THE UNIVERSITY OF QUEENSLAND

AUSTRALIA

The Relationships between Muscle Fibre Composition, Muscle Carnosine and Multiple Sprint (Wingate) Performance

Rick Alexander Dann

Bachelor of Exercise and Sport Science (Honours)

A thesis submitted for the degree of Master of Philosophy at

The University of Queensland, School of Human Movement Studies in 2019

ABSTRACT

Muscle Fibre Composition (MFC) and resting carnosine levels are both important characteristics of skeletal muscle which contribute towards performance and fatigue during high-intensity exercise. It's well documented that type II fibres are fast contracting, fast fatiguing, and are characterised by a high metabolic capacity for anaerobic metabolism. Despite the ability to produce higher forces, individuals with a relatively high proportion of type II muscle fibres are likely to experience more acidosis within the muscle. At the cellular level, type II fibers also have higher resting levels of carnosine. Carnosine, a buffer of hydrogen ions, is therefore important in delaying the rate of fatigue during high-intensity exercise. Higher levels of muscle carnosine found in some athletic individuals has largely been attributed to genetic factors such as type II fibres, however few studies have examined this relationship. Therefore, the main purpose of this thesis was to examine potential relationships between MFC, resting carnosine levels, and multiple sprint (Wingate) performance.

Twenty-six healthy males (25 ± 4 years) were recruited for this study. Each participant had a muscle biopsy taken from the Vastus Lateralis (VL) to determine Muscle Fibre Composition (MFC) and resting muscle carnosine levels. After sufficient recovery from the biopsy procedure, participants completed 3 x 30second sprints (Wingate bouts) on a cycle ergometer; each separated by 4 minutes of rest. Mean Power (MP), anaerobic capacity (AC), peak power (PP), total work (TW) and a fatigue index (FI) was calculated for each Wingate. The statistical analysis included descriptive statistics, Pearson correlation and linear regressions

Resting muscle carnosine levels were not related to percentage of type II fibres ($r= 0.056$; $p= 0.77$), percentage area of type II fibres ($p= 0.83$; $r= 0.048$) or relative fibre area ($p= 0.77$; $r= 0.065$). In addition, resting carnosine levels were not related to any performance or fatigue measures in the repeated Wingate protocol. Significant relationships were found between both percentage area of type II fibres ($p =0.043$; $r = 0.04$) and percentage of type II fibres ($p =0.0432$; $r = 0.4075$) with fatigue index during the third Wingate bout. There were no significant relationships between MFC and fatigue in the first Wingate, however percentage of type II fibres was significantly related to the fatigue profile during the last 15 seconds of the 2nd ($r=-0.42$; $p= 0.036$) and 3rd ($r= -0.44$; $p= 0.028$) Wingate sprints.

In summary, resting carnosine levels were not related to MFC nor any performance measure from the repeated Wingate protocol. This study did show however that individuals with a higher percentage of type II fibres may show greater levels of fatigue and decreased performance in the latter stages of maximal anaerobic exercise.

DECLARATION BY AUTHOR

This thesis is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

I have clearly stated the contribution of others to my thesis as a whole, including statistical assistance, survey design, data analysis, significant technical procedures, professional editorial advice, financial support and any other original research work used or reported in my thesis. The content of my thesis is the result of work I have carried out since the commencement of my higher degree by research candidature and does not include a substantial part of work that has been submitted *to qualify for the award of any* other degree or diploma in any university or other tertiary institution. I have clearly stated which parts of my thesis, if any, have been submitted to qualify for another award.

I acknowledge that an electronic copy of my thesis must be lodged with the University Library and, subject to the policy and procedures of The University of Queensland, the thesis be made available for research and study in accordance with the Copyright Act 1968 unless a period of embargo has been approved by the Dean of the Graduate School.

I acknowledge that copyright of all material contained in my thesis resides with the copyright holder(s) of that material. Where appropriate I have obtained copyright permission from the copyright holder to reproduce material in this thesis and have sought permission from co-authors for any jointly authored works included in the thesis.

Rick Dann

PUBLICATIONS INCLUDED IN THIS THESIS

No publications included

SUBMITTED MANUSCRIPTS DURING CANDIDATURE

No manuscripts submitted for publication

OTHER PUBLICATIONS DURING CANDIDATURE

Conference Abstract: Australian Strength and Conditioning Association (ASCA) International Conference 2019: Abstract for Poster Presentation – "The relationship between Muscle Fibre Composition and fatigue during maximal anaerobic exercise"

CONTRIBUTIONS BY OTHER AUTHORS

Vincent Kelly – Development and design of project, funding, drafting written thesis work

David Jenkins – Drafting thesis writing, interpretation of research data

RESEARCH INVOLVING HUMAN SUBJECTS

Ethics Approval Number: 2019991335

Approving Committee: University of Queensland Human Research Ethics Committee A

See appendix for Ethics Approval Letter

ACKNOWLEDGEMENTS

Firstly, a massive thank you to each and every participant involved in this study! I thank you for your amazing efforts in the Wingate sessions and for your courage during the muscle biopsy procedure. Without your generosity and commitment, this study would not have been possible. I'm extremely grateful for your time, effort and your willingness to donate your muscle for research!

To Dr Vince Kelly, aka Captain Holt; you have been my fearless leader and driving force throughout this MPhil. There's no question that without your support, this thesis would not be possible. I cannot express how thankful I am for your endless support since day 1. Despite the many setbacks and challenges faced over the last 2 years, you've always backed this project and you've always backed me. Your guidance and support has been more than I could have ever asked for. You took the time to understand my 'WHY' at the start of this MPhil and as a result, have developed my knowledge in every avenue of research. I greatly appreciate your persistence (especially during my 900 ethics applications), your patience (while you helped develop my horrible writing ability), but most importantly, I appreciate the large spike in shakas featuring on your social media and your newly perfected technique. Keep up the great work! The skills and lessons you've taught me, will stay with me throughout my whole career. I am extremely grateful for the opportunity to learn from you and I feel very lucky to have had you shape the researcher I've become. I feel truly honoured to have you as my Captain and I know the respect is mutual throughout the whole of the 241st Precinct – Cheers, Boyle

To Associate Professor David Jenkins; the man I owe thanks to for the last 6 years of my academic life. You have without a doubt been the most influential person in my young sport science career. My thanks starts in 2nd year of Undergraduate studies after your class opened my eyes to the true beauty that is Exercise Physiology, which ultimately lead to changing degrees from PE teaching to Sport Science; a change which has allowed me to love what I do every day and pursue a career I'm truly passionate about. Your guidance, enthusiasm, positivity and continuous support has been extremely motivating. Having the opportunity to discuss ideas and have access to your abundance of knowledge over the last 2 years has been invaluable. I am truly thankful for the contribution you've had to not only this thesis, but also my research skills, critical thinking ability, teaching style and leadership skills. Most importantly, you have taught me the influence you can have on people's lives by simply sharing your passion; a lesson which I will hold with me for the rest of my life.

To the 241 Crew (past and present); who have been by my side in the trenches since day 1. Many of you were my role models in my final year of Undergrad and have now become some of my closest friends. The support and encouragement we've shown for each other is something I believe all research groups and offices could learn a lot from. I feel so incredibly grateful coming into the office every day and knowing my favourite people will be there to either share stoke of the little wins or help get through a rough day. The 241 environment has been truly special and I know it's something I'm going to miss like crazy in the future. No other office would be so accepting of a shaka throwing, inappropriate, trash talking, tuppawear hoarding, sober sally, who spends more time sleeping under his desk than working on it. So thank you for putting up with me and my many strengths. Along with all the amazing memories we've had, I'm mostly going to miss Suzy's laughing fits, Marni's snorting, Pete's classical music and Charlie's 'Slippery when Wet' sign. All I have left for you all, is that I love you… and I miss you… and I really want you to call me back… HHHMMMMMMMM

Lastly, to my family; the reason I am the luckiest person on earth. Words can't explain how grateful I am to have you all in my life. Mum, Charlie, Grandma, Grandad, Grogan family and the Anderson family – the support you've shown me during this research is greatly appreciated!

Mum and Charlie; thank you for everything you do. I could spend two more years writing another thesis on how you've helped me and what you've sacrificed to help me get to where I am today. Thank you for your continuous support, not just over the last 2 years, but over my whole 18 years of schooling. Thank you also for trying to understand what my MPhil is about even in times where I had no idea. Through all of the ups and downs you have both been the foundation that has kept me going. You are my motivation to get better every day and the reason I get to follow my passion, so for that, I thank you!

Grandad, this is for you x

FINANCIAL SUPPORT

This research was supported by the 2018 Faculty of Health and Behavioural Sciences Research Collaboration Seeding Grant

KEYWORDS

Carnosine, Muscle Fibre Composition, Wingate, Anaerobic Performance, Fatigue

AUSTRALIAN AND NEW ZEALAND STANDARD RESEARCH CLASSIFICATIONS (ANZSRC)

ANZSRC code: 110602 Exercise Physiology, 100%

FIELDS OF RESEARCH (FOR) CLASSIFICATION

FOR code: 1106, Human Movement and Sports Science, 100%

Table of Contents

1.0 Abbreviations **11**

2.0 General Introduction **12**

3.0 Literature review **14**

3.1 Fatigue 14

3.1.1 Mechanisms of Fatigue: 14

3.2 Muscle Fibre Composition and Fatigue 14

3.2.1 Muscle Fibre Composition and Performance 15

3.3 Determining Muscle Fibre Composition 16

3.3.1 Assessing muscle fibre type from Biopsy Samples 18

3.3.2 MFC Variability and Morphology 19

3.3.3 Different Classifications of Fibres 20

3.3.4 Contractile and Metabolic Characteristics 20

3.4 Muscle Carnosine and Fatigue 22

3.4.1 Carnosine and Wingate-induced Fatigue 22

3.5 Muscle Carnosine 23

3.5.1 Carnosine in type II fibres 23

3.5.2 Dietary effects on resting carnosine levels 24

3.5.3 Training Effects on Resting Muscle Carnosine Levels 25

4.0 The Relationship between Muscle Fibre Composition, Muscle Carnosine and Multiple Sprint (Wingate) Performance **28**

4.1 Introduction 28

4.2 Methods 29

4.2.1 Participants 29

4.2.2 Recruitment 29

4.3 Experimental Overview 29

4.4 Muscle Biopsy Session 30

4.4.1 Pre- Muscle Biopsy 30

4.4.2 Muscle Biopsy Procedure 30

4.4.3 Biopsy Sample Storage 30

4.4.4 Biopsy Analysis 30

4.4.5 Carnosine Analysis 32

4.5 Wingate Testing Session 33

4.5.1 Anthropometric Measures 33

4.5.2 LODE Protocol Setup 33

4.5.3 Bike Setup 33

4.5.4 Testing Instructions 34

4.5.5 Protocol 34
4.6 Data Collection 34
4.6.1 Wingate Outcome Measures 34
4.6.1 Data Analysis: 35
5.0 Results 36
6.0 Discussion 46
6.1 MFC and Resting Carnosine 46
6.2 Carnosine and Sprint Performance 47
6.1.1 Carnosine and Fatigue during Latter Stages of the Wingate Tests 49
6.3 MFC and Sprint Performance 50
6.3.1 MFC and Fatigue in Latter Stages 52
6.4 Wingate Performance and Fatigue 52
6.5 Practical Applications 53
6.6 Limitations and Future Directions 54
7.0 Conclusions 55
8.0 List of References 56
9.0 Appendices 62
9.1 Wingate Mean Cohort Data 62
9.2 Mean Wingate Performance Measures 64
9.3 Mean Wingate Fatigue Graphs 65
9.4 Fatigue Profile and Peak Power 67
10.0 Conference Abstracts 68
10.1 ASCA International Conference Abstract 68
10.1.1 ASCA International Conference Poster 70
10.2 UQ HMNS 2019 Conference Abstract 71
10.3 UQ HMNS 2018 Conference Abstract 72
11.0 Supporting Material 73
10.1 Participant Information Sheet 73
11.2 Informed Consent Form 75
11.3 Muscle Biopsy Information Sheet 76
11.4 Muscle Biopsy Screening Form 77
11.5 Post-Biopsy Care Form 78
11.6 Recruitment Poster 79
11.7 Ethics Approval Letter 80

LIST OF FIGURES AND TABLES

Figures

Figure 1	Histochemical Staining (image)	31
Figure 2	Repeated Wingate Protocol	33
Figure 3	Muscle Carnosine vs % Type II fibres correlation	37
Figure 4	Muscle Carnosine vs % Area occupied by Type II fibres correlation	38
Figure 5	Muscle Carnosine vs Relative Fibre Area correlation	39
Figure 6	Mean Wingate Performance	41
Figure 7	Fatigue Index vs % Type II Fibres correlation	43
Figure 8	Fatigue Index vs % Area occupied by Type II Fibres correlation	44
Figure 9	Mean Power and % Type II fibres (latter stages of W2+W3) correlations	45
Figure 10	Wingate Mean Cohort Data	62
Figure 11,12,13	Mean Wingate Fatigue Graphs x 3	65
Figure 14,15	Fatigue Profile vs Peak Power Correlations	67

Tables

Table 1	Participant Characteristics	29
Table 2	Raw Muscle Biopsy Data	36
Table 3	Performance Data from all 3 Wingate Tests	40
Table 4	Correlations between Carnosine and Wingate measures	42
Table 5,6,7	Mean Wingate Performance Measures x 3	64

1.0 Abbreviations

ADP	Adenosine DiPhosphate
ATP	Adenosine TriPhosphate
AC	Anaerobic Capacity
FI	Fatigue Index
FS	Fatigue Slope
HPLC	High Performance Liquid Chromatography
H+	Hydrogen
^{1}H-MRS	Magnetic Resonance Spectroscopy
MF	Muscle Fibre
MFT	Muscle Fibre Type
MFC	Muscle Fibre Composition
MP	Mean Power
MPP	Mean Peak Power
MRI	Magnetic Resonance Imaging
P	Phosphate
PP	Peak Power
Pcr	Phosphocreatine
TW	Total Work
VL	Vastus Lateralis

2.0 General Introduction

Muscle Fibre Composition (MFC) and resting carnosine levels are both important characteristics of skeletal muscle that contribute towards performance and fatigue during high intensity exercise (Begum, Cunliffe, & Leveritt, 2005; Green, 1997; Sahlin, Alvestrand, Bergstrom, & Hultman, 1977). Although there are various other mechanisms that contribute to fatigue, significant relationships between intramuscular buffer capacity, high intensity exercise performance and fatigue have been identified (Bell & Wenger, 1988; Nevill, Boobis, Brooks, & Williams, 1989). The available evidence suggests that individuals who have the capacity to manage and/or regulate H+ ions that are produced during intense exercise may be able to delay acidosis and potentially improve sprint and high intensity exercise performance.

The rate at which H+ ions are produced in muscles during high intensity exercise is related to the type of muscle fibres recruited. When describing the physiological characteristics of muscle fibres, type I and II fibres each exhibit distinct metabolic and contractile characteristics. Type I muscle fibres produce less force but fatigue less quickly when compared to type II fibres (Linssen et al., 1991; Sargeant & de Haan, 2006).

Bar-Or, Dotan, Inbar, and Rothstein (1980) explored the relationship between muscle fibre composition and fatigue profiles in a 30s sprint (Wingate test). The authors reported that participants who presented with a higher percentage of type II fibres also showed a significantly larger fatigue profile ($r=0.75$, $p=0.001$).

Since the work of Bar-Or et al., (1980), investigations comparing MFC and fatigue profiles during high intensity exercise has been limited. Researchers have either relied on prediction models to determine MFC or have simply estimated MFC profiles from performance measures. However, Inbar, Kaiser, and Tesch (1981) used muscle biopsy samples to determine MFC and compared these data to various performance measures in healthy males. A key finding was that participants with a higher percentage of type II fibres had significantly higher peak power and peak torque values during a Wingate Test; fatigue data was not reported.

Of the several factors that contribute to high intensity exercise and sprint performance (Bell & Wenger, 1988; Bishop, Edge, & Goodman, 2004; Nevill et al., 1989) muscle buffering capacity is particularly important. Carnosine in muscle plays a significant role in the buffering of H+ ions, reducing the rate of metabolic acidosis (Bishop et al., 2004; Suzuki, Ito, Mukai,

Takahashi, & Takamatsu, 2002). Several studies have examined the relationship between resting carnosine levels and fatigue resulting from high-intensity exercise (Derave et al., 2007; Lievens, 2017; Suzuki et al., 2002).

Suzuki et al. (2002) measured resting carnosine levels from muscle biopsy samples and compared these with fatigue profiles from Wingate tests. Positive and significant correlations between resting carnosine levels, percentage of type II fibres and mean power output throughout the 30 seconds and last 10 seconds were found. This suggests that carnosine influences the fatigue profile of a Wingate test, particularly during the last 10 seconds of exercise. Derave et al. (2007) confirmed this relationship in their study that examined fatigue in response to five sets of 30 maximal voluntary isokinetic knee extensions; participants with higher levels of resting carnosine showed less fatigue in the latter stages of exercise.

A more recent study by (Lievens, 2017) used ^{1}H-MRS as a non-invasive predictor of MFC and related these data to fatigue profiles of healthy males who completed three Wingate tests in succession. They found that participants with higher levels of resting carnosine, as determined by ^{1}H-MRS, had a higher fatigue index within each Wingate. This high fatigue index represents a greater reduction in power output and performance over time. They concluded that estimated MFC from the gastrocnemius muscle is a reliable predictor of the fatigue profile and fatigue index from repeated Wingate tests.

Though researchers have investigated MFC, carnosine levels and fatigue profiles in separate studies for over thirty years (Alen, Hakkinen, & Komi, 1984; Campbell, Bonen, Kirby, & Belcastro, 1979; Costill et al., 1976; Kaiser, Rossner, & Karlsson, 1981), research is yet to examine the relationship between MFC and resting carnosine levels determined from muscle biopsy and fatigue profiles in response to multiple sprint exercise. Evidence by (Inbar et al., 1981; Suzuki et al., 2002) demonstrate variance in fatigue profiles amongst individuals however it is still unknown whether muscle fibre composition and carnosine levels play a role in the rate at which individuals fatigue during high intensity exercise. The aim of this thesis is to extend the work of (Inbar et al., 1981; Lievens, 2017; Suzuki et al., 2002) and examine potential relationships between MFC, resting carnosine levels, and multiple sprint (Wingate) performance.

