forces during fatigued and nonfatigued sprinting. *Med Sci Sports Exerc* 26: 605-609, 1994.

149. Paavolainen L, Nummela A, Rusko H and Hakkinen K. Neuromuscular characteristics and fatigue during 10 km running. *Int J Sports Med* 20: 516-521, 1999.

150. Pandolf KB. Differentiated ratings of perceived exertion during physical exercise. *Med Sci Sports Exerc* 14: 397-405, 1982.

151. Papapetropoulou V, Tsolakis J, Terzis S, Paschalis C and Papapetropoulos T. Neurophysiologic studies in peripheral arterial disease. *J Clin Neurophysiol* 15: 447-450, 1998.

152. Parr BM. *Exercise training in patients with peripheral vascular disease* (Dissertation). University of Cape Town, 2003.

153. Pasquet B, Carpentier A, Duchateau J and Hainaut K. Muscle fatigue during concentric and eccentric contractions. *Muscle Nerve* 23: 1727-1735, 2000.

154. Pattichis CS, Schofield I, Merletti R, Parker PA and Middleton LT. Introduction to this special issue. Intelligent data analysis in electromyography and electroneurography. *Med Eng Phys* 21: 379-388, 1999.

155. Pedrinelli R, Marino L, Dell'Omo G, Siciliano G and Rossi B. Altered surface myoelectric signals in peripheral vascular disease: correlations with muscle fiber composition. *Muscle Nerve* 21: 201-210, 1998.

156. Perakyla T, Tikkanen H, von Knorring J and Lepantalo M. Poor reproducibility of exercise test in assessment of claudication. *Clin Physiol* 18: 187-193, 1998.

157. Portney LG and Watkins MP. *Foundations of clinical research:Applications to practice*. New Jersey: Prentice Hall Health, 1990.

158. Potvin JR. Effects of muscle kinematics on surface EMG amplitude and frequency during fatiguing dynamic contractions. *J Appl Physiol* 82: 144-151, 1997.

159. Rainoldi A, Bullock-Saxton JE, Cavarretta F and Hogan N. Repeatability of maximal voluntary force and of surface EMG variables during voluntary isometric contraction of quadriceps muscles in healthy subjects. *J Electromyogr Kinesiol* 11: 425-438, 2001.

160. Rainoldi A, Galardi G, Maderna L, Comi G, Lo CL and Merletti R. Repeatability of surface EMG variables during voluntary isometric contractions of the biceps brachii muscle. *J Electromyogr Kinesiol* 9: 105-119, 1999.

161. Rainoldi A, Melchiorri G and Caruso I. A method for positioning electrodes during surface EMG recordings in lower limb muscles. *J Neurosci Methods* 134: 37-43, 2004.

162. Ricard MD, Hills-Meyer P, Miller MG and Michael TJ. The effects on bicycle frame geometry on muscle activation and power during a Wingate Anaerobic test. *J Sports Sci Med* 5: 25-32, 2006.

163. Ross WD and Marfell-Jones MJ. Kinanthropometry. In: Physiological testing of the high performance athlete, edited by MacDougall JD, Wenger HA and Green HJ. Champaign, Human Kinetics, 1991, p. 223-308.

164. Roy SH, De Luca CJ and Schneider J. Effects of electrode location on myoelectric conduction velocity and median frequency estimates. *J Appl Physiol* 61: 1510-1517, 1986.

165. Sarre G, Lepers R, Maffiuletti N, Millet G and Martin A. Influence of cycling cadence on neuromuscular activity of the knee extensors in humans. *Eur J Appl Physiol* 88: 476-479, 2003.

166. Scherer S. Peripheral arterial disease: effects on skeletal muscle. *Cardiopulm Phys Ther* 17: 3-8, 2006**.**

167. Scrimgeour AG, Noakes TD, Adams B and Myburgh K. The influence of weekly training distance on fractional utilisation of maximum aerobic capacity in marathon and ultramarathon runners. *Eur J Appl Physiol Occup Physiol* 55: 202-209, 1986.