3.0 Literature review

3.1 Fatigue

A commonly accepted definition of fatigue was proposed by Edwards and colleagues, stating that 'fatigue is failure to maintain the required or expected force (or power output)' (1983). Fatigue experienced by athletes occurs as a result of impaired muscle function and/or a decrease in cognitive performance (R. H. T. Edwards, Porter, & Whelan, 2009), Irrespective of the origin, fatigue during exercise results in a decline in performance (Derman, Hawarden, & Schwellnus, 2010).

3.1.1 Mechanisms of Fatigue:

During high intensity exercise, a reduction in Adenosine TriPhosphate (ATP), Phosphocreatine (PCr) and glycogen, and the accumulation of Adeonosine DiPhosphate (ADP), Phosphate (P) and Hydrogen (H+) irons are believed to each contribute to fatigue (Begum et al., 2005; Green, 1997; Sahlin et al., 1977). The contribution of H+ ions to fatigue is undecided, however their accumulation results in a fall in intramuscular pH; the rate and extent of metabolic acidosis (Juel, 1997; Parkhouse & McKenzie, 1984) is related to fatigue and performance during high intensity exercise performance (Bell & Wenger, 1988; Nevill et al., 1989). Therefore, individuals who are able to regulate H+ ions or simply produce less of them during high intensity exercise will potentially present with smaller and less severe fatigue profiles during 'all-out' anaerobic sprint exercise.

3.2 Muscle Fibre Composition and Fatigue

Cell size in addition to the sarcoplasmic reticulum, capillary density, oxidative capacity and type of myosin ATPase vary between skeletal muscle fibre types; these differences are collectively responsible for differences in function between fibres (Cormie, McGuigan, & Newton, 2011; Scott, Stevens, & Binder–Macleod, 2001; Robert S. Staron, 1997). Type II skeletal muscle fibres are faster to contract and produce greater force compared to type I fibres. However, type II fibres fatigue faster during high intensity exercise (Linssen et al., 1991; Sargeant & de Haan, 2006). Compared to type I fibres, type II fibres have a greater capacity for anaerobic metabolism (Karlsson, Sjodin, Jacobs, & Kaiser, 1981) and differ in H+ ion production and handling, sodium/potassium pump function, ATPase activity and calcium resorption/handling by the sarcoplasmic reticulum (Allen, Lamb, & Westerblad, 2008; Fitts, 1994; Green, 1997; Karlsson et al., 1981).

One of the first studies to examine a potential relationship between muscle fibre composition and high intensity exercise performance was conducted by Bar-Or et al. (1980). They determined MFC from a biopsy taken from the Vastus Lateralis (VL) and used a single Wingate test (30s cycle sprint) to assess performance and fatigue. Changes in power output were compared with the percentage of type II fibres and fibre cross sectional area. Participants with a higher percentage of type II fibres showed higher average power ($r=0.63$, $p=0.0$; $n=19$) and larger fatigue profiles ($r=0.75$, $p=0.001$; $n=19$).

Thorstensson and Karlsson (1976) also investigated the relationship between fatigue and muscle fibre type. Muscle fibre composition of the VL muscle was determined from muscle biopsies in ten healthy male subjects between 25 and 40 years of age. Fatigue was measured by a reduction in force production during the repeat isokinetic knee extension protocol. A linear relationship was found between fatigue and the percent of type II muscle fibres ($r= 0.86$, $p= <0.01$; $n=10$).

3.2.1 Muscle Fibre Composition and Performance

Various investigations (Douris et al., 2006; Foster, Costill, Daniels, & Fink, 1978; Fry et al., 2003; Inbar et al., 1981; Methenitis et al., 2016) have shown that muscle fibre composition is a significant determinant of performance across a variety of different exercise tasks. Muscle fibre type influences aerobic (Foster et al., 1978), strength (Douris et al., 2006; Inbar et al., 1981), and power (Fry et al., 2003; Methenitis et al., 2016) performance.

Inbar et al. (1981) examined the relationship between muscle fibre composition in both aerobic and anaerobic performance capacities. Twenty-nine healthy males [8 sedentary, 5 physically active, 16 trained runners (7 long-distance and 9 short distance)] completed a Wingate test (30s cycle sprint), an isokinetic single leg test, a 40 metre sprint, a 300 metre run and a 2000 metre run. Muscle was biopsied from the VL muscle and fibre composition was determined. Peak power and maximal peak torque during the Wingate test were significantly ($p < 0.001$) related to type II fibre percentage. Significant relationships were also found between the percentage of type II muscle fibre and 40m sprint and 2000m performances (positive and negative relationships respectively). The authors concluded that muscle fibre composition influenced performance of both short term and endurance based exercises.

An investigation by Fry et al. (2003) compared muscle fibre characteristics and composition with performance in male Olympic weightlifters. Muscle was biopsied from the VL muscle of six highly trained weightlifters. The 'snatch' movement and countermovement jump were used as measures of power whilst the clean and jerk was used as a strength dominant exercise. The weightlifters (n=7) possessed a larger percentage of type II fibers compared to the untrained group (n=7). The weightlifting and vertical jump performances in the trained group were related to the estimated number of their type IIa fibres. Performance in the snatch and vertical jump was positively related to the estimated number of type IIa fibres whilst the clean and jerk performance correlated with a smaller area of IIb fibres. The authors suggested that the proportions of type IIa and IIb fibres were the dominant factors relating to performance in the clean & jerk, snatch and countermovement jump performance of trained men.

A more recent investigation by Methenitis et al. (2016) examined the relationships between conduction velocity, muscle fibre composition and power performance in subjects with different training backgrounds. Subjects were assigned to one of four groups; sedentary (n=10), endurance athletes (n=9), power trained (n=10) and strength trained (n=9). The power trained athletes performed significantly better in a countermovement jump test and had a significantly higher rate of force development and percentage of cross section area of type II fibres than the other groups ($P < 0.001$). It was concluded that the neural signal patterns and the size and percentage of type II fibres present in the VL contributed towards performance in multi-joint explosive movements.

Muscle fibre typology is clearly related to different performance measures. Type II fibres in particular have been consistently related to performance increases in powerful movements such as sprinting and countermovement jumps. There is limited research however of the importance of type I fibres and their contribution towards similar exercise testing. Whether a relationship exists between muscle fibre type (and muscle carnosine levels) and performance in physical tests that assess muscular strength, power and endurance, is yet to be determined.

3.3 Determining Muscle Fibre Composition

Skeletal muscle tissue has been examined in clinical and experimental populations since the 1860's (Costill et al., 1976). Early researchers sampled muscle post mortem; an advantage of

this being that large samples could be examined. The first biopsy procedure conducted on a living subject was performed by the French Neurologist, Guillaume-Benjamin-Amand Duchenne (Patel, Cooper, & Aihie Sayer, 2012). Duchenne's original method was refined by others and the procedure became safer and more effective (O'Rourke & Ike, 1995).

There have been many different variations in the needle biopsy but the use of percutaneous needles has been strongly favoured over the open biopsy method. Percutaneous biopsy needles leave smaller scars, are relatively cheap to use and different parts and depths of the muscle belly can be sampled (R. Edwards, Young, & Wiles, 1980; O'Rourke & Ike, 1995). In 1962 Jonas Bergstrom introduced his version of a percutaneous biopsy needle and this revolutionised the way skeletal muscle tissue is extracted (Bergstrom, 1975). The Bergstrom needle consists of a sharp trocar, cutting cannula and a pushing rod which extracts between 25-75 mg of muscle tissue. The introduction of this needle allowed scientists to examine changes in muscle metabolites in response to acute and chronic exercise for the first time. Determination of fibre type composition (i.e., numbers and sizes of slow and fast contracting fibres) was also possible. Fibre typology involves taking a sample of muscle tissue then trimming the sample for the cross sectional area to be exposed for analysis. The tissue sample is then stained for myofibrillar ATPase which differentiates slow (type I) and fast (type II) contracting fibres (Donselaar, Eerbeek, Kernell, & Verhey, 1987; Meunier, Picard, Astruc, & Labas, 2010).

Since the introduction of the Bergstrom needle, numerous investigators have made adjustments to maximise the size of muscle sampled and reduce discomfort for the subject. Variations have included the use of suction (Hennessey, Chromiak, Della Ventura, Guertin, & MacLean, 1997) which was first established in 1982 and has been shown to increase tissue yield between two-to-five fold whilst ensuring trauma to the subject remains minimal (Evans, Phinney, & Young, 1982).

Gollnick, Armstrong, Saubert, Piehl, and Saltin (1972) were the first to investigate the enzyme activity and fibre composition of healthy, trained and untrained men using the biopsy samples. They found that the endurance trained athletes had a significantly higher percentage of type I fibres (60%) compared to the non-trained participants (44%). The oxidative capacity of both type I and II muscle fibres present in the endurance athletes also showed a higher oxidative capacity than the untrained participants. Prince, Hikida, and Hagerman (1976) also reported that elite distance runners had a higher percentage of type I fibres and fewer type II fibres

compared to the untrained participants and power athletes. They reported that the endurance group possessed on average 83% type I fibres compared to 55% in the power lifter group. This difference in percentage was consistent with the findings of Costill and colleagues; their endurance (long distance) runners had on average 69.4 ± 4.8% type I fibres compared to sprinters who had 27.4 ± 3.2%. Both of these research groups sampled muscle from the VL muscle and determined muscle fibre type composition from myosin ATPase activity.

Tesch, Thorsson, and Kaiser (1984) examined potential differences in capillary supply and fibre type characteristics between elite power lifters, endurance athletes and non-athletes. The percentage of type II fibres for power lifters was 70 ± 6%, while for endurance athletes it was 40 ± 11% and for non-athletes it was 61 ± 10%. Ricoy, Encinas, Cabello, Madero, and Arenas (1998) have since shown that sprinters and power-lifters also possesses higher percentages of type II fibres. Their investigation reported the histochemical data of VL muscle of 16 women and 66 men (72 of whom were athletes in various events). They also determined that the largest percentage of type I fibres was present in long distance runners. In contrast to elite power and endurance athletes, the muscle fibre composition of healthy (untrained) individuals is usually close to 50% type I and 50% type II (Elder, Bradbury, & Roberts, 1982; Gollnick et al., 1973; Howald, Hoppeler, Claassen, Mathieu, & Straub, 1985; E. Jansson, Sjodin, & Tesch, 1978; Johnson, Polgar, Weightman, & Appleton, 1973).

3.3.1 Assessing muscle fibre type from Biopsy Samples

Although the percutaneous needle biopsy technique is relatively simple and cheap, it is invasive. There is the possibility of formation of scare tissue around the wound and a degree of discomfort and risk, albeit small, of nerve damage and internal localised bleeding. While recovery time following the procedure is generally acceptable with recreationally active individuals, it is less suitable for use in athletic populations. Due to these collective limitations, the use of muscle biopsies is not generally welcomed by athletes and support/coaching staff of athletic populations.

In addition to the above mentioned limitations, there is evidence that the percentage of type I and II fibres may vary at different depths within the muscle belly (Elder et al., 1982; Jennekens, Tomlinson, & Walton, 1971). Therefore, muscle sampled using the needle biopsy technique may not provide an accurate representation of an individual's whole-muscle fibre composition.

3.3.2 MFC Variability and Morphology

Studies that have examined differences in skeletal muscle fibre composition between individuals have generally biopsied muscle from the gastrocnemius or VL. These muscles have more of a 'mixed muscle fibre composition' profile compared to other muscles. Other muscles such as the triceps which is predominantly comprised of type II fibres (67.3%) or the soleus, which is predominantly comprised of type I fibres (68%) show less variation between individuals (Elder et al., 1982; Gollnick et al., 1972; Johnson et al., 1973).

A number of studies from the 1970s have examined whether the metabolic and/or contractile characteristics of skeletal muscle respond to training. Gollnick et al. (1972) investigated enzyme activity and fibre composition of 74 healthy men; muscle was biopsied from the deltoid and VL muscles. The subject population varied from untrained to trained athletes in various different sports (swimmers, runners, weightlifters, canoers and cyclist). The authors reported the untrained group averaged 46% type I muscle fibres and 57% type II in the deltoid muscle. Despite the average composition being approximately neutral (51.5%), the range of this groups' composition varied from possessing 14 – 60% type I muscle fibers. A similar trend was also found after examining the VL muscle. The young and middle aged participants combined presented with an average of 40% type I muscle fibres. In comparison to the untrained group, the endurance based athletes had on average a significantly higher percentage of type I fibres (72.5%).

One year after Gollnick and colleagues released their findings, Johnson et al. (1973) published an article which examined the muscle fibre type composition in 36 different human muscles. Six male autopsy subjects aged between 17 and 30 years old were investigated. Muscle fibre type was determined from 50 sites across 36 different muscles in each subject. There was a wide variation in fibre type composition in the majority of muscles across subjects. In 41 of the 50 sites, there was a significant difference in composition between each subject. A notable finding was that tonic muscles (iliopsoas and erector spinae) had a relatively high percentage of type I fibres while phasic muscles (gluteus maximus, biceps femoris and rectus femoris) had a high percentage of type II fibres. Muscles such as soleus and tibialis anterior which are predominantly postural muscles had on average 87% and 73% type I fibres respectively. Adductor Pollicis had a significantly high percentage of type I fibres (80%) which could be possibly explained by the contribution of this muscle in isometric grip strength involved in many day-to-day activities. This premise was also consistent with muscles which are

predominantly phasic in nature. Orbicularis Oculi (84.6%), sternocleidomastoid (64.8%), triceps brachii (67.5%) and rectus femoris (70%) were all identified as muscles with the highest percentage of type II fibres. Their primary functions include blinking, head movements, extension of elbow and flexion of knee respectively.

3.3.3 Different Classifications of Fibres

There are different ways of classifying muscle fibres from biopsy samples. While researchers have generally classified samples as being 'type I and II', some have employed a more detailed histochemical classification: type I, IC, IIC, IIAC, IIA, IIAB and IIB (Scott:2001, Meunier:2010). R. S. Staron et al. (2000) also used the same '6 category' approach for classification and presented muscle fibre data collected over a ten year period. Their findings highlighted a gender difference when examining area occupied by each of these specific fibre types. Men presented with IIA>I>IIB whilst women possessed I>IIA>IIB. A staining process was used for the identification of each fibre type. Scott et al. (2001) explain how histochemical staining can result in a more accurate and precise analysis of myosin ATPase. By identifying the different intensity of staining, the characteristics of muscle cells can be further understood and therefore more accurate representation of the muscle fibre type composition can be made. However, the majority of the current literature uses three classifications of muscle fibre type (type I, IIa, IIb) which is considered to be sufficient in the classification of fibre type.

3.3.4 Contractile and Metabolic Characteristics

The variability in contractile characteristics of muscle is largely genetically determined, and there is little influence from training (Komi et al., 1977; Simoneau & Bouchard, 1989; Simoneau et al., 1986). Simoneau and Bouchard (1989) investigated the influence of genetics on determining an individual's fibre composition compared to other external factors. This investigation examined the muscle fibre composition of 418 biopsies taken from the VL muscle of 270 healthy sedentary and 148 physically active participants. Overall, 15% of the total variance in fibre composition in humans was due to sampling and technical error, 40% due to environmental factors and the remaining 45% due to genetics alone.

Komi et al. (1977) also examined genetic influence on fibre composition and conducted an investigation aimed at exploring skeletal muscle fibre composition and muscle enzyme activity in monozygous and dizygous male and female twins. Muscle was sampled from the VL muscle from 31 pairs of twins. When comparing both types of twins and both sexes, each exhibited a

similar muscle fibre type composition. This particular investigation concluded that genetics is the predominant influence on muscle fibre type composition. A separate twin study by Bouchard and colleagues (1986) compared non-twin brothers (n=32), dizygotic (n=26) and monozygotic (n=35) twins to examine the genetic effect on muscle fibre type composition. Biopsies from the VL were taken which showed that the genetic effect contributes to around 25% – 50% of the total muscle fibre type composition variation after being adjusted for age and sex differences.

Although there is strong evidence to suggest genetics plays a leading role in determining the contractile characteristics of muscle fibres, a muscle fibre's metabolic characteristics are highly responsive to training (Holloszy & Coyle, 1984; Howald et al., 1985; Eva Jansson, Sjödin, & Tesch, 1978; Trappe, Costill, & Thomas, 2001). Some have taken the nurture approach and suggest there is reason to believe that training or exercise interventions play a role in determining muscle fibre composition. Endurance training can result in significant adaptations to skeletal muscle tissue and the respective muscle fibres (Holloszy & Coyle, 1984). This review of the literature suggests that there is a common increase in mitochondrial content and oxidative capacity of type I and IIa muscle fibres after endurance training. Additionally, there has been a greater dependence on fat oxidisation, slower use of muscle glycogen and less lactate production as a result of endurance training. While these changes are metabolic in nature, the contractile characteristics of each muscle fibre type stays relatively stable in response to training. Howald et al. (1985) examined changes in metabolic characteristics in muscle fibres with endurance training in 10 sedentary previously sedentary subjects. The six week intervention consisted of 30 minute sessions on cycle ergometers, five times a week. The muscle fibre types were identified using metabolic enzyme activity. Both fibre types showed significant metabolic changes with a 12% increase (type I) and 24% reduction (type IIb) in oxidative capacity. The investigators concluded that high intensity endurance training promotes development of the oxidative capacity (in particular, the mitochondrial activity) in both fibre types in untrained males. There were no contractile fibre characteristics examined in the methods of this study.

Despite this clear change in metabolic properties of fibres in response to training, various investigators have shown that the contractile characteristics are less responsive to a training intervention (Andersen & Aagaard, 2006; Zierath & Hawley, 2004). Time to peak tension, Ca^{2+}myosin ATPase and mg^{2+} actomyosin ATPase have been examined in both type I and II

muscle fibres with only subtle changes detected after training. A study by Trappe et al. (2001) examined the effects of a 21 day reduction in training volume (taper) on muscle function and muscle fibre type. The authors examined metabolic enzyme activity to determine muscle fibre type whilst also examining changes in contractile characteristics such as force production. Both type I and type II fibres were examined from muscle biopsies of six young (20 ± 1 years) college swimmers throughout the duration of a tapering period. Type IIa fibres had a much greater response to tapering after 6 months of high volume swim training. A significant difference (0.63Mn ± 0.04 – 0.82± 0.05) was also found in the peak force production of the type IIa fibres post- swim taper. It was proposed that these findings were due to the increase in size (diameter 11%) of the IIa muscle fibres. Although the changes in contractile characteristics were minimal in this study, the authors concluded that there is still some changes due to an exercise or lack of exercise stimulus, especially towards type IIa fibres (Trappe et al., 2001). Therefore, there may be a shift in contractile characteristics between types IIa and IIb but not between I and II.

In summary, studies have shown muscle fibre composition is mostly genetically determined, with a possibility of subtle and minimal changes from intense training bouts or exercise interventions. While metabolic characteristics of fibres can change with different training backgrounds and interventions, the contractile speeds of type I and type II muscle fibres remain unchanged with training. There is evidence of contractile changes between subdivisions of the type II fibres (type IIa and IIb) but not between types I and II.

3.4 Muscle Carnosine and Fatigue

Muscle carnosine influences buffering capacity and has thus been identified as significant contributor to high intensity exercise performance and fatigue (Bell & Wenger, 1988; Bishop et al., 2004; Nevill et al., 1989; Suzuki et al., 2002). A number of studies have examined the relationship between resting carnosine levels and fatigue during high-intensity exercise (Derave et al., 2007; Lievens, 2017; Suzuki et al., 2002).

3.4.1 Carnosine and Wingate-induced Fatigue

Suzuki et al. (2002) examined relationships between muscle carnosine, fibre composition and high-intensity exercise performance (2002). It is important to note that the muscle carnosine values and muscle fibre composition were both estimated from the VL muscle using needle

biopsy. Muscle data were compared with peak power, mean power and fatigue derived from a Wingate cycle test. The authors found a significant correlation ($r=0.65$, $p<0.05$; $n=11$) between resting carnosine levels and the percentage of type IIx fibres. There also was a significant correlation between carnosine levels and mean power ($r=0.79$, $p<0.01$; $n=11$) and also between carnosine levels and power output over the final 10 seconds of the sprint ($r=0.66$, $p<0.05$; $n=11$). It was concluded that resting carnosine values were related to Wingate test performance and fatigue.

A study by Derave et al. (2007) found similar results when the effects of beta-alanine supplementation on isokinetic and isometric fatigue were examined. Beta alanine supplementation has been shown to increase resting levels of muscle carnosine concentrations. The authors' protocol required participants to complete five sets of 30 maximal voluntary isokinetic knee extensions at 180 degrees. Participants who had higher levels of carnosine due to Beta alanine supplementation performed better ($p=0.05$) in the latter stages of the test. These results support Suzuki's findings and indicate that carnosine loading may significantly improve the latter stages of high-intensity exercise.

A recent investigation by Lievens (2017) examined the relationship between percentage of type II fibres and fatigue during a Wingate test. Twenty-one healthy males had their gastrocnemius MFC assessed using ^{1}H-MRS. Six participants presented with mainly Type II fibres whilst six were mainly Type I. These 12 participants then performed three Wingate tests, each separated by four minutes of passive recovery. Peak power, average power and fatigue index were calculated for each sprint. It was found that the type II dominant group had on average a higher fatigue index within each Wingate test and for the three combined tests. The mean power however did not change between groups. It was concluded that despite the consistency of mean power, there were significant relationships between muscle fibre composition, the fatigue profile and the fatigue index from repeated Wingate sprints.

3.5 Muscle Carnosine

3.5.1 Carnosine in type II fibres

Muscle carnosine is a dipeptide typically found in high concentrations in type II muscle fibres (Blancquaert, Everaert, & Derave, 2015; Harris, Dunnett, & Greenhaff, 1998; Harris et al., 2006). Although the main physiological roles of carnosine are not completely clear, researchers

have identified it as a pH buffer in skeletal muscle (Mannion, Jakeman, Dunnett, Harris, & Willan, 1992; Smith, 1938). Harris et al. (1998) were the first to identify a relationship between carnosine and type II fibres. They took muscle biopsies from the VL muscle with four participants. ATP-ase activity (to determine muscle fibre type) and carnosine were measured. On average, type II muscle fibres had more than twice as much carnosine (23.3mM) than type I fibres (10.5mM). Research in the field since the work of (Harris et al., 1998) has consistently shown that type II fibres in inactive, active and athletic populations all have around two-fold carnosine content than type I fibres.

3.5.2 Dietary effects on resting carnosine levels

Given that the best dietary sources of carnosine are meat, a number of studies have examined the relationship between dietary behaviours and resting carnosine levels. Baguet, Everaert, De Naeyer, et al. (2011) reported a vegetarian diet resulted in significantly lower resting muscle carnosine levels. ^{1}H-MRS was used to estimate carnosine concentrations in 149 healthy participants (94 men and 55 women); levels in the soleus, gastrocnemius and tibialis anterior muscles were recorded and dietary records analysed. Amongst the 94 men, there were 15 vegetarians who had a 26% lower carnosine level in the gastrocnemius when compared to the rest of the men.

Various investigators (Derave et al., 2007; Harris et al., 2006; Hill et al., 2007) have examined the influence of beta-alanine supplementation on resting carnosine levels. Harris et al. (2006) supplemented participants over four weeks with 3.2 g.d and 6.4 g.d of beta-alanine and found 42.1 and 64.2% increases in resting carnosine levels respectively. Derave et al. (2007) conducted a placebo- controlled, double blind study to examine changes in muscle carnosine concentrations in sprint athletes (n=15) over four weeks of beta-alanine supplementation. Carnosine levels were measured using proton MRS before and after the supplementation period. After four weeks, the beta-alanine group increased their carnosine levels in the gastrocnemius and soleus by 37% and 47% respectively. Hill et al. (2007) examined thirteen individuals who also followed a beta-alanine supplementation program and twelve individuals who received a placebo condition for up to 10 weeks. Muscle biopsies from the VL were analysed for carnosine; carnosine levels significantly increased by 58% and 80% after four and 10 weeks respectively in the beta-alanine supplementation group. There were no increases in carnosine levels in the control group.

In their paper that reviewed the determinants of muscle carnosine content, (Harris et al., 2012) concluded that muscle fibre type, species, beta-alanine intake and training all influence resting carnosine levels. Consistent with this, a number of studies (Parkhouse, McKenzie, Hochachka, & Ovalle, 1985; Tallon, Harris, Boobis, Fallowfield, & Wise, 2005) have found that resistance trained individuals have higher than average muscle carnosine levels.

Baguet et al. (2009) identified carnosine as a relatively stable metabolite within skeletal muscle after examining the washout of beta-alanine and carnosine following a period of beta-alanine supplementation. Fifteen untrained males were assigned either to a supplementation or placebo group for 6 weeks. In this investigation, the control group (5.9± 0.9) only experienced a 9-15% change in muscle carnosine levels over a 3 month wash-out period. The authors suggested this amount of variability is small and that these results indicate that carnosine is a stable molecule (Baguet et al., 2009). However, it is important to consider the limitations of using carnosine concentrations to estimate muscle fibre composition. To elicit the same changes in carnosine concentrations through the diet, 4kg of red meat or 1.2kg of turkey meat would need to be consumed daily (Wolos, Piekarska, Pilecka, Ciereszko, & Jablonowska, 1983). Thus, diet alone is not likely going to change carnosine levels to the same extent of beta-alanine supplementation.

The collective weight of available evidence suggests that carnosine levels differ between populations with different training practices. However, a number of factors (eg., muscle fibre composition, dietary intake of meat, beta-alanine supplementation) will also influence these levels to varying degrees.

3.5.3 Training Effects on Resting Muscle Carnosine Levels

A number of investigations have examined the influence of training on muscle carnosine levels. Most studies suggest there is no significant change in muscle carnosine concentrations in response to training. Kendrick et al. (2008) and Mannion, Jakeman, and Willan (1994) used sprint training in their interventions which took place over 4-16 weeks. Kendrick et al. (2008) prescribed whole-body training and cycle training in two different studies. The first training intervention consisted of resistance training 4 days/week for 10 weeks (n=26). The four sessions each week were divided into 2 x upper body and 2 x lower body with the aim of

increasing whole-body strength and overall muscle mass. After 10 weeks of resistance training, there was no change (29.2 mM +- 9.8 mM – 27.3 mM +- 9.5mM) in muscle carnosine levels.

Mannion et al. (1994) had three groups complete isokinetic knee extension 3 days/week for 16 weeks. Group 1 (n=13) performed the knee extensions at 4.19 $rad.s^{-1}$ for six sets of 25 repetitions with 30 seconds rest, Group 2 (n=10) performed the knee extensions at 1.05$rad.s^{-1}$ for six sets of 15 repetitions with 40 seconds rest and Group 3 (control group; n=10) did not perform any knee extensions. Despite an increase in work capacity after the 16 weeks of training, there was no significant change (p=0.37) in muscle carnosine levels assessed through muscle biopsy tissue samples.

A recent investigation by Baguet, Everaert, De Naeyer, et al. (2011) examined potential changes in muscle carnosine levels in response to a training intervention with participants following either a mixed or a vegetarian diet. The subjects completed 2-3 sessions of sprint training each week for five weeks. No significant increases in muscle carnosine were found for the training and mixed diet group and no changes were found between the training and vegetarian diet group. Muscle carnosine was measured by ^{1}H-MRS pre- and post-exercise intervention.

Although the majority of the current literature shows that skeletal muscle carnosine concentrations remain unchanged in response to training, an investigation by (Y. Suzuki, Ito, Takahashi, & Takamatsu, 2004) found that sprint training increased carnosine levels (measured using muscle biopsies), in athletic individuals. The exercise intervention involved a total of 28 Wingate tests over the entire duration of the study. The authors observed a doubling of muscle carnosine levels in six participants. It is worth noting that one participant's baseline carnosine concentration was 3.4 mmol.kg-1 and this increased to 13.6mmol.kg-1 after the training intervention. Limitations of this study include the low number of participants (n=6) and a low total duration (14 minutes) of training. It is questionable that such a low training volume can produce an increase in resting carnosine levels as reported; others have not shown similar findings.

A recent study by Painelli et al. (2018) investigated the effects of high-intensity interval training on resting muscle carnosine levels. Resting muscle carnosine levels were determined through chromatography on 20 recreationally active males. The HIIT group (n=10) performed

HIIT training on a cycle ergometer 3 days a week for 12 weeks. The HIIT group followed a progressive protocol which started as six bouts of 2 minute sprints (140% of lactate threshold) in week 1 and progressed to 12 bouts (170% of lactate threshold) in week 12. Muscle carnosine levels increased in both type I (from 14.4 ± 5.9 to 16.8 ± 7.6; p= 0.047) and II fibres (from 18.8 ± 6.1 to 20.5 ± 6.4; p= 0.067) for the HIIT group. This suggests there is potential for an increase in resting carnosine with 12 weeks of HIIT training.

In conclusion, MFC, resting carnosine levels and fatigue profiles have been examined in a number of studies for over 30 years (Alen et al., 1984; Campbell et al., 1979; Costill et al., 1976; Kaiser et al., 1981). However, investigations that have explored the relationships between them have generally been limited to MFC prediction methods. Evidence by (Inbar et al., 1981; Suzuki et al., 2002) demonstrate variance in Wingate-induced fatigue profiles amongst individuals, however it is still unknown whether MFC and carnosine play a significant role in the way an individual fatigues during high intensity exercise. Therefore, the aim of this thesis is to examine the potential relationships between MFC, resting carnosine levels and multiple sprint (Wingate) performance.

It is hypothesised that percentage of type II fibres will have a positive relationship with resting muscle carnosine levels and repeated sprint performance. The secondary hypothesis is that participants with higher carnosine levels will perform better (fatigue less) in the latter stages of a 30-second sprint whilst also in the subsequent 2nd and 3rd Wingate tests.

4.0 The Relationship between Muscle Fibre Composition, Muscle Carnosine and Multiple Sprint (Wingate) Performance

4.1 Introduction

While a number of mechanisms influence performance during high intensity exercise, acidosis resulting from an accumulation of hydrogen ions has long been implicated in the fatigue process (Juel, 1997; Parkhouse & McKenzie, 1984). In turn, muscle fibre composition and resting carnosine levels have consistently been related to both the production and buffering of hydrogen ions respectively (Bell & Wenger, 1988; Parkhouse et al., 1985).

Compared to type I skeletal muscle fibres, type II fibres are fast contracting and fast fatiguing; they are also characterised by a high metabolic capacity for anaerobic metabolism (Staron:1997, Scott:2001, Cormie:2011). Thus, individuals with a high relative proportion of type II muscle fibres generate higher forces, more rapidly. However, in producing more energy through anaerobic metabolism to achieve these higher forces, there is likely more rapid acidosis within the muscle.

Type II skeletal muscle fibres have higher resting levels of carnosine than type I skeletal muscle fibres (Harris et al., 1998). Carnosine is a buffer of hydrogen ions and is thus a factor identified by researchers as being important in delaying the rate of fatigue during high intensity exercise. Indeed, sprinters are characterised by higher resting levels of carnosine and this has been attributed to genetic factors (eg. a higher % of type II fibres) in addition to potential adaptations resulting from training (Baguet, Everaert, Hespel, et al., 2011). Diet and supplementation with beta alanine have also been shown to influence resting muscle carnosine concentrations.

In addition to those studies that have shown a positive and significant relationship between type II muscle fibres and resting carnosine concentrations, other investigations have shown a relationship between carnosine concentrations and high intensity exercise performance (Lievens, 2017; Suzuki et al., 2002). However, few studies have examined the relationship between muscle fibre type, muscle carnosine concentrations derived from muscle biopsies and Wingate-induced fatigue with the same cohort of participants. The aim of this study was to extend the recent work of (Inbar et al., 1981; Lievens, 2017; Suzuki et al., 2002) to better describe the relationships between MFC, resting carnosine levels and Wingate-induced fatigue using a large heterogeneous group of trained males. Exploring these relationships may provide

valuable information for prescribing training interventions for Type I or II predominant individuals, whilst also providing evidence for athletes currently supplementing with β-Alanine.

4.2 Methods

4.2.1 Participants

Thirty (n=30) healthy males (25 ± 4 years) were recruited for this study. The primary exclusion criteria were vegetarians and/or those who had supplemented with beta-alanine in the previous three months. The study was approved by an ethics committee at The University of Queensland (AN: 2019001335).

Table 1. Participant Characteristics

No. of Participants	Age (years)	Height (m)	Body Mass (kg)
30	25 ± 4	1.75 ± 0.11	74.0 ± 9.8

Mean ± standard deviation.

4.2.2 Recruitment

Participants were recruited from the student population, local rugby league clubs, triathlon squads, rowing clubs and a high performance academy.

4.3 Experimental Overview

The study required each participant to attend one muscle biopsy session and complete one Wingate testing session. An Adult Pre-Exercise Screening (APSS) Form was sent to each participant prior to confirming their place in the study. Participants were stratified as 'Low Risk' to ensure they were safe to conduct the high intensity session. Each underwent the muscle biopsy procedure in their first session; muscle was sampled from the right VL. Muscle tissue was cleaned, prepared for its respective analysis and then placed directly in a freezer. A total of 30 participants completed the biopsy procedure. Participants were asked to refrain from strenuous and moderate intensity exercise for 24 hours prior to the Wingate session. The Wingate session comprised of a standardised warm-up of low intensity cycling and dynamic stretches. Three x 30 second cycle sprints were then completed, with each sprint separated by 4 minutes of rest. Of the 30 participants who completed the biopsy procedure, three were unable

to complete the Wingate session due to injury. Twenty-six of the 27 participants were able to complete the full repeat Wingate protocol, with one reporting light-headedness and nausea after the second Wingate. Thus, 26 participants completed the full study protocol.

4.4 Muscle Biopsy Session

4.4.1 Pre- Muscle Biopsy

Prior to muscle sampling, each participant was provided with a 'Biopsy Information Sheet' and 'Biopsy Care Form' for detailed explanation of all safety considerations associated with the procedure. Participants were asked to avoid vigorous exercise involving the legs for 48 hours prior to the biopsy session.

4.4.2 Muscle Biopsy Procedure

Each participant had muscle sampled from the VL muscle in the right thigh using a sterile hollow needle. The whole insertion region on the thigh was carefully cleaned before a small amount of local anaesthetic was injected under the skin (5-10mL of 10mg.mL Xylocaine). A small ~10 mm incision was made into the skin and underlying fascia to create an opening for the biopsy needle. The biopsy needle was then inserted through the incision into the muscle belly of the VL muscle and a small piece of muscle (~200 mg) was removed. The needle was then immediately removed. Following the biopsy, the incision was closed with sterile tape (steri-strips) and wrapped with a tensor bandage.

4.4.3 Biopsy Sample Storage

In preparation of the Immunohistochemical analysis (fibre typology), each sample was quickly washed in 0.9% saline before any fat, connective tissue or blood was removed. The sample was then weighed and frozen in Isopentane before being cooled on dry ice. Samples destined for carnosine analysis were snap frozen immediately in liquid nitrogen. All samples were stored in a freezer at -80 ^{0}C until later analysis.

4.4.4 Biopsy Analysis

The MFC analysis took place in Norway (Norwegian School of Sport Sciences) where standardised published procedures were followed to identify type I and type II muscle fibre counts and their corresponding cross-sectional areas. Muscle biopsies were orientated and cut at 8 μm-thickness at −20 °C using a microtome (CM1860, Leica, Germany). The sections were

mounted on microscope slides (Superfrost Plus, Thermo Scientific, MA, USA), air-dried, and stored at −80 °C. After thawing, sections were blocked in 1% bovine serum albumin (BSA) in PBS containing 0.05% Tween 20 (PBS-t; BSA, A4503, Sigma Life Science, St. Louis, MO, USA; PBS, 524650, Calbiochem, EMD Millipore, Darmstadt, Germany; Tween 20, 437082Q, VWR International, Radnor, PA, USA) for 60 min at room temperature prior to incubating with anti-dystrophin (ab15277, AbCam, Cambridge, UK) and anti-myosin heavy chain 1 (BA-D5, DSHB, Iowa City, IA, USA; deposited to the DSHB by Schiaffino, S.) antibodies overnight at 4˚C. After incubating overnight, the sections were washed three times for 10 min in PBS-t and incubated with appropriate secondary antibodies (concentration 1:500) (A11005 or A11001, Molecular Probes, Life technologies, Carlsbad, CA, USA) for 60 min at room temperature. After three 10-min washes in PBS-t, the muscle sections were covered with a coverslip, mounted using ProLong Gold Antifade Reagent with DAPI (P36935, Molecular Probes).

Sections were imaged using a high-resolution camera (DP72, Olympus Corp., Japan) mounted on a microscope (BX61, Olympus) with a fluorescence light source (X-Cite, 120PCQ, EXFO Photonic Solutions Inc., Mississauga, ON, Canada), using a 4× 0.13 NA air objective (UPlanFL N, Olympus) for CSA analyses. Individual muscle fiber CSA and fiber type composition were analyzed and measurements were calculated using TEMA software (Checkvision, Hadsund, Denmark). CSA and fiber type composition analysis included 124 (range, 16–287) type 1 fibers and 180 (range, 23–318) type 2 fibers. Fibers with abnormal shape, e.g. longitudinal or oblique, were excluded from the CSA analyses. There is no minimum number of fibres for this type of analysis, however it has been recommend that around 150 fibres is ideal (Adams, 1968).