168. Shiavi R, Bourne J and Holland A. Automated extraction of activity features in linear envelopes of locomotor electromyographic patterns. *IEEE Trans Biomed Eng* 33: 594-600, 1986.

169. Shiavi R, Bugle HJ and Limbird T. Electromyographic gait assessment, Part 1: Adult EMG profiles and walking speed. *J Rehabil Res Dev* 24: 13-23, 1987.

170. Sleivert GG and Wenger HA. Reliability of measuring isometric and isokinetic peak torque, rate of torque development, integrated electromyography, and tibial nerve conduction velocity. *Arch Phys Med Rehabil* 75: 1315-1321, 1994.

171. Sloniger MA, Cureton KJ, Prior BM and Evans EM. Lower extremity muscle activation during horizontal and uphill running. *J Appl Physiol* 83: 2073-2079, 1997.

172. Soderberg GL and Knutson LM. A guide for use and interpretation of kinesiologic electromyographic data. *Phys Ther* 80: 485-498, 2000.

173. St Clair GA, Lambert ML and Noakes TD. Neural control of force output during maximal and submaximal exercise. *Sports Med* 31: 637-650, 2001.

174. St Clair GA and Noakes TD. Evidence for complex system integration and dynamic neural regulation of skeletal muscle recruitment during exercise in humans. *Br J Sports Med* 38: 797-806, 2004.

175. St Clair GA, Schabort EJ and Noakes TD. Reduced neuromuscular activity and force generation during prolonged cycling. *Am J Physiol Regul Integr Comp Physiol* 281: R187-R196, 2001.

176. Strandness DE. Peripheral arterial system. In: Duplex scanning in vascular disorders, New York: Raven Press, 1993, p. 159-195.

177. Tan KH, De Cossart L and Edwards PR. Exercise training and peripheral vascular disease. *Br J Surg* 87: 553-562, 2000.

178. Taylor AD and Bronks R. Reproducibility and validity of the quadriceps muscle integrated electromyogram threshold during incremental cycle **ergometry. *Eur J Appl Physiol Occup Physiol* 70: 252-257, 1995.**

179. Tesch PA, Dudley GA, Duvoisin MR, Hather BM and Harris RT. Force and EMG signal patterns during repeated bouts of concentric or eccentric muscle actions. *Acta Physiol Scand* 138: 263-271, 1990.

180. Todd G, Taylor JL and Gandevia SC. Measurement of voluntary activation of fresh and fatigued human muscles using transcranial magnetic stimulation. *J Physiol* 551: 661-671, 2003.

181. Trontelj JV, Jabre J and Mihelin M. Needle and Wire Detection Techniques. In: Electromyography: physiology, engineering, and noninvasive applications, edited by Merletti R and Deluca CJ. New Jersey: John Wiley & Sons, Inc, 2004, p. 27-46.

182. Tucker R, Kayser B, Rae E, Raunch L, Bosch A and Noakes T. Hyperoxia improves 20 km cycling time trial performance by increasing muscle activation levels while perceived exertion stays the same. *Eur J Appl Physiol* 101: 771-781, 2007.

183. Tucker R, Rauch L, Harley YX and Noakes TD. Impaired exercise performance in the heat is associated with an anticipatory reduction in skeletal muscle recruitment. *Pflugers Arch* 448: 422-430, 2004.

184. Ulmer HV. Concept of an extracellular regulation of muscular metabolic rate during heavy exercise in humans by psychophysiological feedback. *Experientia* 52: 416-420, 1996.

185. Vandewalle H, Maton B, Le Bozec S and Guerenbourg G. An electromyographic study of an all-out exercise on a cycle ergometer. *Arch Int Physiol Biochim Biophys* 99: 89-93, 1991.

186. Vigreux B, Cnockaert JC and Pertuzon E. Factors influencing quantified surface EMGs. *Eur J Appl Physiol Occup Physiol* 41: 119-129, 1979.

187. Viitasalo JH and Komi PV. Signal characteristics of EMG with special reference to reproducibility of measurements. *Acta Physiol Scand* 93: 531-539, 1975.