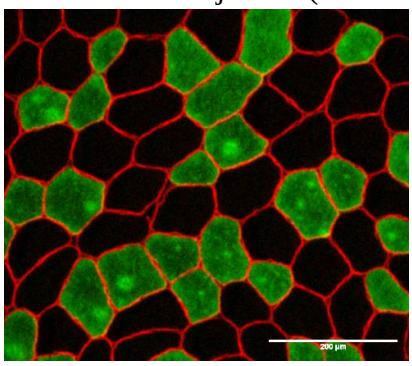


Figure 1: Histochemical Staining

Figure 1 shows a magnified view of how the MFC was determined from each participant. Stained fibers showing green were positive for MHC1 whilst the unstained black fibers were positive for MHC2. The red lining was positive staining against dystrophin (visualizing the cell membrane).

Fiber-type distribution was studied on immunohistochemical serial preparations using anti-fast IIa myosin heavy-chain N2.261 and anti-slow myosin heavy-chain A4.951 monoclonal antibodies (concentration 1:500) (Alexis Biochemicals, San Diego, California, USA). The

fibers were designated as Type I and II. For each subject, 100 - 500 fibers were analyzed, and each fiber type was expressed as a percentage of the total number counted

4.4.5 Carnosine Analysis

Carnosine analysis was completed in the biochemistry laboratory of the School of Human Movement and Nutritional Sciences at the University of Queensland. The samples were analysed using the method of (Mora, Sentandreu, & Toldra, 2007) with the modifications described below.

Each sample wet weight was measured using electronic scales (AND GRG – 202, Japan). Each sample then had 5 times the weight of 10m M Hydrochloric acid (HCl) added to the sample. The sample was homogenised using a 'BeadBug Microtube Homogenizer (Benchmark Scientific, Inc Edison, NJ 08818). Each sample used three stainless steel 3mm beads and was homogenized for 3 bouts of 20 seconds. Samples were placed on ice before and between bouts to maintain temperature. A 20 µl aliquot of the homogenate was removed and placed in the freezer for protein analysis.

The remaining homogenate was then centrifuged at 14 000 rpm for 10 minutes. 70µl of the supernatant was removed for HPLC analysis. This was deproteinized using 3 volumes (210 µl) of acetonitrile before being mixed (vortex-genie, Scientific Industries) for 10 seconds and then resting on ice for 20 minutes. These samples were then centrifuged (Mikro 200, Hettich) again at 14 000 rpm for 10 minutes.

4.4.6 High performance liquid chromatography (HPLC) analysis

Working standard solutions were prepared over the appropriate concentration range by dilution of stock solutions. All stock solutions were stored at -20 °C until use. The carnosine standard solutions (Sigma-Aldrich) were set at 0.5 mM, 0.25 mmol and 0.125 mmol through diluting stock solutions with distilled water.

Carnosine was analysed using HPLC (High Pressure Liquid Chromatography) in the biochemistry laboratory at the University of Queensland. Twenty microliters of each sample was injected into the HPLC system. The chromatographic separation was carried out using a Kinetex 5µm HILIC 100A 250 x 4.6mm LC column (Phenomenex, USA) at 27°C. Mobile phases consisted of solvent A, containing 0.65 mM ammonium acetate, pH 5.5, in water/acetonitrile (25:75), and solvent B, containing 4.55 mM ammonium acetate, pH 5.5, in

water/ acetonitrile (70:30). The solvents were filtered through a 0.22 µm membrane filter and degassed prior to the analytical run. The separation conditions were a linear gradient from 0 to 100% of solvent B in 13 min, then held at 100% B for 2 min before returning to 100% A in 3 min at a flow rate of 1.2 mL/min. The column was equilibrated for 3 min under the initial conditions before each injection. The separation was monitored using a diode array detector at a wavelength of 214 nm. Peak areas were correlated to compound concentration by interpolation in the corresponding calibration curve.

4.5 Wingate Testing Session

4.5.1 Anthropometric Measures

The body mass of each participant was taken without shoes and with exercise attire. Each measurement was obtained by the same electronic scales (Avery Weigh-Tronix) for each participant. Height was measured using a fixed (Seca 217) stadiometer.

4.5.2 LODE Protocol Setup

A LODE cycle ergometer (Excalibur Sport 925900) was used for each Wingate testing session. Excalibur Sport LODE software was used to record and analyse Wingate data. Resistance was internally loaded in the LODE program. The duration and resistance for each stage of the test was pre-set into the program. Resistance was set at 25 Watts (absolute load) for warm up whilst a torque factor of 0.7 was used for each Wingate phase.

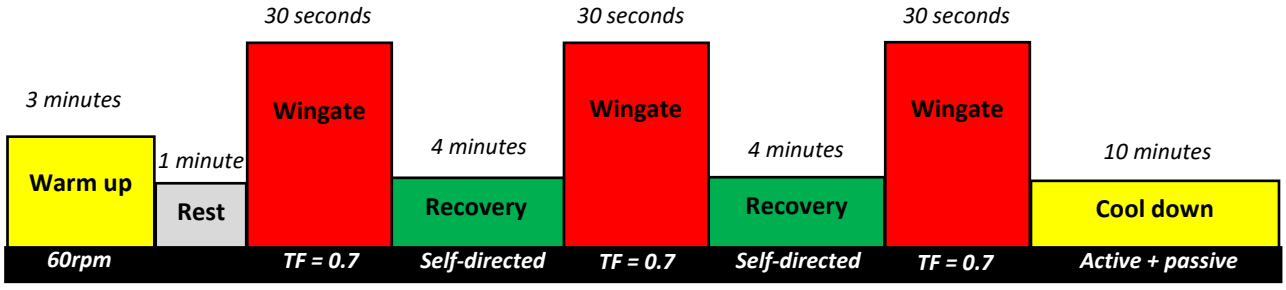


Figure 2: Repeated Wingate Protocol

4.5.3 Bike Setup

The seat height, handlebar height and distance away was all adjusted for each participant. They were instructed to find the most comfortable and powerful position (by changing the seat and handlebar positions). This self-selected position was kept constant for all 3 Wingate tests. Live data during the Wingate test was not in view of the participants. The warm up, Wingate tests,

rest periods and cool down were all programmed into the software and the same protocol was used every time.

4.5.4 Testing Instructions

The testing protocol was explained to each participant before the commencement of the warm-up to ensure they were familiar with the test. The following points were repeated and made extremely clear to each participant:

1) *"Each Wingate test needs to be a maximal and all-out effort. This means there should be no pacing (100% effort from the very first second).*

2) *You will start each Wingate in a stationary position with your dominant leg at 90 degrees*

3) *You must keep your bottom on the seat at all times during each Wingate*

4) *You should not stop the Wingate test until I have instructed you to do so.*

4.5.5 Protocol

Prior to performing the Wingate tests, each participant filled out an Adult Pre-Exercise Screening (APSS) Form to ensure they were safe to conduct the high intensity session. A 24 hour dietary recall was also taken to ensure they followed the pre-test requirements. Height and weight were also measured and used to make allow relative measurements to be calculated.

A standardised warm up was prescribed directly before the commencement of each Wingate testing session. This was broken up into dynamic stretches (leg swings, waking lunges, bum kicks, high knees, body weight squats) and low intensity cycling (60rpms @ 25 W).

Each participant then had 60 seconds of passive rest before the commencement of the first 30 second all out sprint (Wingate). This was then followed by 4 minutes of passive rest. This was then repeated 2 more times (Wingate + rest). After the 3rd Wingate was completed, a self-directed cool down was completed. All participants were provided with standardised encouragement throughout each Wingate bout.

4.6 Data Collection

4.6.1 Wingate Outcome Measures

Various performance and fatigue measures were recorded from the LODE cycle ergometer (Excalibur Sport 925900) software during the Wingate protocol. The following measures were determined; Mean Power (MP), Anaerobic Capacity (AC), Peak Power (PP), Relative Peak

Power (RPP), Total Work (TW), Relative Total Work, (RTW), Fatigue Index (FI) and Fatigue Profile (FS).

For the purpose of this study the following definitions of each measure are as follows: Mean Power is the average power across the 30 seconds; Anaerobic Capacity is the average power divided by body mass; Peak Power is the maximal power; Relative Peak Power is the maximal power divided by body mass; Total Work is the average power multiplied by time; Relative Total Work is the total work divided by body mass. Fatigue Index was determined by calculating the percentage difference between maximal and minimal power. This is measured as a percentage.

$$\frac{(Peak\ Power - MIN\ Power\ Output)}{Peak\ Power}\ X\ 100$$

The Fatigue Profile was determined by dividing the difference between the maximal and minimum power output values by the respective amount of time. This was measured in Watts/second.

$$\frac{(Peak\ Power - MIN\ Power\ Output)}{Time}$$

4.6.1 Data Analysis:

Data were analysed using SPSS statistics software package (version 23.0, SPSS, Inc. Chicago, IL). The analysis included the use of standard descriptive statistics, interclass correlation, bland Altman plots, Pearson's correlation coefficients, regression equations or the comparable non-parametric tests as necessary. Pearson's Correlation was used to determine the relationships between Carnosine and percentage of type II fibres, fibre area and relative fibre area. Mean and standard deviations were determined for all muscle tissue and Wingate testing analysis. All measures were checked for normality. For all tests, an alpha level 0f 0.05 was set as the standard for statistical significance.

5.0 Results

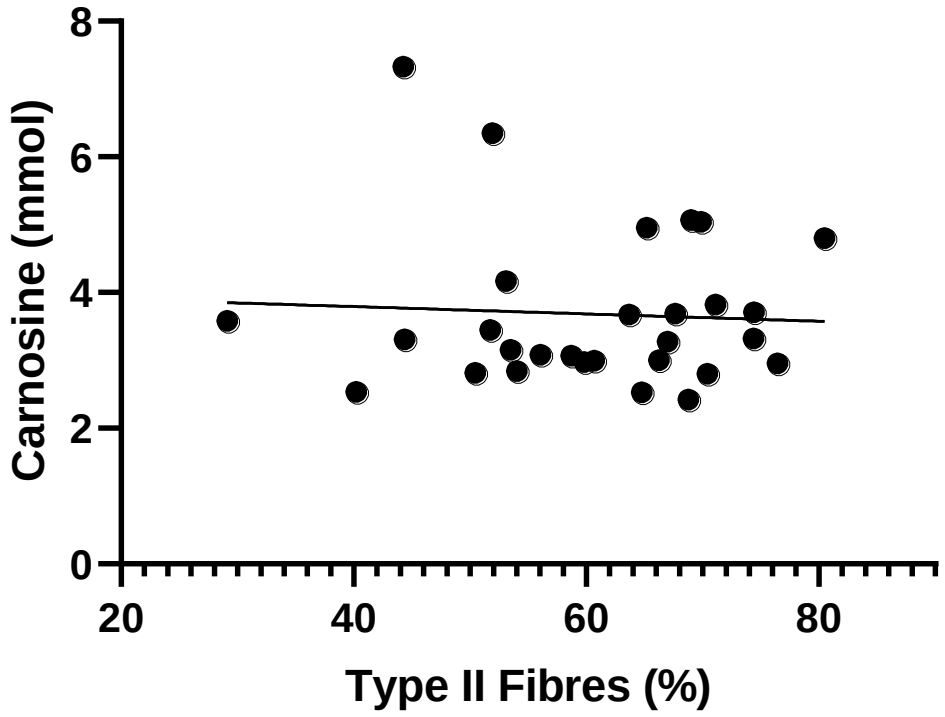
Table 2 shows the results from the Histochemistry and HPLC analysis of the biopsy samples (n = 29). Each type of analysis is presented as a mean and standard deviation. All data were normally distributed.

Analysis Type	Mean	SD +/-	Range
Carnosine (mmoL·kg^{-1}dm**)**	3.58	1.2	2.0 - 7.3
Type II (%)	60.6	11.9	29.1 – 80.4
Type I (%)	39.4	11.9	19.6 – 70.9
Type II CSA (%)	62.8	11.3	36.35 – 83.0
Type I CSA (%)	42.2	15.3	17.05 – 77.0
$\frac{Average\ Type\ II\ CSA}{Average\ Type\ I\ CSA}$	1.23	0.1	0.89 – 1.6

Table 2. Raw Muscle Biopsy Data

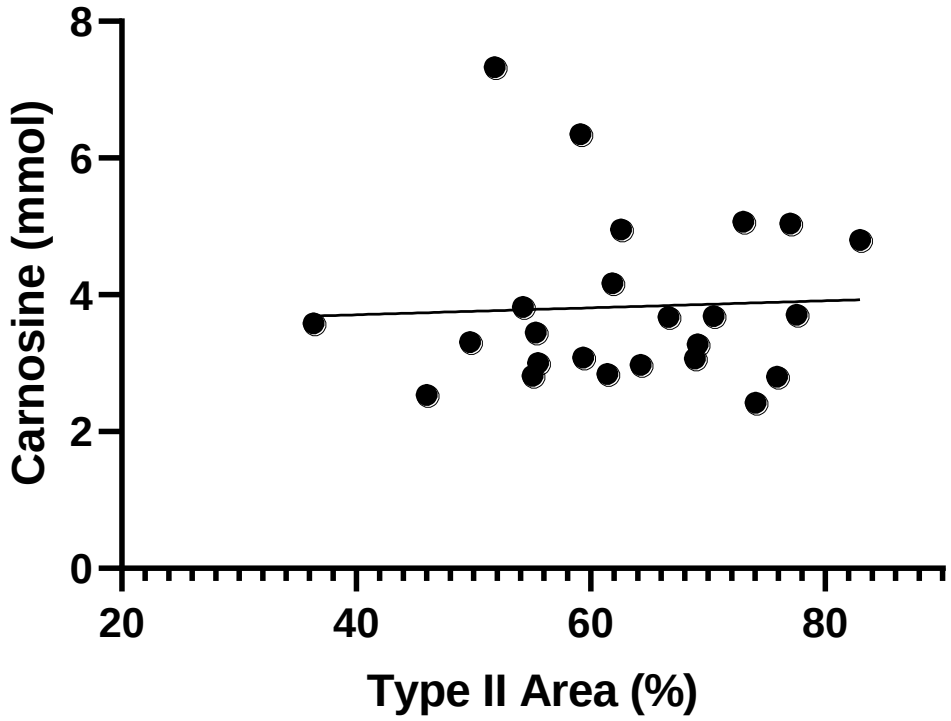
All 30 muscle samples were successfully analysed for resting carnosine levels. Some of the samples however received freezing damage in transit to colleagues in Norway prior to MFC analysis. Therefore, 29 samples were successfully analysed for percentage type I and II fibres and 24 samples could be analysed for CSA percentage.

Figure 3 shows the correlation between resting muscle carnosine level and percentage of type II fibres (r = -0.056; p= 0.77; n=29).



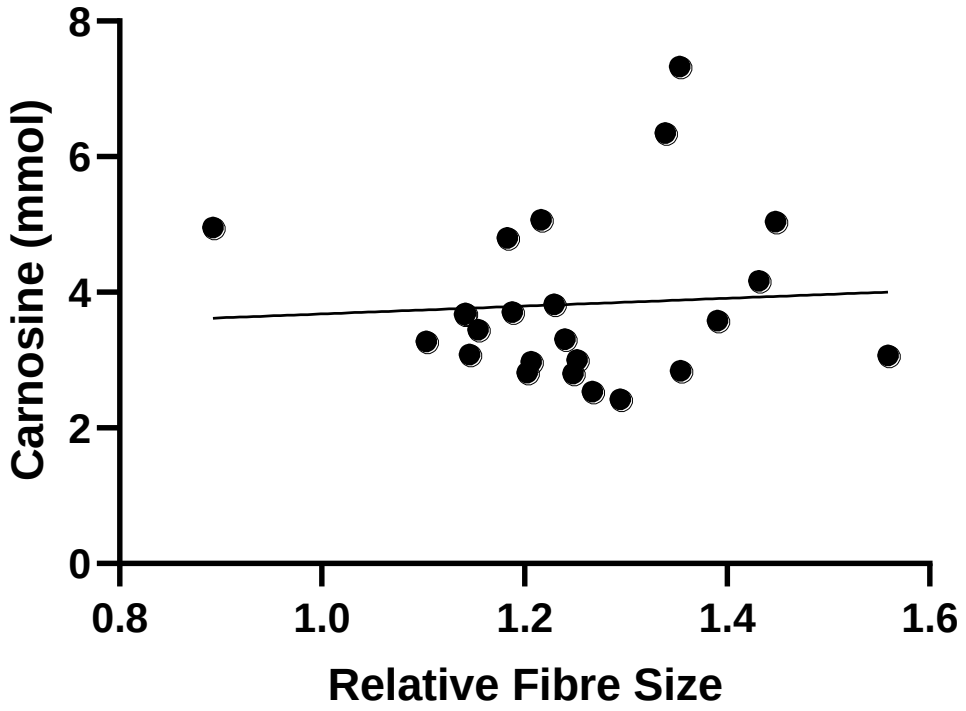
There was no significant relationship (very weak) between carnosine concentrations and percentage of type II fibres. Both variables were obtained from the VL muscle in each participant.

Figure 4 shows the correlation between resting muscle carnosine level and percentage area of type II fibres (r = 0.048; p= 0.838; n= 24).



There was no significant relationship (very weak) between carnosine and percentage area of type II fibres (n=24). Both variables were obtained from the VL muscle in each participant. During transit to colleague who conducted the Histochemisty analysis, five muscle samples were damaged and were unable to be analysed for CSA.

Figure 5 shows the correlation between resting muscle carnosine level and relative fibre size (r= 0.07; p= 0.77; n=24).



There was no significant relationship (very weak) between carnosine concentrations and relative fibre (n=24). Both variables were obtained from the VL muscle in each participant. During transit to colleague who conducted the Histochemisty analysis, five muscle samples were damaged and were unable to be analysed for relative fibre area.