188. Viitasalo JH and Komi PV. Signal characteristics of EMG during fatigue. *Eur J Appl Physiol Occup Physiol* 37: 111-121, 1977.

189. Vuorimaa T, Virlander R, Kurkilahti P, Vasankari T and Hakkinen K. Acute changes in muscle activation and leg extension performance after different running exercises in elite long distance runners. *Eur J Appl Physiol* 96: 282-291, 2006.

190. Wagenmakers AJ. Role of amino acids and ammonia in mechanisms of fatigue. In: Muscle fatigue mechanism in exercise and training, edited by Marconnet P. Basel, Switzerland: Karger. Med & Sport Science, 1992, p. 69-87.

191. Weir JP, Beck TW, Cramer JT and Housh TJ. Is fatigue all in your head? A critical review of the central governor model. *Br J Sports Med* 40: 573-586, 2006.

192. Weitz JI, Byrne J, Clagett GP, Farkouh ME, Porter JM, Sackett DL, Strandness DE, Jr. and Taylor LM. Diagnosis and treatment of chronic arterial insufficiency of the lower extremities: a critical review. *Circulation* 94: 3026-3049, 1996.

193. Westing SH, Cresswell AG and Thorstensson A. Muscle activation during maximal voluntary eccentric and concentric knee extension. *Eur J Appl Physiol Occup Physiol* 62: 104-108, 1991.

194. Winter DA. *The biomechanics and motor control of human gait: Normal, elderly and pathological*. Waterloo: University of Waterloo Press, 1991.

195. Winter DA, Fuglevand AJ and Archer SE. Crosstalk in surface electromyography: theoretical and practical estimates. *J Electromyogr Kinesiol* 4: 15-26, 1994.

196. Winter DA and Yack HJ. EMG profiles during normal human walking: stride-to-stride and inter-subject variability. *Electroencephal Clin Neurophysiol* 67: 402-411, 1987.

197. Yang JF and Winter DA. Electromyography reliability in maximal and submaximal isometric contractions. *Arch Phys Med Rehabil* 64: 417-420, 1983.

198. Yang JF and Winter DA. Electromyographic amplitude normalisation methods: improving their sensitivity as diagnostic tools in gait analysis. *Arch Phys Med Rehabil* 65: 517-521, 1984.

199. Zazula D, Karlson S and Doncarli C. Advanced signal processing techniques. In: Electromyography: physiology, engineering, and noninvasive

applications, edited by Merletti R and Parker PA. New Jersey: John Wiley & Sons Inc, 2004, p. 259-304.

200. Zipp P. Recommendations for the standardisation of lead positions in surface electromyography. *Eur J Appl Physiol* 50: 41-54, 1982.

201. Zuniga EN, Truong XT and Simons DG. Effects of skin electrode position on averaged electromyographic potentials. *Arch Phys Med Rehabil* 51: 264-272, 1970.

APPENDIX

Informed Consent for Running Trials

Department Of Human Biology, Faculty Of Health Sciences
University Of Cape Town

UCT/MRC Research Unit of Exercise Science and Sports Medicine

Measurement of EMG activity during maximal running

Informed consent

The MRC / UCT Research Unit for Exercise Science and Sports Medicine will be conducting a study to measure EMG activity of the active leg muscles during running till exhaustion. This means that we want to find out how your various muscle groups are recruited during maximal tests until exhaustion occurs.

In order to do this, we will require you to perform three maximal tests until exhaustion. You will be required to come into the Sports Science Institute of South Africa on 4 occasions.

On the first visit

PRELIMINARY TESTING

- You will be required to fill out a Quality of Health Questionnaire, this questionnaire will ask you questions about your physical function etc ;
- We will measure your weight, height and percentage of body fat, determined by measuring 7 skinfolds, including triceps, biceps, suprailiac, subscapular, calf, thigh and abdomen. This is a non-invasive test for the estimation of body fat.
- You will be familiarised with the treadmill and then you have to perform a progressive treadmill test to determine your peak treadmill running speed (PTRS). The gradient will be set at zero and the treadmill speed will start at a speed of 10 $km.h^{-1}$. Every 30 seconds, treadmill speed will be increased by 0.5 $km.h^{-1}$ until you are unable to maintain the pace of the treadmill. The PTRS is defined as the fastest running speed you can maintain for 30 seconds.

- You will practise performing maximal voluntary contractions of your quadricep, hamstring and calf muscles of your right leg. We will measure you **maximal voluntary contraction** using the Biodex Dynamometer 3 (Biodex Medical Systems, New York) **–** where you will be required to push against a non-moving arm attachment using muscles in your leg. Your hips, thighs and upper body will be firmly strapped to the seat of the dynamometer. Your arm position for this test is standardised, where you have to cross your arms over your chest. This test will be conducted with your knee resting at a 60° angle from full leg extension (straightened leg) for the thigh and hamstring muscles. Whereas for the calf muscles, the leg will be elevated forming a 30° angle at the knee between the upper and lower leg and the foot firmly strapped against a foot-plate. You have to push against the foot-plate using your calf muscles. A standardise warm up before the maximal voluntary contraction includes four contractions of the knee and calf extensors at 50 %, 60 %, 70 % followed by 80 % of your subjective maximum. The test included three maximum voluntary contractions (MVC) of 5 seconds each separated by 60 second intervals. You will be verbally motivated so as to encourage you to achieve your maximum potential. The purpose of the MVC allows measurement of muscle recruitment patterns during the running test to be expressed as a percentage of this method.

- You will be familiarised with the pacing lights in the gym. You have to keep up with speed of the lights which are placed on the inner circumference of the track at an interval of 7 m and can be set to blink sequentially at a given running velocity. The pacing lights will be set to mimic the Peak Treadmill Running Speed test where the speed will start at 10 $km.h^{-1}$. Every 30 seconds, the pacing light speed will be increased by 0.5 $km.h^{-1}$ until exhaustion. The peak speed maintained 30 seconds prior to test termination will be taken as the PRS.

EXPERIMENTAL TESTING

After preliminary testing, the actual study, consisting of 3 trials separated by 5-7 days (in which you have to maintain your training and no hard training within 24hrs of testing). The protocol for all trials are the exact same

When you arrive at the lab for the trials the following will be **measured prior to the start of the exercise test**

- **EMG electrodes will be placed on your 6 muscles**- Two electrodes which measures your muscle activity will be placed onto the skin surface of each of the following lower limb muscles during all the tests; Vastus lateralis (VL), Vastus medialis (VM), Rectus femoris (RF), Biceps femoris (BF) and Medial and Lateral gastrocnemius (MG and LG respectively). Prior to placing the electrodes on the skin, the skin over the muscle will be shaved and cleaned with alcohol. Two electrodes will be was placed on the belly of each muscle with an inter-electrode distance of 10 mm and carefully taped.

- We will measure you **maximal voluntary contraction** using the Biodex Dynamometer 3 (Biodex Medical Systems, New York) – where you will be required to push against a non-moving arm attachment using muscles in your leg. Your hips, thighs and upper body will be firmly strapped to the seat of the dynamometer. Your arm position for this test is standardised, where you have to cross your arms over your chest. This test will be conducted with your knee resting at a 60° angle from full leg extension (straightened leg) for the thigh and hamstring muscles. Whereas for the calf muscles, the leg will be elevated forming a 30° angle at the knee between the upper and lower leg and the foot firmly strapped against a foot-plate. You have to push against the foot-plate using your calf muscles. A standardise warm up before the maximal voluntary contraction includes four contractions of the knee and calf extensors at 50 %, 60 %, 70 % followed by 80 % of your subjective maximum. The test included three maximum voluntary contractions (MVC) of 5 seconds each separated by 60 second intervals. You will be verbally motivated so as to encourage you to achieve your maximum potential. The purpose of the MVC allows measurement of muscle recruitment patterns during the GTET test to be expressed as a percentage of this method.