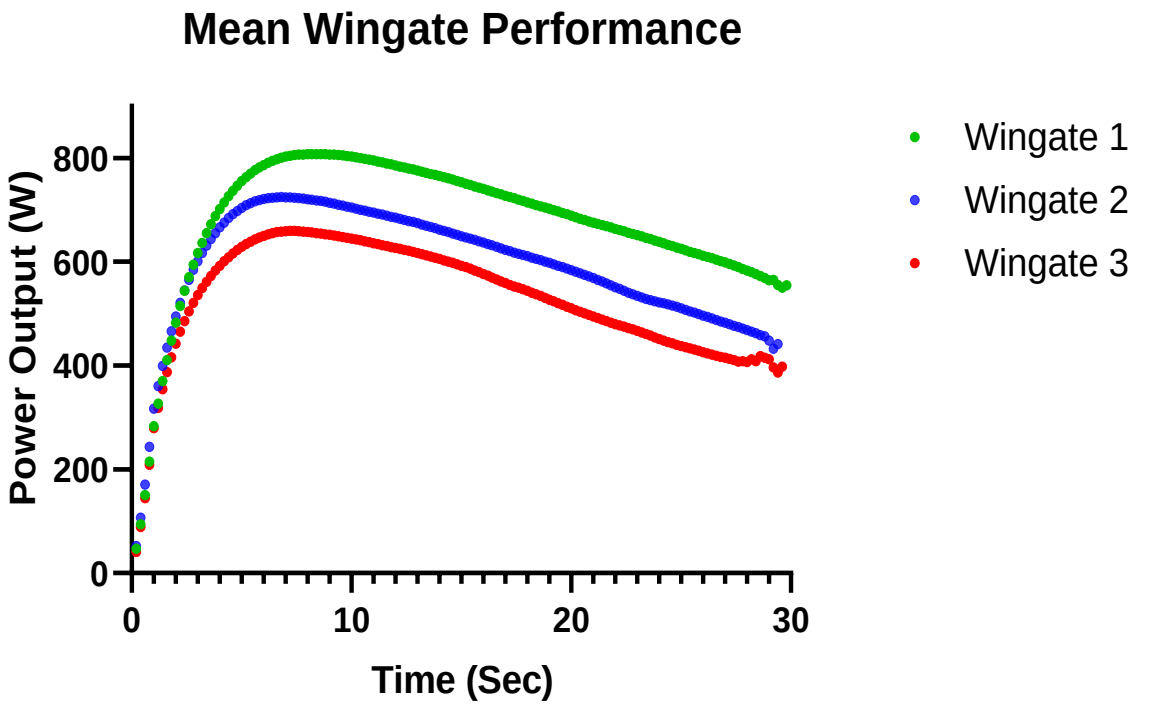
Table 3 summarises the performance and fatigue data for all participants (n=26) who completed the full protocol. The table shows each Wingate measure as an accumulation over the three Wingate sprints.

Wingate Measure	Mean	SD	Range
Mean Power (W)	632.2	78.2	879.0 – 496.3
Anaerobic Capacity (W·kg^{-1}BM)	8.52	1.03	6.9 - 10.7
Peak Power (W)	1163.8	206.7	788.1 - 1,775.2
Anaerobic Power (W·kg^{-1}BM)	15.6	2.0	11.34 - 18.9
Total Work (J)	56543.7	6980.1	44,427.5 - 78,507.6
Relative Total Work (J·kg·bm^{-1})	224.0	29.9	161.3 - 270.1
Fatigue Index (%)	37.4	12.8	16.1 - 70.8
Fatigue Profile (W·sec^{-1})	36.8	8.9	22.7 - 61.0

Table 3. Performance data from all three Wingate tests

Out of the 30 participants, 27 were able to attend and attempt the Wingate testing session. Only one participant was unable to complete the full protocol due to feelings of light-headedness and nauseousness. This individual's data were not included in the analysis; data for the 26 participants who successfully completed the full study protocol are thus shown. Further details and measures from the Wingate tests are and can be found in appendix 2 (Section 9.2).

Figure 6 shows the mean power output profile during each Wingate test (n=26). There was a 25% reduction in mean power, 24% reduction in anaerobic capacity and a 41% increase in the fatigue profile observed between the first and third Wingate tests.



There were a number of significant relationships found within and across variables in each Wingate test. The mean power showed significant decreases between each Wingate (W1 – W2; p= 0.004, W2 – W3; p= 0.0027) and also throughout the whole repeated protocol (W1 – W3; p= <0.0001).

Anaerobic capacity also showed the same significant differences with (W1 – W2; p= 0.0004, W2 – W3; p= 0.0023) whilst also throughout the whole repeated protocol (W1 – W3; p= <0.0001).

Total work also showed significant decreases between each Wingate (W1 – W2; p= 0.005, W2 – W3; p= 0.0022) and throughout the whole protocol (W1 – W3; p=<0.0001). Relative total work showed similar significant decreases with (W1 – W2; p= 0.0119, W2 – W3; p= 0.0014) and throughout the whole protocol (W1 – W3; p=<0.0001)

Peak Power significantly decreased between Wingate tests 1-3 (p=<0.0001) and 2-3 (p=0.0101). Relative peak power showed the same trend with a significant decreases between Wingate tests 1-3 (p=<0.0001) and 2-3 (p=0.0015).

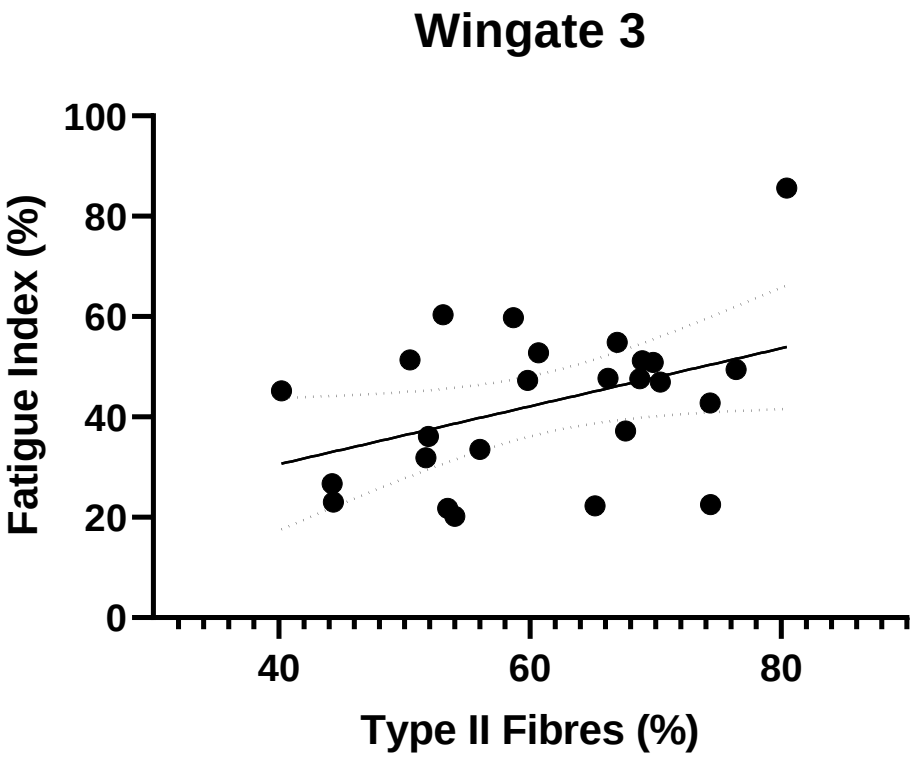
Table 4 shows the r and p values of Pearson's correlations run between resting carnosine levels and each Wingate measure within each Wingate bout.

Pearson's correlations between Carnosine and Wingate measures from each Wingate

Wingate Measure	Wingate 1	Wingate 2	Wingate 3
Mean Power (W)	-0.07437 (p=0.7124)	0.1700 (p=0.3966)	0.1553 (p=0.4487)
Anaerobic Capacity (W/kg.bm)	-0.099 (p=0.6270)	0.2220 (p=0.2657)	0.1952 (p=0.3392)
Peak Power (W)	-0.1084 (p=0.5905)	0.1523 (p=0.4483)	0.1950 (p=0.3398)
Anaerobic Power (W/kg.bm)	-0.08687 (p=0.6666)	0.2526 (p=0.2036)	0.2555 (p=0.2078)
Total Work (J)	-0.06892 (p=0.7327)	0.1680 (p=0.4022)	0.1535 (p=0.4540)
Relative Total Work (J/kg.bm)	0.1694 (p=0.3983)	0.2734 (p=0.1676)	0.2267 (p=0.2655)
Fatigue Index (%)	-0.1409 (p=0.4833)	-0.2283 (p=0.2520)	-0.07584 (p=0.7127)
Fatigue Profile (W/sec)	0.07965 (p=0.6929)	0.005933 (p=0.9766)	0.1980 (p=0.3322)

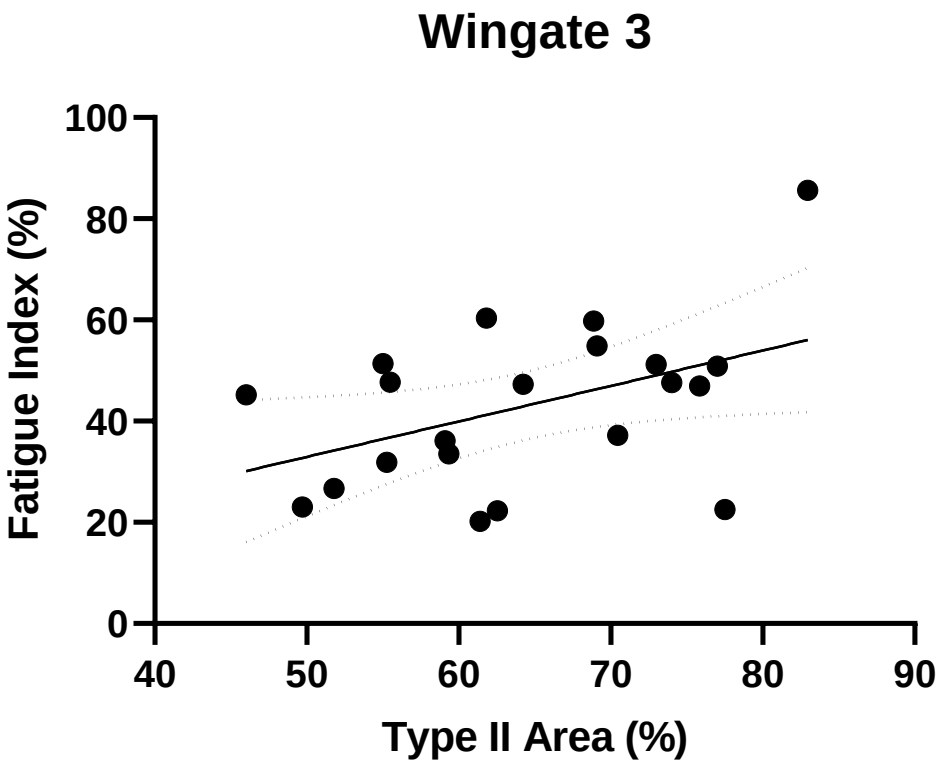
There were no significant relationships between resting carnosine levels and any of the performance or fatigue measures in any of the Wingate bouts.

Figure 7 shows the relationship between the Fatigue Index in the third Wingate bout and the percentage of type II fibres.



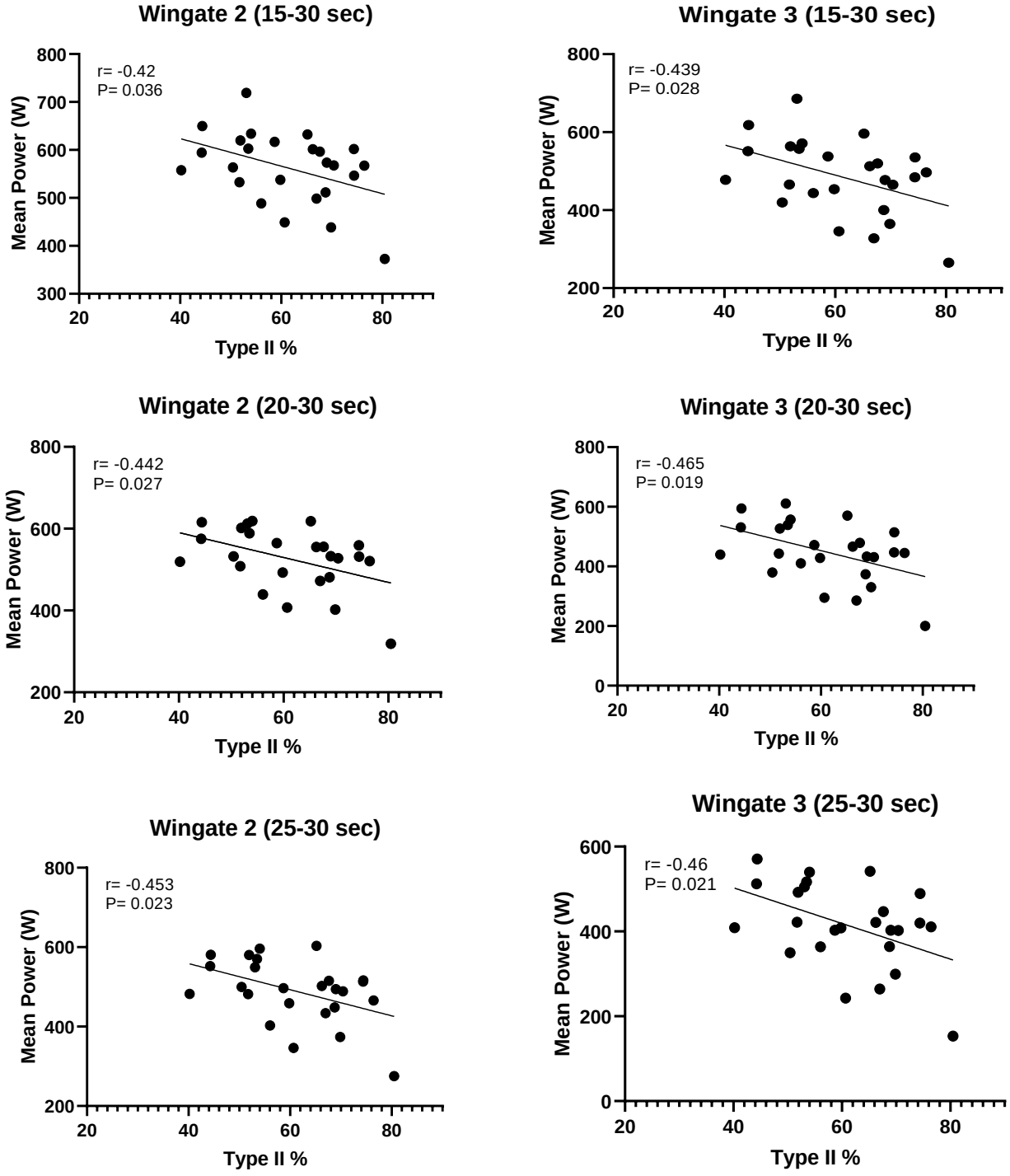
This shows a moderately strong, positive correlation (r= 0.4075; r^2= 0.166; p =0.0432) between the percentage of type II and Fatigue Index from the last Wingate bout (n=26).

Figure 8 shows the relationship between the Fatigue Index in the third Wingate bout and the percentage area of type II fibres.



There was moderately strong and positive correlation (p =0.043; r^2 = 0.199; r = 0.426) between the percentage area of type II and Fatigue Index from the last Wingate bout (n=26).

Figure 9 shows a breakdown of the last 15 seconds of the 2nd and 3rd Wingate bouts. Each graph examines the relationship between Mean Power and Percentage of Type II fibres. The 'r' and 'p' values for each correlation is provided on the graphs.



All six graphs show significant and negative correlations between percentage of type II fibres and mean power output. The correlations are stronger on average in the 3rd Wingate test compared to the 2nd test. The r and p values show that the correlations are also stronger in the 25-30 second time period compared with the last 15 seconds.

6.0 Discussion

The present study examined relationships between skeletal muscle fibre composition (MFC), carnosine levels and multiple sprint (Wingate) performance. It was hypothesised that percentage of type II fibres would be related to carnosine levels and better repeated sprint performance. It was also hypothesised that participants with higher carnosine levels would perform better (fatigue less) in the latter stages of a 30 second sprint while also in the subsequent 2nd and 3rd Wingate tests.

The primary finding from this study was that resting carnosine levels were not related to percentage of type II fibres, percentage area of type II fibres or relative fibre area. In addition, resting carnosine levels were not related to any performance or fatigue measures in the repeated Wingate protocol. There were however relationships found between fibre type and multiple sprint performance; percentage of type II fibres and percentage area of type II fibres were both related to power and to fatigue in the latter stages of the protocol.

6.1 MFC and Resting Carnosine

The current study found no significant correlations between percentage of type II fibres ($r= 0.056$; $p= 0.77$; $n=29$), percentage area of type II fibres ($r= 0.048$; $p= 0.83$; $n=24$) or relative fibre area ($r= 0.065$; $p= 0.77$; $n=24$) and resting carnosine levels. This finding stands apart from the work of Harris et al who reported that type II fibres possess 200% more carnosine than type I fibres (Harris et al., 1998). The current study determined MFC using MHC antibodies, whilst Harris and colleagues used ATPase. It remains unclear if the different methods would contribute towards the variance.

However, the present findings agree with the those of (Suzuki et al., 2002) who reported no significant relationship between resting carnosine and myosin ATPase determined type II fibres. Their investigation determined carnosine and MFC from the VL muscle and provided very similar ranges (1.29 – 7.14 mmol; 34.7 – 68.2%) to the current study. Suzuki et al., also found a significant correlation ($r= 0.65$ $p= <0.05$; $n= 11$) between resting carnosine and percentage of type IIx fibres. It's unclear whether the current study would have supported or disagreed with these findings as the subtype type IIx was not examined.

An investigation by Baguet, Everaert, Hespel, et al. (2011) found a significantly positive correlation ($r^2 = 0.50$; $p = 0.009$) between percentage area of type II fibres and resting carnosine levels. Interestingly this study had very similar ranges of carnosine concentrations (1.82-6.25

mM) and percentage type II fibre area (29-62%) to the current study and also those data reported by (Suzuki et al., 2002). It is important to note that Baguet, Everaert, Hespel, et al. (2011) sampled muscle from the gastrocnemius muscle whilst muscle sampled by Suzuki et al. (2002), similar to the current study, was from the VL. It is generally accepted that these two muscles are similar in terms of MFC (Elder et al., 1982; Gollnick et al., 1972; Johnson et al., 1973) however, correlations between MFC and resting carnosine between these muscles are yet to be compared. Major differences between the present study and the investigation by Baguet et al (2011a) was that they used ^{1}H-MRS to estimate resting carnosine and they had relatively low participant numbers (n=12). The same authors published a different paper (2011b) which found a significant correlation between ^{1}H-MRS and carnosine measured using HPLC (r^2= 0.517; p= 0.002). This shows that although there is a significant correlation, the correlation coefficient does not account for around half of the variance and could question the validity of their carnosine measure.

An investigation by da Eira Silva et al. (2019) questioned the validity of using magnetic resonance spectroscopy (^{1}H-MRS) to determine resting muscle carnosine levels. There was no significant relationship found between ^{1}H-MRS determined carnosine and HPLC determine carnosine (r=0.27; p=0.38; n=16). The authors noted that various mechanisms such as; magnetic interaction with neighbouring nuclei and spin-spin coupling can cause high baseline noise (da Eira Silva et al., 2019). Due to the small amplitude of carnosine in ^{1}H-MRS, this noise can significantly affect the accuracy and reliability of the reading, particularly with individuals who have low levels of carnosine. They concluded by suggesting these methodological issues must be considered when using ^{1}H-MRS to interpret resting muscle carnosine values (da Eira Silva et al., 2019).

The present findings in relation to MFC and resting carnosine levels do not deviate much from those available in the related literature. The mean and ranges from the current study's cohort data are similar to those of previously published work. However, the methods to determine MFC and resting carnosine were different and this could potentially explain disparities.

6.2 Carnosine and Sprint Performance

The present study found no significant relationships between carnosine and mean power, anaerobic capacity, peak power, relative peak power, total work, relative total work, fatigue profile and fatigue index. This does not support the study's hypothesis and suggests the involvement of carnosine as a hydrogen ion buffer did not have a significant effect on either

performance or fatigue in this cohort. Though evidence from the available literature is mixed, the majority, on balance, show that higher carnosine levels are associated with improved Wingate performance (de Salles Painelli et al., 2014; Sale, Saunders, & Harris, 2010; Suzuki et al., 2002; Tobias et al., 2013).

An investigation by Suzuki et al. (2002) reported very similar resting muscle carnosine concentrations and sprint performance to those found in the current study. The resting carnosine concentrations (3.66 mmol ± 1.87 vs 3.58 mmol ± 1.17) and Mean Power (8.4 W ± 0.4 vs 8.52 W ± 1.03) from a single Wingate bout were significantly related ($r=0.785$, $p= <0.01$; $n=11$) in their study (Suzuki et al., 2002). This may be explained by their participants being all untrained; participants in the current study ranged from untrained to trained.

An abstract published by Lievens (2017), examined performance and fatigue from three repeated Wingate tests in pre-selected individuals with extremely high (>0.5 z-score; n=6) and low (>0.5 z-score; n=6) levels of resting muscle carnosine. They reported no significant differences in mean power between groups (Lievens, 2017). They did however observe significant differences in fatigue within and across each Wingate. The high carnosine group presented a higher fatigue index (51% ±5) within each Wingate and across the whole protocol (18%±10) when compared to the low carnosine group (36%±5 and 7%±6 respectively). These findings differ from the work of Suzuki and colleagues, however their use of ^{1}H-MRS for determining muscle carnosine and small participant numbers may explain this.

It is accepted that carnosine is one of several compounds that contributes to buffering and in turn contributes towards anaerobic performance and fatigue (Hill et al., 2007; Sale et al., 2010) Many studies have also found that in addition to carnosine, there are numerous other buffering mechanisms that can influence sprint and multiple sprint performance. These mechanisms include the action of phosphates, proteins and dipeptides in the cytosol after a change in pH (Bogdanis, Nevill, Lakomy, & Boobis, 1998; Hill et al., 2007; Sale et al., 2010). Bogdanis et al. (1998) suggested that the reduction in intramuscular pH during maximal anaerobic exercise does not tend to affect performance or fatigue during a single Wingate bout due to the short exercise duration. However, they suggest that the fall in pH significantly affects subsequent bouts. This is also supported by their earlier work in 1995 which showed that the fall in pH did not have a significant effect on the power output following two Wingate tests (Bogdanis, Nevill, Boobis, Lakomy, & Nevill, 1995).

An investigation by Baguet, Everaert, De Naeyer, et al. (2011) examined changes in resting carnosine levels and buffering capacity after 5 weeks of sprint training. Despite finding no significant differences after training, they found a significant correlation (r= 0.343; p= 0.07) between resting carnosine levels and overall buffering capacity (Baguet, Everaert, De Naeyer, et al., 2011). The authors suggested that carnosine plays a large role in the intracellular buffering capacity, however it also suggests that other buffers also affect performance. There is still considerable unexplained variance suggesting that factors in addition to resting muscle carnosine concentrations influence sprint performance.

Another potential explanation for the non-significant relationship between carnosine and fatigue in the present study may be the range in training status of the participants in the cohort. The current study recruited both recreationally active and athletic males. As described earlier by (Sale et al., 2010), intracellular buffering capacity is responsive to training and therefore the variety in training status may have masked the carnosine buffering contribution.

Carnosine was also compared to the relative peak power decrease between each Wingate. There was a significant and positive relationship (r^2= 0.151; p= 0.0497) between carnosine and the percentage decrease in relative peak power from Wingate tests 1-2. This decrease ranged from 31.52% to -7.74%, with a total of seven participants increasing relative peak power in their 2nd Wingate. A possible explanation for the increase in relative peak power for the second Wingate might be due to the variance in fatigue profiles during the first Wingate. When grouped, these seven participants' fatigue profiles were found to be significantly less (r^2= 0.21; p= 0.0184) than the remainder of the cohort.

This relationship also runs true for the seven participants with the highest percentage decrease in relative peak power. When compared to the remainder of the cohort, these seven participants present significantly (r^2= 0.259; p= 0.01) higher fatigue index's. These findings suggest that participants who demonstrate a higher fatigue index are significantly less likely to re-produce high relative peak power values for the second Wingate test.

6.1.1 Carnosine and Fatigue during Latter Stages of the Wingate Tests

There were no significant relationships found between carnosine and fatigue index or performance indicators in the latter stages of any of the Wingate test. There was a common trend for the performance to gradually increase and the fatigue profile to gradually decrease

with higher levels of resting carnosine. Despite this, no statistical significance was found in the 15-30, 20-30 or 25-30 second periods in any Wingate bout.

It was expected that participants with higher carnosine levels would perform better (fatigue less) in the latter stages of each Wingate and particularly in the last 5 and 10 seconds; Previous research has shown trends in the latter stages of anaerobic exercise and the general role carnosine has in the intramuscular buffering capacity.

Multiple authors have suggested there is a relationship between carnosine and the latter stages of maximal sprint exercise (Derave et al., 2007; Suzuki et al., 2002). Suzuki et al. (2002) measured resting carnosine levels from muscle biopsy samples and compared these with fatigue profiles from one single Wingate test. Positive and significant correlations between resting carnosine levels, percentage of type II fibres and mean power output throughout the 30 seconds and last 10 seconds were found (Suzuki et al., 2002).

An investigation by Derave et al. (2007) found similar relationships when examining fatigue in response to five sets of 30 maximal voluntary isokinetic knee extensions. The individuals with higher levels of resting carnosine showed less fatigue in the latter stages of the test which resulted in a significant ($p= 0.05$; $n=15$) and negative correlation between relative peak power and resting carnosine levels (Derave et al., 2007).

An explanation for the different findings in the current study may be due the duration of the repeated sprint protocol used. Due to the accumulation of hydrogen ions being the most likely mechanism involved, it could be suggested that the present duration of the protocol was simply not long enough to induce a significant difference in $H+$ ion accumulation and consequently a significant decrease in performance.

The absence of any relationship between carnosine and the performance or fatigue measures in the current study, suggests there are other mechanisms with greater significance as buffers that contribute towards repeated high intensity performance. Though it is commonly agreed that carnosine is a buffer, the significance this has on the performance of recreationally active individuals may be blunted by other mechanisms.

6.3 MFC and Sprint Performance

Significant relationships were found between percentage area of type II fibres ($r= 0.04$; $p= 0.043$), percentage of type II fibres ($r= 0.408$; $p =0.0432$) and fatigue index during the third

Wingate bout. There were no significant relationships between MFC and fatigue in the first Wingate, whilst some significant relationships were found in the latter stages of the 2nd sprint. These findings are in line with the hypothesis that a higher percentage of type II fibres is associated with greater fatigue.

Individuals with predominantly type II fibres show greater signs of fatigue in the latter stages of maximal anaerobic exercise (Bar-Or et al., 1980; Karlsson et al., 1981; P. Tesch & Karlsson, 1978; Thorstensson & Karlsson, 1976). Despite this consistency, the modes used to elicit fatigue vary. Percentage of type II fibres has been correlated with maximal knee extensions, 40m sprint performance and a single Wingate test.

Bar-Or and colleagues found similar findings with a single Wingate bout. They found that 56% of the variance in power decrease (fatigue) could be explained by relative fibre size. No significant correlations were found between percentage area ($p=0.38$) or percentage of type II fibres ($p=0.22$) with a decrease in power output.

In contrast to these findings, the current study did not find a relationship between percentage of type II fibres ($r= 0.3061$; $p=0.14$), percentage area of type II fibres ($r= 0.2661$; $p=0.24$) or relative fibre area ($r= 0.2561$; $p=0.21$) with fatigue during the 1st Wingate test. Although this generally agrees with the work of (Bar-Or et al., 1980), the variety in training status of participants in the present study may explain the differences. However, a single Wingate sprint may not be enough to differentiate between type I and type II fibre dominant individuals; a single Wingate sprint may not result in a significant increase in H+ ion production in type II dominant individuals to result in a significant decrease in performance.

Interestingly, the current study found no significant relationships between percentage of type II fibres with Peak Power ($p=0.8$; $p=0.54$; $p=0.48$), Relative Peak Power ($p=0.9$; $p=0.74$; $p=0.81$), Mean Power ($p=0.37$; $p=0.1$; $p=0.12$), or Relative Mean Power ($p=0.7$; $p=0.38$; $p=0.37$) across any of the Wingate tests. It was expected that type II fibres would be related to higher peak power during each Wingate. Investigations by (Bar-Or et al., 1980; Suzuki et al., 2002) agree with this and reported correlations between percentage type II fibres and peak power ($p= <0.05$; $p=<0.01$) whilst also no significant findings with mean power. Both of these papers are very similar to the current study, however the difference in participant training status between investigations may in part explain inconsistencies.

6.3.1 MFC and Fatigue in Latter Stages

The percentage of type II fibres was significantly related to the fatigue profile during the last 15 seconds of the 2nd (r= -0.42; p= 0.036) and 3rd (r= -0.44; p= 0.028) Wingate sprints. These relationships become stronger when the last 10 seconds and last 5 seconds in both the 2nd (r= -0.44; p=0.027 and r=-.045; p=0.02) and 3rd (r= -0.47; p=0.02 and r=-0.046; p=0.02) sprints were considered. All six graphs (Figure 6) demonstrate significant and negative correlations between percentage of type II fibres and mean power output. It was hypothesised that individuals with more type II fibres would fatigue faster in the latter stages of each Wingate bout. This was based on the idea that type II fibres at a cellular level fatigue at a significantly faster rate compared with type I.

There are various mechanisms that might explain why individuals with predominantly type II fibres showed greater fatigue in latter stages of maximal anaerobic exercise. One reason might be the production and accumulation of lactate in the exercising muscles. It's been shown that high levels of H+ production in maximal anaerobic exercise has been correlated with higher performance (Thorstensson & Karlsson, 1976). Although this is the case, a long duration of maximal exercise will end up accumulating these H+ ions and consequently decreasing performance (Thorstensson & Karlsson, 1976). This premise supports the current findings as the significant differences in MFC relate fatigue is exclusive to the latter stages of the protocol. It could be assumed that the accumulation of H+ ions in type II dominant individuals became a significant factor in performance in the last Wingate and latter stages of the 2nd, due to a higher production rate. It seems reasonable to propose that the contribution of H+ ions as a result of a high proportion of type II fibres would be partially responsible for the fatiguing during anaerobic endurance capacity.

6.4 Wingate Performance and Fatigue

The mean cohort fatigue index showed a significant increase (p=0.0048) between the first and last Wingate sprints. The mean fatigue profile showed small decreases, but there were no significant differences across any of the Wingate tests. The lack of significant differences between Wingate trials for fatigue profile, may be explained by the significant decreases in peak power across each Wingate. Due to the reduction in peak power in Wingate sprints 1 and 2, there will be a small decrease in power output even if there is a higher percentage of fatigue. This is also confirmed by the significant correlation (r= 0.75; p=< 0.0001) shown in Appendix 4 (Section 9.4). This shows that participants who recorded a high original peak power, also

fatigued the greatest during the sprint. This trend also stays consistent when compared with the fatigue index in the last 10 seconds. There was a significant and positive correlation (r= 0.61; p= 0.001) between peak power and the fatigue profile from the last 10 seconds.

The performance data from the current study is consistent with studies that have used recreationally active males of a similar demographic (Forbes, Candow, Little, Magnus, & Chilibeck, 2007; Greer, McLean, & Graham, 1998; Inbar et al., 1981; Watt, Hopkins, & Snow, 2002). As seen in Appendix 1, there were significant decreases in peak power, mean power, anaerobic capacity, anaerobic power and total work across each Wingate (Section 9.1). There were also significant increases in the fatigue index and slope across each Wingate test. All of the present cohort sprint data are consistent with those from other studies that have examined the same variables during a 3 x Wingate protocol with 4 minute rest periods (Greer et al., 1998; Inbar et al., 1981; Lievens, 2017; McCartney et al., 1986; Spriet, Lindinger, McKelvie, Heigenhauser, & Jones, 1989; Suzuki et al., 2002).

6.5 Practical Applications

The current study has reported that MFC influences multiple sprint performance, particularly in the latter stages of exercise. Strength and conditioning coaches and high performance staff can use this knowledge to make subtle changes to training programs for individuals who may possess high percentages of a certain fibre type. It could be proposed that type II dominant athletes may need longer rest periods and shorter exercise duration when prescribing anaerobic exercise to account for the likelihood of high levels of fatigue in the latter stages.

The lack of variance reported in the 1st Wingate bout can also show practitioners that a single Wingate test may not provide an accurate understanding and representation of maximal anaerobic performance and fatigue. It would be suggested that if coaching staff require a comprehensive understanding of how an individual performs and fatigues during maximal anaerobic exercise, a repeated Wingate protocol similar to this study should be implemented.

The performance in the latter stages of this protocol can also provide practitioners with an estimation of an individual's MFC. The significant correlation with type II fibres and fatigue in last 15 seconds of the 2nd and the whole 3rd Wingate bout, suggested that individuals who show grater signs of fatigue may also present with a higher percentage of type II fibres. Therefore, examining performance in these latter stages (observing or using a regression model) may assist in the estimation of MFC for athletic individuals.

Various recommendations can be made for individuals who show greater signs of fatigue in the latter stages of anaerobic exercise. It may be beneficial to extend rest periods between sets in resistance training and anaerobic conditioning drills. Due to the higher chance of H+ ion accumulation, these individuals may require longer rest periods to ensure performance on the subsequent sets are of quality. To optimise performance, it also may be beneficial for these individuals to limit maximal anaerobic bouts of exercise to short durations (less than 20 seconds). If longer durations of maximal effort are required during competition, it would be recommended that extended rest is taken directly after.

6.6 Limitations and Future Directions

A limitation in any study that involves muscle biopsies is the size of the sample collected and method of analysis. The muscle sample used to determine the MFC of the VL muscle in the present study was less than 1% of the entire muscle. The muscle biopsy procedure is commonly accepted as the gold standard procedure when it comes to MFC, however the sample does not necessarily represent the whole muscle belly. It would be recommended that multiple samples from different portions of the VL muscle are taken to ensure there is no grouping or localised fibre composition effects on the reading.

Another possible limitation is the lack of a familiarisation session for the Wingate testing. Completing a single Wingate bout prior to the actual testing protocol could allow the participant to become familiar with the maximal nature of the task. This would mentally prepare each individual for the task, whilst also giving the researcher information to see if pacing is present. Although it was made extremely clear prior to the test that is was to be an all-out, maximal effort, the demanding nature of the test suggests a cognitive/mental component may have a subtle impact on the fatigue profile.

It is recommended that future research should focus on identifying the influence of high percentages of MFC or extreme levels of muscle carnosine on maximal anaerobic performance. Although the current study had a good range of MFC and resting muscle carnosine values, it would be of benefit to examine a large number of individuals who possess high percentages of either type I or II fibres or particularly high and low carnosine levels. This could further develop the understanding of anaerobic performance in athletic populations and could warrant changes in the way coaching staff individualise exercise programs.

Given that muscle carnosine may not play a significant role in performance during maximal anaerobic exercise, the use of supplements aimed at increasing resting carnosine concentrations

(eg. beta-alanine) is questionable; further research could include supplementation within an experiment that includes the same procedures as those used in the present study.

7.0 Conclusions

The present investigation found no significant relationships between MFC and resting muscle carnosine levels. Contrary to the hypothesis, there were no significant relationships between resting carnosine levels and any performance or fatigue measures in the repeated Wingate protocol.

There were however relationships found between fibre type and multiple sprint performance; percentage of type II fibres and percentage area of type II fibres were both related to an increase in fatigue during the latter stages of the protocol. This may be explained by the increased production of H+ ions and the inability to regulate them in the latter stages of the protocol.

This study has furthered the current research and highlighted the relationships and roles of MFC and carnosine in maximal anaerobic exercise. In summary, this investigation showed no relationship between MFC and resting muscle carnosine levels, whilst also demonstrating that individuals with a relatively higher percentage of type II fibres may show greater levels of fatigue in the latter stages of maximal anaerobic exercise.

8.0 List of References

Adams, R. (1968). Report of a subcommittee on the quantitation of muscle biopsy findings. *J. Neurol. Sci, 6*, 179-189.