- Perform a 2 X 20 m maximal sprint on the gym track

- Perform the peak running speed (PRS) test on the gym track, where the speed of the pacing light will start at 10 $km.h^{-1}$. Every 30 seconds, the pacing light speed will be increased by 0.5 $km.h^{-1}$ until exhaustion. The peak speed maintained 30 seconds prior to test termination will be taken as the PRS.

- Following a 20 minute resting period the runners will then perform a submaximal run on the track at 70% of PRS for 5 minutes.

- Heart rate (Polar ® Heart Rate Monitors, Polar Electro OY) will be measured throughout the trial

Because each trial is a maximum all-out effort, you will be requested to refrain from doing any high intensity exercise the day before testing. You should also make sure that you eat and drink as you normally do before the trials. Also, you should attempt to keep your own personal training routine constant for the time it takes to complete the 3 trials.

Possible risks of participation

There are no risks involved in the measurement of heart rate and EMG.

The exercises itself carries no major risks other and is no more risky than performing recreational exercise at a high intensity. Additionally, each trial will be supervised and aspects such as your heart rate will constantly be recorded and monitored by means of sophisticated equipment.

Statement of understanding and consent:

I confirm that the exact procedure and techniques, and possible complications of the above tests have been thoroughly explained to me. I am free to withdraw from the study at any time, should I choose to do so. I understand that I may ask questions at any time during the testing procedure. I know that the personal information required by the researchers and derived from the testing procedure will remain strictly confidential and will only be revealed as a number in statistical analysis.
I have carefully read this form and understand the nature, purpose and procedures of this study. I agree to participate in this research project of the MRC / UCT Research Unit for Exercise Science and Sports Medicine

Name of volunteer: ……..

Signature of volunteer: ……

Name of investigator: ……..

Signature of investigator: ……..

Date: ……

Department Of Human Biology, Faculty of Health Sciences
University of Cape Town, South Africa
UCT/MRC Research Unit for Exercise Science and Sports Medicine

INFORMED CONSENT

"Skeletal muscle in patients with peripheral vascular disease before and after angioplasty"

Dear Subject

The UCT/MRC Research Unit for Exercise Science and Sports Medicine is conducting a study to investigate the effect angioplasty on skeletal muscle recruitment during a graded treadmill exercise test until exhaustion (maximal test). This simply means that we want to examine how your muscle activity changes before and after blood flow is restored to your lower limb.

In order to examine "how your skeletal muscle and body responds" to the restoration of blood flow, we will take the following measurements before, during and after your tests:

PRELIMINARY TESTING

- Medical history recorded by the medical practitioner who is a co-investigator of this study, based at the Sports Science Institute of South Africa
- Anthropometrical measurements including body weight, height and skinfolds (triceps, biceps, suprailiac, subscapular, calf, thigh and abdomen);
- Familiarisation to treadmill running
- Familiarisation to rating on the Borg category ratio scale (Rating of Perceived Exertion Scale)
- Familiarisation to rating on the Perception of Pain Scale
- Measurement of the ankle brachial blood pressure index meaning we will measure your blood flow at the ankle and calculate ankle brachial blood pressure index
- Measure heart rate using electrocardiogram (ECG)

- Measure blood flow non-invasively in affected limb using the Colour Duplex Doppler at Kingsbury Hospital

Experimental Testing

- **Trial 1: Graded treadmill exercise test till exhaustion (GTET)**

  Please note the test will be supervised by a qualified medical doctor from our department.

  Prior to exercise test the following will be performed*:*

  - Measure ankle blood pressure to calculate ankle brachial index
  - Shave your leg to attach the EMG electrodes to your skin so as to measure muscle activity during the maximal voluntary contraction and the exercise test
  - Resting venous blood sample (two millilitres) will be drawn from the forearm vein

  During the GTET the following measurements will also be performed:

  - Muscle activity of the 6 muscles mentioned above (using the EMG system)
  - Heart rate will be recorded continuously using the electrocardiogram monitor;
  - Effort perception (RPE) and perception of pain every 2 minutes;
  - Blood pressure every 2 minutes
  - Heart rate measures every 2 minutes
  - Measure the amount of oxygen you breath in and the carbon dioxide you breath out
  - Pain free walking distance
  - Maximal walking distance