Alen, M., Hakkinen, K., & Komi, P. V. (1984). Changes in neuromuscular performance and muscle fiber characteristics of elite power athletes self-administering androgenic and anabolic steroids. *Acta Physiol Scand, 122*(4), 535-544. doi:10.1111/j.1748-1716.1984.tb07542.x

Allen, D. G., Lamb, G. D., & Westerblad, H. (2008). Skeletal muscle fatigue: cellular mechanisms. *Physiol Rev, 88*(1), 287-332. doi:10.1152/physrev.00015.2007

Andersen, L. L., & Aagaard, P. (2006). Influence of maximal muscle strength and intrinsic muscle contractile properties on contractile rate of force development. *Eur J Appl Physiol, 96*(1), 46-52. doi:10.1007/s00421-005-0070-z

Baguet, A., Everaert, I., De Naeyer, H., Reyngoudt, H., Stegen, S., Beeckman, S., . . . Derave, W. (2011). Effects of sprint training combined with vegetarian or mixed diet on muscle carnosine content and buffering capacity. *Eur J Appl Physiol, 111*(10), 2571-2580. doi:10.1007/s00421-011-1877-4

Baguet, A., Everaert, I., Hespel, P., Petrovic, M., Achten, E., & Derave, W. (2011). A new method for non-invasive estimation of human muscle fiber type composition. *PLoS One, 6*(7), e21956. doi:10.1371/journal.pone.0021956

Baguet, A., Reyngoudt, H., Pottier, A., Everaert, I., Callens, S., Achten, E., & Derave, W. (2009). Carnosine loading and washout in human skeletal muscles. *J Appl Physiol (1985), 106*(3), 837-842. doi:10.1152/japplphysiol.91357.2008

Bar-Or, O., Dotan, R., Inbar, O., & Rothstein, A. (1980). *Anaerobic Capacity and Muscle Fiber Type Distribution in Man* (Vol. 01).

Begum, G., Cunliffe, A., & Leveritt, M. (2005). Physiological Role of Carnosine in Contracting Muscle. *15*(5), 493. doi:10.1123/ijsnem.15.5.493

Bell, G. J., & Wenger, H. A. (1988). The effect of one-legged sprint training on intramuscular pH and nonbicarbonate buffering capacity. *European Journal of Applied Physiology and Occupational Physiology, 58*(1), 158-164. doi:10.1007/bf00636620

Bergstrom, J. (1975). Percutaneous Needle-Biopsy of Skeletal-Muscle in Physiological and Clinical Research. *Scandinavian Journal of Clinical & Laboratory Investigation, 35*(7), 606-616.

Bishop, D., Edge, J., & Goodman, C. (2004). Muscle buffer capacity and aerobic fitness are associated with repeated-sprint ability in women. *Eur J Appl Physiol, 92*(4-5), 540-547. doi:10.1007/s00421-004-1150-1

Blancquaert, L., Everaert, I., & Derave, W. (2015). Beta-alanine supplementation, muscle carnosine and exercise performance. *Curr Opin Clin Nutr Metab Care, 18*(1), 63-70. doi:10.1097/MCO.0000000000000127

Bogdanis, G. C., Nevill, M. E., Boobis, L. H., Lakomy, H. K., & Nevill, A. M. (1995). Recovery of power output and muscle metabolites following 30 s of maximal sprint cycling in man. *J Physiol, 482 (Pt 2)*, 467-480. doi:10.1113/jphysiol.1995.sp020533

Bogdanis, G. C., Nevill, M. E., Lakomy, H. K., & Boobis, L. H. (1998). Power output and muscle metabolism during and following recovery from 10 and 20 s of maximal sprint exercise in humans. *Acta Physiol Scand, 163*(3), 261-272. doi:10.1046/j.1365-201x.1998.00378.x

Campbell, C. J., Bonen, A., Kirby, R. L., & Belcastro, A. N. (1979). Muscle fiber composition and performance capacities of women. *Med Sci Sports, 11*(3), 260-265.

Cormie, P., McGuigan, M., & Newton, R. (2011). Developing Maximal Neuromuscular Power (Vol. 41, pp. 17-38). Auckland: Springer Science & Business Media.

Costill, D. L., Daniels, J., Evans, W., Fink, W., Krahenbuhl, G., & Saltin, B. (1976). Skeletal muscle enzymes and fiber composition in male and female track athletes. *J Appl Physiol, 40*(2), 149-154.

da Eira Silva, V., de Salles Painelli, V., Shinjo, S. K., Cilli, E. M., Sale, C., Gualano, B., . . . Artioli, G. G. (2019). Is proton magnetic resonance spectroscopy a valid method to quantify muscle carnosine in humans? *bioRxiv*, 568923. doi:10.1101/568923

de Salles Painelli, V., Saunders, B., Sale, C., Harris, R. C., Solis, M. Y., Roschel, H., . . . Lancha, A. H., Jr. (2014). Influence of training status on high-intensity intermittent performance in response to beta-alanine supplementation. *Amino Acids, 46*(5), 1207-1215. doi:10.1007/s00726-014-1678-2

Derave, W., Ozdemir, M. S., Harris, R. C., Pottier, A., Reyngoudt, H., Koppo, K., . . . Achten, E. (2007). beta-Alanine supplementation augments muscle carnosine content and attenuates fatigue during repeated isokinetic contraction bouts in trained sprinters. *J Appl Physiol (1985), 103*(5), 1736-1743. doi:10.1152/japplphysiol.00397.2007

Derman, E. W., Hawarden, D., & Schwellnus, M. P. (2010). Allergic rhinoconjunctivitis in athletes - mechanisms of impaired performance and implications for management. *Current Allergy & Clinical Immunology, 23*(2), 59-62.

Donselaar, Y., Eerbeek, O., Kernell, D., & Verhey, B. A. (1987). Fibre sizes and histochemical staining characteristics in normal and chronically stimulated fast muscle of cat. *J Physiol, 382*, 237-254.

Douris, P. C., White, B. P., Cullen, R. R., Keltz, W. E., Meli, J., Mondiello, D. M., & Wenger, D. (2006). The relationship between maximal repetition performance and muscle fiber type as estimated by noninvasive technique in the quadriceps of untrained women. *J Strength Cond Res, 20*(3), 699-703. doi:10.1519/17204.1

Edwards, R., Young, A., & Wiles, M. (1980). Needle biopsy of skeletal muscle in the diagnosis of myopathy and the clinical study of muscle function and repair. *N Engl J Med, 302*(5), 261-271. doi:10.1056/NEJM198001313020504

Edwards, R. H. (1983). Biochemical bases of fatigue in exercise performance: catastrophe theory of muscular fatigue. *Biochemistry of exercise, 13*, 3-28.

Edwards, R. H. T., Porter, R., & Whelan, J. (2009). *Human Muscle Fatigue*: Novartis Foundation Symposia.

Elder, G. C., Bradbury, K., & Roberts, R. (1982). Variability of fiber type distributions within human muscles. *Journal of Applied Physiology, 53*(6), 1473-1480. doi:10.1152/jappl.1982.53.6.1473

Evans, W. J., Phinney, S. D., & Young, V. R. (1982). Suction applied to a muscle biopsy maximizes sample size. *Med Sci Sports Exerc, 14*(1), 101-102.

Fitts, R. H. (1994). Cellular mechanisms of muscle fatigue. *Physiol Rev, 74*(1), 49-94. doi:10.1152/physrev.1994.74.1.49

Forbes, S. C., Candow, D. G., Little, J. P., Magnus, C., & Chilibeck, P. D. (2007). Effect of Red Bull energy drink on repeated Wingate cycle performance and bench-press muscle endurance. *Int J Sport Nutr Exerc Metab, 17*(5), 433-444.

Foster, C., Costill, D. L., Daniels, J. T., & Fink, W. J. (1978). Skeletal muscle enzyme activity, fiber composition and VO2 max in relation to distance running performance. *Eur J Appl Physiol Occup Physiol, 39*(2), 73-80.

Fry, A. C., Schilling, B. K., Staron, R. S., Hagerman, F. C., Hikida, R. S., & Thrush, J. T. (2003). Muscle fiber characteristics and performance correlates of male Olympic-style weightlifters. *J Strength Cond Res, 17*(4), 746-754.

Gollnick, P. D., Armstrong, R. B., Saltin, B., Saubert, C. W. t., Sembrowich, W. L., & Shepherd, R. E. (1973). Effect of training on enzyme activity and fiber composition of human skeletal muscle. *J Appl Physiol, 34*(1), 107-111.

Gollnick, P. D., Armstrong, R. B., Saubert, C. W. t., Piehl, K., & Saltin, B. (1972). Enzyme activity and fiber composition in skeletal muscle of untrained and trained men. *J Appl Physiol, 33*(3), 312-319. doi:10.1152/jappl.1972.33.3.312

Green, H. J. (1997). Mechanisms of muscle fatigue in intense exercise. *J Sports Sci, 15*(3), 247-256. doi:10.1080/026404197367254

Greer, F., McLean, C., & Graham, T. E. (1998). Caffeine, performance, and metabolism during repeated Wingate exercise tests. *J Appl Physiol (1985), 85*(4), 1502-1508. doi:10.1152/jappl.1998.85.4.1502

Harris, R. C., Dunnett, M., & Greenhaff, P. L. (1998). Carnosine and taurine contents in individual fibres of human vastus lateralis muscle. *Journal of Sports Sciences, 16*(7), 639-643.

Harris, R. C., Tallon, M. J., Dunnett, M., Boobis, L., Coakley, J., Kim, H. J., . . . Wise, J. A. (2006). The absorption of orally supplied beta-alanine and its effect on muscle carnosine synthesis in human vastus lateralis. *Amino Acids, 30*(3), 279-289. doi:10.1007/s00726-006-0299-9

Harris, R. C., Wise, J. A., Price, K. A., Kim, H. J., Kim, C. K., & Sale, C. (2012). Determinants of muscle carnosine content. *Amino Acids, 43*(1), 5-12. doi:10.1007/s00726-012-1233-y

Hennessey, J. V., Chromiak, J. A., Della Ventura, S., Guertin, J., & MacLean, D. B. (1997). Increase in percutaneous muscle biopsy yield with a suction-enhancement technique. *J Appl Physiol (1985), 82*(6), 1739-1742.

Hill, C. A., Harris, R. C., Kim, H. J., Harris, B. D., Sale, C., Boobis, L. H., . . . Wise, J. A. (2007). Influence of beta-alanine supplementation on skeletal muscle carnosine concentrations and high intensity cycling capacity. *Amino Acids, 32*(2), 225-233. doi:10.1007/s00726-006-0364-4

Holloszy, J. O., & Coyle, E. F. (1984). Adaptations of skeletal muscle to endurance exercise and their metabolic consequences. *J Appl Physiol Respir Environ Exerc Physiol, 56*(4), 831-838. doi:10.1152/jappl.1984.56.4.831

Howald, H., Hoppeler, H., Claassen, H., Mathieu, O., & Straub, R. (1985). Influences of endurance training on the ultrastructural composition of the different muscle fiber types in humans. *Pflugers Arch, 403*(4), 369-376.

Inbar, O., Kaiser, P., & Tesch, P. (1981). Relationships between leg muscle fiber type distribution and leg exercise performance. *Int J Sports Med, 2*(3), 154-159. doi:10.1055/s-2008-1034603

Jansson, E., Sjodin, B., & Tesch, P. (1978). Changes in muscle fibre type distribution in man after physical training. A sign of fibre type transformation? *Acta Physiol Scand, 104*(2), 235-237. doi:10.1111/j.1748-1716.1978.tb06272.x

Jansson, E., Sjödin, B., & Tesch, P. (1978). Changes in muscle fibre type distribution in man after physical training: A sign of fibre type transformation? *Acta Physiologica Scandinavica, 104*(2), 235-237. doi:10.1111/j.1748-1716.1978.tb06272.x

Jennekens, F. G., Tomlinson, B. E., & Walton, J. N. (1971). Data on the distribution of fibre types in five human limb muscles. An autopsy study. *J Neurol Sci, 14*(3), 245-257.

Johnson, M. A., Polgar, J., Weightman, D., & Appleton, D. (1973). Data on the distribution of fibre types in thirty-six human muscles. An autopsy study. *J Neurol Sci, 18*(1), 111-129.

Juel, C. (1997). Lactate-proton cotransport in skeletal muscle. *Physiol Rev, 77*(2), 321-358. doi:10.1152/physrev.1997.77.2.321

Kaiser, P., Rossner, S., & Karlsson, J. (1981). Effects of beta-adrenergic blockade on endurance and short-time performance in respect to individual muscle fiber composition. *Int J Sports Med, 2*(1), 37-42. doi:10.1055/s-2008-1034582

Karlsson, J., Sjodin, B., Jacobs, I., & Kaiser, P. (1981). Relevance of muscle fibre type to fatigue in short intense and prolonged exercise in man. *Ciba Found Symp, 82*, 59-74. doi:10.1002/9780470715420.ch4

Kendrick, I. P., Harris, R. C., Kim, H. J., Kim, C. K., Dang, V. H., Lam, T. Q., . . . Wise, J. A. (2008). The effects of 10 weeks of resistance training combined with beta-alanine supplementation on whole body strength, force production, muscular endurance and body composition. *Amino Acids, 34*(4), 547-554. doi:10.1007/s00726-007-0008-3

Komi, P. V., Viitasalo, J. H., Havu, M., Thorstensson, A., Sjodin, B., & Karlsson, J. (1977). Skeletal muscle fibres and muscle enzyme activities in monozygous and dizygous twins of both sexes. *Acta Physiol Scand, 100*(4), 385-392.

Lievens, E. B., T. Derave, W. (2017). Can non-invasively determined muscle typology predict fatigue and recovery profile? *Book of abstracts : 22nd annual congress of the European College of Sport Science.*

Linssen, W. H., Stegeman, D. F., Joosten, E. M., Merks, H. J., ter Laak, H. J., Binkhorst, R. A., & Notermans, S. L. (1991). Force and fatigue in human type I muscle fibres. A surface EMG study in patients with congenital myopathy and type I fibre predominance. *Brain, 114 (Pt 5)*, 2123-2132.

Mannion, A. F., Jakeman, P. M., Dunnett, M., Harris, R. C., & Willan, P. L. (1992). Carnosine and anserine concentrations in the quadriceps femoris muscle of healthy humans. *Eur J Appl Physiol Occup Physiol, 64*(1), 47-50.

Mannion, A. F., Jakeman, P. M., & Willan, P. L. (1994). Effects of isokinetic training of the knee extensors on high-intensity exercise performance and skeletal muscle buffering. *Eur J Appl Physiol Occup Physiol, 68*(4), 356-361.

McCartney, N., Spriet, L. L., Heigenhauser, G. J., Kowalchuk, J. M., Sutton, J. R., & Jones, N. L. (1986). Muscle power and metabolism in maximal intermittent exercise. *J Appl Physiol (1985), 60*(4), 1164-1169. doi:10.1152/jappl.1986.60.4.1164

Methenitis, S., Karandreas, N., Spengos, K., Zaras, N., Stasinaki, A. N., & Terzis, G. (2016). Muscle Fiber Conduction Velocity, Muscle Fiber Composition, and Power Performance. *Med Sci Sports Exerc, 48*(9), 1761-1771. doi:10.1249/MSS.0000000000000954

Meunier, B., Picard, B., Astruc, T., & Labas, R. (2010). Development of image analysis tool for the classification of muscle fibre type using immunohistochemical staining. *Histochem Cell Biol, 134*(3), 307-317. doi:10.1007/s00418-010-0733-7

Mora, L., Sentandreu, M. A., & Toldra, F. (2007). Hydrophilic chromatographic determination of carnosine, anserine, balenine, creatine, and creatinine. *J Agric Food Chem, 55*(12), 4664-4669. doi:10.1021/jf0703809

Nevill, M. E., Boobis, L. H., Brooks, S., & Williams, C. (1989). Effect of training on muscle metabolism during treadmill sprinting. *J Appl Physiol (1985), 67*(6), 2376-2382. doi:10.1152/jappl.1989.67.6.2376

O'Rourke, K. S., & Ike, R. W. (1995). Muscle biopsy. *Curr Opin Rheumatol, 7*(6), 462-468.

Painelli, D. S., Nemezio, K. M., Pinto, A. J., Franchi, M., Andrade, I., Riani, L. A., . . . Artioli, G. G. (2018). High-Intensity Interval Training Augments Muscle Carnosine in the Absence of Dietary Beta-alanine Intake. *Med Sci Sports Exerc, 50*(11), 2242-2252. doi:10.1249/MSS.0000000000001697

Parkhouse, W. S., & McKenzie, D. C. (1984). Possible contribution of skeletal muscle buffers to enhanced anaerobic performance: a brief review. *Med Sci Sports Exerc, 16*(4), 328-338.

Parkhouse, W. S., McKenzie, D. C., Hochachka, P. W., & Ovalle, W. K. (1985). Buffering capacity of deproteinized human vastus lateralis muscle. *J Appl Physiol (1985), 58*(1), 14-17. doi:10.1152/jappl.1985.58.1.14

Patel, H., Cooper, C., & Aihie Sayer, A. (2012). *Percutaneous Muscle Biopsy: History, Methods and Acceptability*.

Prince, F. P., Hikida, R. S., & Hagerman, F. C. (1976). Human muscle fiber types in power lifters, distance runners and untrained subjects. *Pflugers Arch, 363*(1), 19-26.

Ricoy, J. R., Encinas, A. R., Cabello, A., Madero, S., & Arenas, J. (1998). Histochemical study of the vastus lateralis muscle fibre types of athletes. *J Physiol Biochem, 54*(1), 41-47.

Sahlin, K., Alvestrand, A., Bergstrom, J., & Hultman, E. (1977). Intracellular pH and bicarbonate concentration as determined in biopsy samples from the quadriceps muscle of man at rest. *Clin Sci Mol Med, 53*(5), 459-466.

Sale, C., Saunders, B., & Harris, R. C. (2010). Effect of beta-alanine supplementation on muscle carnosine concentrations and exercise performance. *Amino Acids, 39*(2), 321-333. doi:10.1007/s00726-009-0443-4

Sargeant, A. J., & de Haan, A. (2006). Human muscle fatigue: the significance of muscle fibre type variability studied using a micro-dissection approach. *J Physiol Pharmacol, 57 Suppl 10*, 5-16.