  After the GTET the following measurements will also be performed:

  - Measure ankle brachial blood pressure
  - Measure ankle brachial index
  - venous blood sample (two millilitres) will be drawn from the forearm vein at 3, 4 and 5 minutes post exercise test

- **Trial 2: Graded treadmill exercise test till exhaustion (GTET)**
- **To be conducted 3 days after angioplasty**

  Please note the test will be supervised by a qualified medical doctor

- The test will follow 3 days after angioplasty

***Prior to exercise test the following measurements will be performed**:*

-Blood flow using the Color Duplex Doppler at Kingsbury Hospital

- Measure ankle blood pressure to calculate ankle brachial index
- Shave your leg to attach the EMG electrodes to your skin so as to measure muscle activity during the maximal voluntary contraction and the exercise test
- Resting venous blood sample (two millilitres) will be drawn from the forearm vein

During the GTET the following measurements will also be performed:

- Muscle activity of the 6 muscles mentioned above (using the EMG system)
- Heart rate will be recorded continuously using the electrocardiogram monitor;
- Effort perception (RPE) and perception of pain every 2 minutes;
- Blood pressure every 2 minutes
- Heart rate measures every 2 minutes
- Measure the amount of oxygen you breath in and the carbon dioxide you breath out
- Pain free walking distance
- Maximal walking distance

After the GTET the following measurements will also be performed:

- Measure ankle brachial blood pressure
- Measure ankle brachial index
- venous blood sample (two millilitres) will be drawn from the forearm vein at 3, 4 and 5 minutes post exercise test

Possible risks participating in this trial

The complications associated with exercise testing are relatively low (ACSM, 2000), however you need to be aware that you are at an increased risk when performing exercise. There are possible risks associated with exercise testing; you may experience arrhythmia (irregular, fast or slow heart rhythm), possible plaque rupture, acute thrombosis, drop in blood pressure, ischaemia (decreased oxygen to the

heart), pain and/or cramps in lower limbs, claudication, fatigue, shortness of breath and in very rare instances heart attack, stroke or death. Every effort will be made to minimize these risks by evaluation of preliminary information relating to your health and fitness and specifically throughout observations during your testing and by ensuring trained medical supervision throughout the trial. In addition to minimising the above mentioned risks, emergency equipment and trained personnel will be available to deal with unusual situations should they arise.

Blood will be sampled by means of placing a cannula in the forearm vein and will flush with sterile saline solution after each blood sampling time-point. Sterile techniques will be used, and the cannula will be inserted by a medical doctor. Risks associated with the drawing of blood include discomfort, infection (wound sepsis), muscle bruising (haematoma formation) and numbness (peripheral subcutaneous nerve injury). The risks will be minimized by cleaning the area around the vein with a disinfectant alcohol swap before the blood will be drawn. Immediately afterwards a protective plaster will be applied.

There are no risks involved in the measurement of heart rate and EMG, except as otherwise stated in association with exercise.

I,_____clearly understand the nature of the study and the possible risks associated with the trial. I also understand the extend of each procedure as described in the information sheet and listed above;

I confirm that the exact procedure and techniques, and possible complications of the research trial have been thoroughly explained to me. I am aware that participation in this research study is absolutely voluntary and that I am free to withdraw from the study at any time without stating a reason and without prejudice. I am also aware that the doctor or researcher can withdraw me from the study. I am aware that there will be trained medical supervision throughout the trial. In addition to minimising the above mentioned risks, emergency equipment and trained personnel will be available to deal with unusual situations should they arise.

I know that any personal information required by the researchers and derived from the testing procedure will remain strictly confidential and will only be revealed as a number in statistical analysis.

I have carefully read this form together with the information sheet and understand the nature, purpose and procedures of this study. I agree to participate in this research project of the UCT/MRC Research Unit for Exercise Science and Sports Medicine.

Name of volunteer: ……..

Signature of volunteer: ……

Name of investigator: ……..

Signature of investigator: ……..

Date: ……

Modified Physical Activity Readiness Questionnaire (PAR-Q)

Name			**Date**
DOB	**Age**	**Home Phone**	**Work Phone**

Regular exercise associated with many health benefits, yet any change of activity may increase the risk of injury. Completion of this questionnaire is a first step when planning to increase the amount of physical activity in your life. Please read each question carefully and answer every question honestly:

Yes	No	1) Has a physician ever said you have a heart condition and you should only do physical activity recommended by a physician?
Yes	No	2) When you do physical activity, do you feel pain in your chest?
Yes	No	3) When you were not doing physical activity, have you had chest pain in the past month?
Yes	No	4) Do you ever lose consciousness or do you lose your balance because of dizziness?
Yes	No	5) Do you have a joint or bone problem that may be made worse by a change in your physical activity?
Yes	No	6) Is a physician currently prescribing medications for your blood pressure or heart condition?
Yes	No	7) Are you pregnant?
Yes	No	8) Do you have insulin dependent diabetes?
Yes	No	9) Are you 69 years of age or older?
Yes	No	10) Do you know of any other reason you should not exercise or increase your physical activity?

If you answered yes to any of the above questions, talk with your doctor BEFORE you become more physically active. Tell your doctor your intent to exercise and to which questions you answer yes.

Anthropometrical and Information Work Sheet for cyclist

Name: _____Date:_____

DOB: _____

Weight: _____Height: _____

EMG & Battery: Day 1_____ Day 2:_____ Day 3:_____

Skinfold Measurements:

Anatomical Areas	**1**	**2**	**Avg**
Triceps **Biceps** **Subscap** **Supra iliac** **Abdomen** **Thigh** **Calf** **Mid-Thigh circumference** **Upper_____, Middle_____,** **Lower_____**			

PPO 1 (__/___/_): File Name_____ Watts:_____

PPO 2 (__/___/_): File Name_____ Watts:_____

PPO 3(__/___/_): File Name_____ Watts:_____

Chair Settings:

Chair length:Dyno length:

Chair height:Dyno height:

Leg attachment length:Bike Settings: seat height-

Ankle length:chair height-Handle bar-

Anthropometrical and Information Work Sheet for Runners

Name: _____Date:_____

DOB: _____

Weight: _____Height: _____

Skinfold Measurements:

Anatomical Areas	**1**	**2**	**Avg**
Triceps **Biceps** **Subscap** **Supra iliac** **Abdomen** **Thigh** **Calf** **Mid-Thigh circumference** **Upper_____, Middle_____,** **Lower_____** **Length of quad _____**			

Peak treadmill running speed: _____, Time: _____

Biodex Chair Settings:

Chair length:Dyno length:

Chair height:Dyno height:

Leg attachment length:

Ankle length:Leg rest-across:

- down:

	Sprint Trial 1 (s)		**Trial 2 (s)**		**Trial 3 (s)**	
10 m						
20 m						

Anthropometrical and Information Work Sheet for patients with PVD

Name: _____Date:_____

DOB: _____

Weight: _____Height: _____

Trial 1:Trial 2

ABI preABI pre

ABI post ABI post

Skinfold Measurements:

Anatomical Areas	**1**	**2**	**Avg**
Triceps **Biceps** **Subscap** **Supra iliac** **Abdomen** **Thigh** **Calf** **Mid-Thigh circumference** **Upper_____, Middle_____,** **Lower_____**			

Chair Settings:

Seat length

Chair length:Dyno length:

Chair height:Dyno height:

Leg attachment length:

Down

Across

Ankle length:

Leg Support:

PAIN SCALE

0	No pain
1	Very Very Slight
2	Very Slight
3	Slight
4	Mild
5	Moderate
6	Moderate to Severe
7	Severe
8	Very Severe
9	Very Very Severe
10	Maximum

BORG SCALE OF PERCEIVED EXERTION

6	
7	Very, Very Light
8	
9	Very Light
10	
11	Fairly Light
12	
13	Somewhat Hard
14	
15	Hard
16	
17	Very Hard
18	
19	Very, Very Hard
20	