Scott, W., Stevens, J., & Binder–Macleod, S. A. (2001). Human Skeletal Muscle Fiber Type Classifications. *Physical Therapy, 81*(11), 1810-1816. doi:10.1093/ptj/81.11.1810

Simoneau, J. A., & Bouchard, C. (1989). Human variation in skeletal muscle fiber-type proportion and enzyme activities. *Am J Physiol, 257*(4 Pt 1), E567-572. doi:10.1152/ajpendo.1989.257.4.E567

Simoneau, J. A., Lortie, G., Boulay, M. R., Marcotte, M., Thibault, M. C., & Bouchard, C. (1986). Inheritance of human skeletal muscle and anaerobic capacity adaptation to high-intensity intermittent training. *Int J Sports Med, 7*(3), 167-171. doi:10.1055/s-2008-1025756

Smith, E. C. (1938). The buffering of muscle in rigor; protein, phosphate and carnosine. *J Physiol, 92*(3), 336-343. doi:10.1113/jphysiol.1938.sp003605

Spriet, L. L., Lindinger, M. I., McKelvie, R. S., Heigenhauser, G. J., & Jones, N. L. (1989). Muscle glycogenolysis and H+ concentration during maximal intermittent cycling. *J Appl Physiol (1985), 66*(1), 8-13. doi:10.1152/jappl.1989.66.1.8

Staron, R. S. (1997). Human Skeletal Muscle Fiber Types: Delineation, Development, and Distribution. *Canadian Journal of Applied Physiology, 22*(4), 307-327. doi:10.1139/h97-020

Staron, R. S., Hagerman, F. C., Hikida, R. S., Murray, T. F., Hostler, D. P., Crill, M. T., . . . Toma, K. (2000). Fiber type composition of the vastus lateralis muscle of young men and women. *J Histochem Cytochem, 48*(5), 623-629. doi:10.1177/002215540004800506

Suzuki, Ito, O., Mukai, N., Takahashi, H., & Takamatsu, K. (2002). High level of skeletal muscle carnosine contributes to the latter half of exercise performance during 30-s maximal cycle ergometer sprinting. *Jpn J Physiol, 52*(2), 199-205.

Suzuki, Y., Ito, O., Takahashi, H., & Takamatsu, K. (2004). The Effect of Sprint Training on Skeletal Muscle Carnosine in Humans. *International Journal of Sport and Health Science, 2*, 105-110. doi:10.5432/ijshs.2.105

Tallon, M. J., Harris, R. C., Boobis, L. H., Fallowfield, J. L., & Wise, J. A. (2005). The carnosine content of vastus lateralis is elevated in resistance-trained bodybuilders. *J Strength Cond Res, 19*(4), 725-729. doi:10.1519/041018.1

Tesch, Thorsson, A., & Kaiser, P. (1984). Muscle capillary supply and fiber type characteristics in weight and power lifters. *J Appl Physiol Respir Environ Exerc Physiol, 56*(1), 35-38. doi:10.1152/jappl.1984.56.1.35

Tesch, P., & Karlsson, J. (1978). Isometric strength performance and muscle fibre type distribution in man. *Acta Physiol Scand, 103*(1), 47-51. doi:10.1111/j.1748-1716.1978.tb06189.x

Thorstensson, A., & Karlsson, J. (1976). Fatiguability and fibre composition of human skeletal muscle. *Acta Physiol Scand, 98*(3), 318-322. doi:10.1111/j.1748-1716.1976.tb10316.x

Tobias, G., Benatti, F. B., de Salles Painelli, V., Roschel, H., Gualano, B., Sale, C., . . . Artioli, G. G. (2013). Additive effects of beta-alanine and sodium bicarbonate on upper-body intermittent performance. *Amino Acids, 45*(2), 309-317. doi:10.1007/s00726-013-1495-z

Trappe, S., Costill, D., & Thomas, R. (2001). Effect of swim taper on whole muscle and single muscle fiber contractile properties. *Medicine and science in sports and exercise, 33*(1), 48-56. doi:10.1097/00005768-200101000-00009

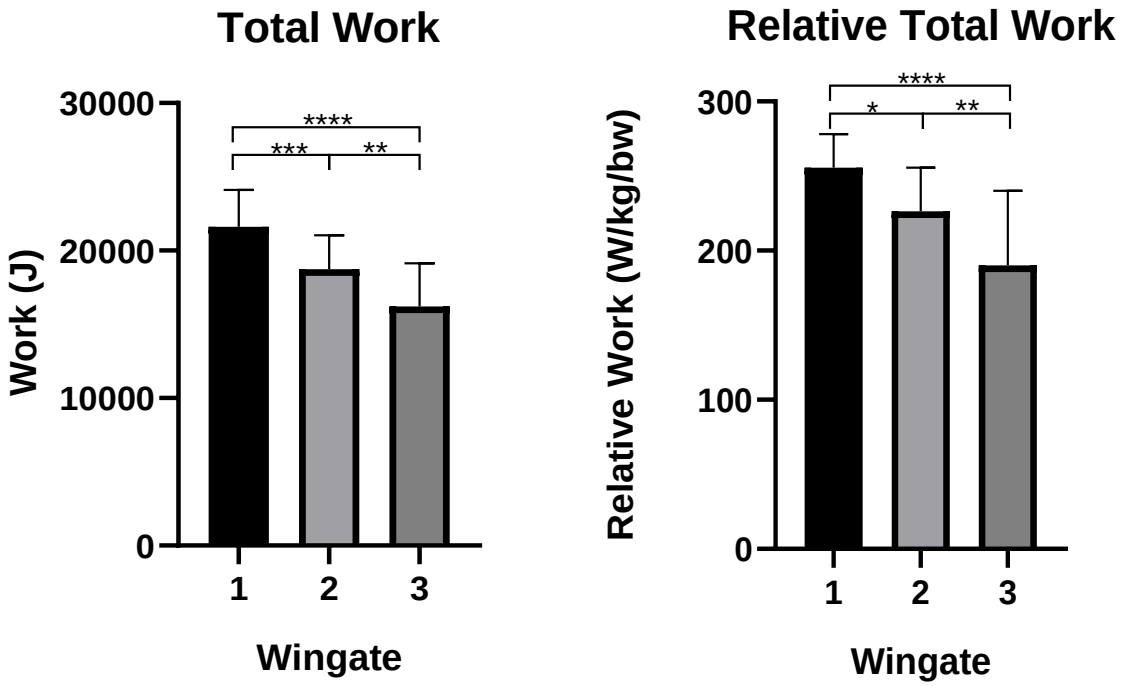
Watt, K. K., Hopkins, W. G., & Snow, R. J. (2002). Reliability of performance in repeated sprint cycling tests. *J Sci Med Sport, 5*(4), 354-361.

Wolos, A., Piekarska, K., Pilecka, T., Ciereszko, A., & Jablonowska, C. (1983). A new rapid method for determination of anserine and carnosine in muscles. *Comp Biochem Physiol B, 74*(3), 623-626.

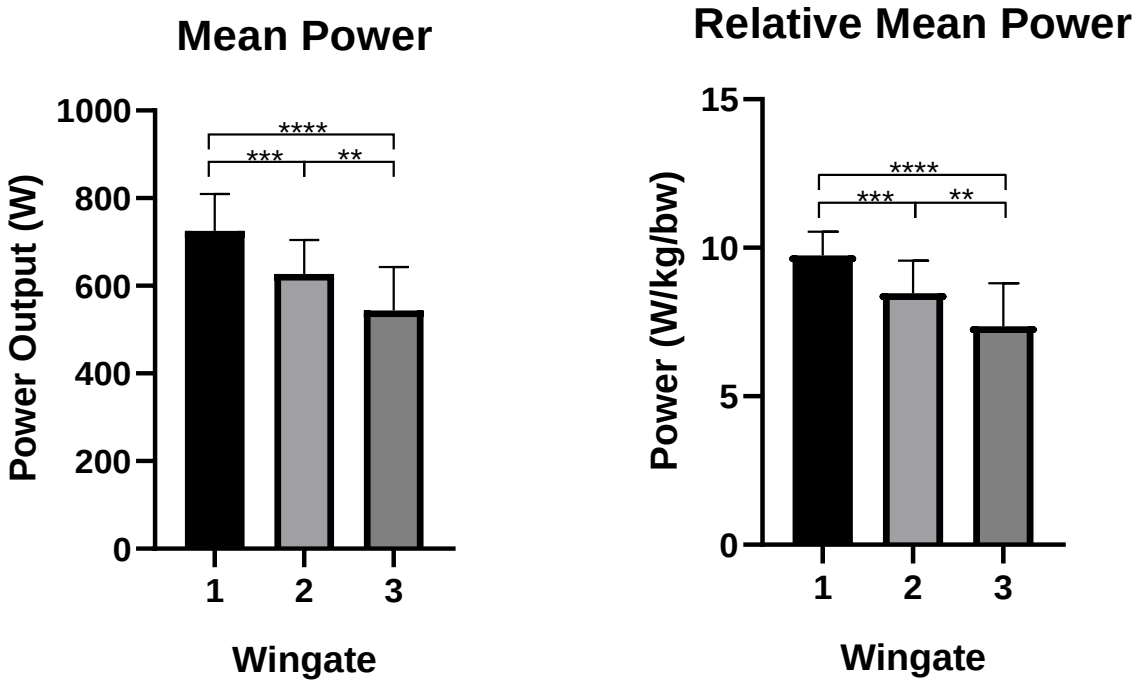
Zierath, J. R., & Hawley, J. A. (2004). Skeletal muscle fiber type: influence on contractile and metabolic properties. *PLoS Biol, 2*(10), e348. doi:10.1371/journal.pbio.0020348

9.0 Appendices

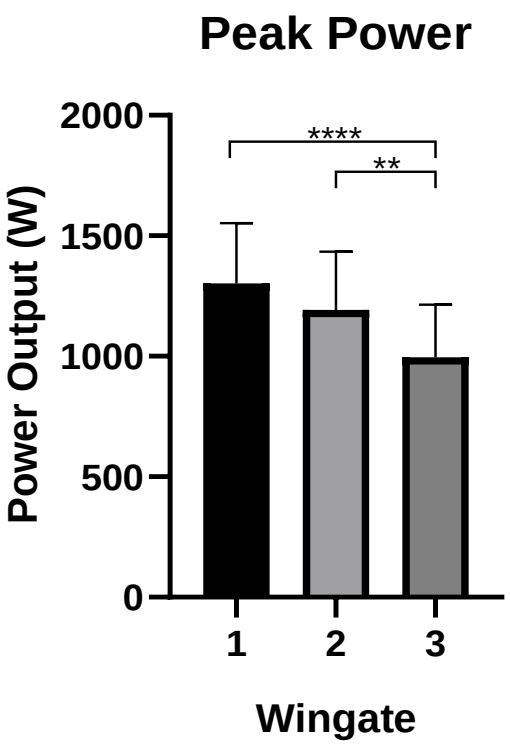
9.1 Wingate Mean Cohort Data.

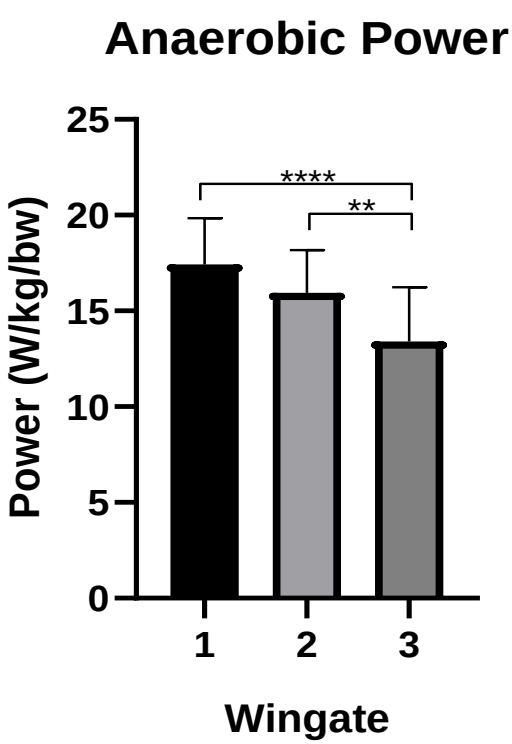


The mean and standard deviations for each measure are shown for each Wingate bout. Total Work and Relative Total Work both significantly decrease across each Wingate test.

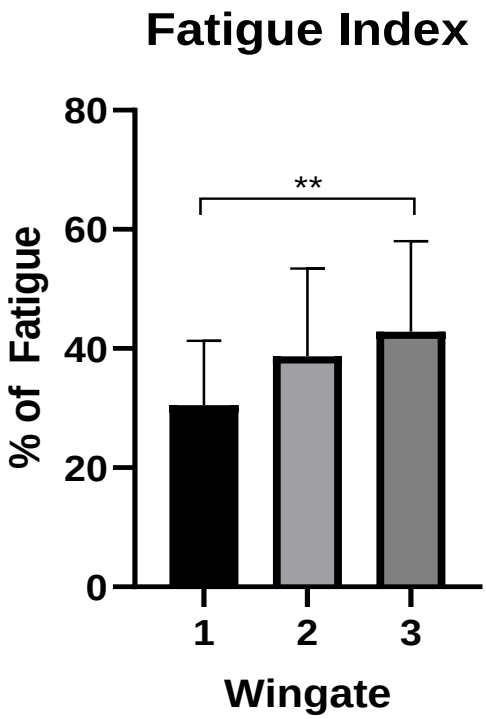


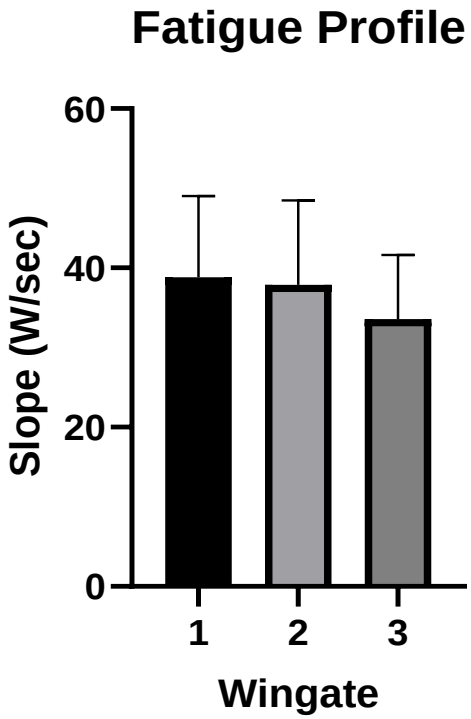
Mean and Relative Mean Power both significantly decrease across each Wingate test.





Peak Power and Anaerobic Power both significantly decrease across each Wingate test.





Fatigue Index shows a significant increase from the first to third Wingate tests. Fatigue Profile did not show any significant changes throughout the Repeated Wingate Protocol.

9.2 Mean Wingate Performance Measures

Table 5. Wingate 1

Wingate Measure	Mean	SD	Range
Mean Power	725.12	84.23	1014.96- 589.25
Anaerobic Capacity	9.75	0.8	10.83 - 8.06
Peak Power	1303.25	249.6	1849.46 - 796.08
Anaerobic Power	17.42	2.42	22.48 - 11.50
Total Work	21602.73	2520.51	30259.88 - 17529.56
Relative Total Work	255.74	22.33	291.46 - 217.17
Fatigue Index	30.51	10.81	57.30 - 9.81
Fatigue Slope	38.85	10.2	61.21 - 18.50

Table 5 describes the Wingate measures exclusively from the first Wingate test (n=27). This shows the mean and standard deviations for each Wingate measure.

Table 6. Wingate 2

Wingate Measure	Mean	SD	Range
Mean Power	627.27	77.63	848.24 - 475.49
Anaerobic Capacity	8.46	1.1	10.69 - 6.68
Peak Power	1192.55	240.92	1910.13 - 808.41
Anaerobic Power	15.94	2.24	19.48 - 11.68
Total Work	18735.69	2291.64	25220.65 - 14263.64
Relative Total Work	226.41	29.32	279.40 - 154.65
Fatigue Index	38.72	14.7	69.36 - 14.04
Fatigue Slope	37.88	10.6	67.35 - 21.71

Table 6 describes the Wingate measures exclusively from the second Wingate test (n=27). This shows the mean and standard deviations for each Wingate measure.

Table 7. Wingate 3

Wingate Measure	Mean	SD	Range
Mean Power	544.09	98.61	773.66 - 346.92
Anaerobic Capacity	7.35	1.45	10.47 - 4.84
Peak Power	995.62	218.25	1565.89 - 513.13
Anaerobic Power	13.41	2.84	18.26 - 7.33
Total Work	16205.28	2943.23	23027.13 - 10325.60
Relative Total Work	190.01	50.12	257.95 - 80.23
Fatigue Index	42.86	15.15	85.59 - 20.24
Fatigue Slope	33.57	8.03	54.54 - 23.08

Table 7 describes the Wingate measures exclusively from the third Wingate test (n=27). This shows the mean and standard deviations for each Wingate measure.

9.3 Mean Wingate Fatigue Graphs

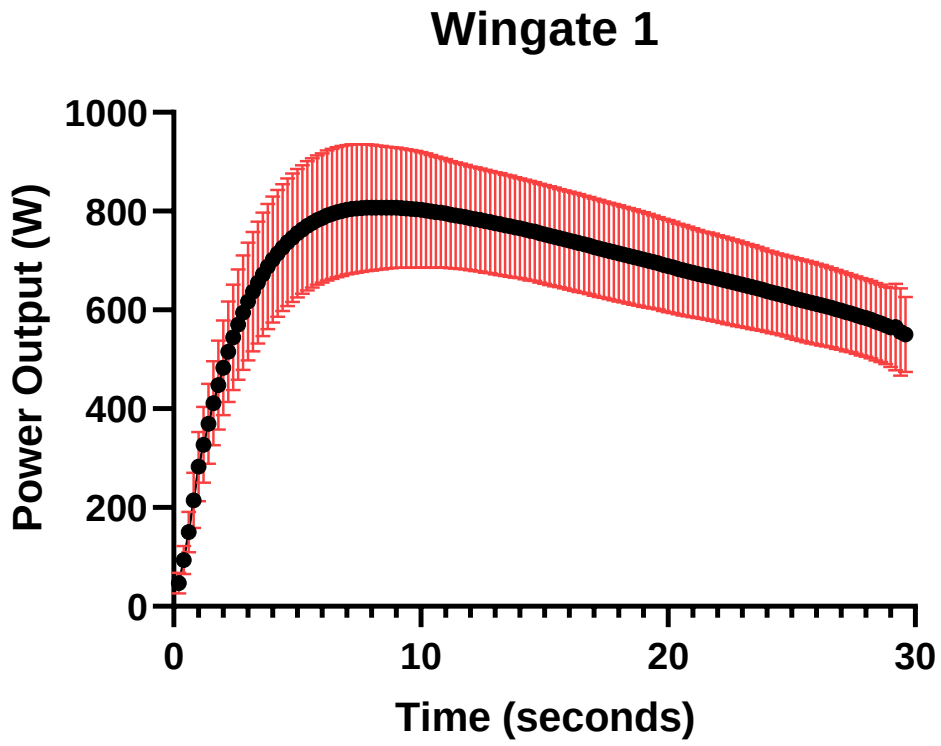


Figure 11 shows the mean power output profile of the first Wingate test. Mean power 725.12 ± 78.2, peak power 1163.81 ± 206.71 and fatigue index 30.51 ± 10.81

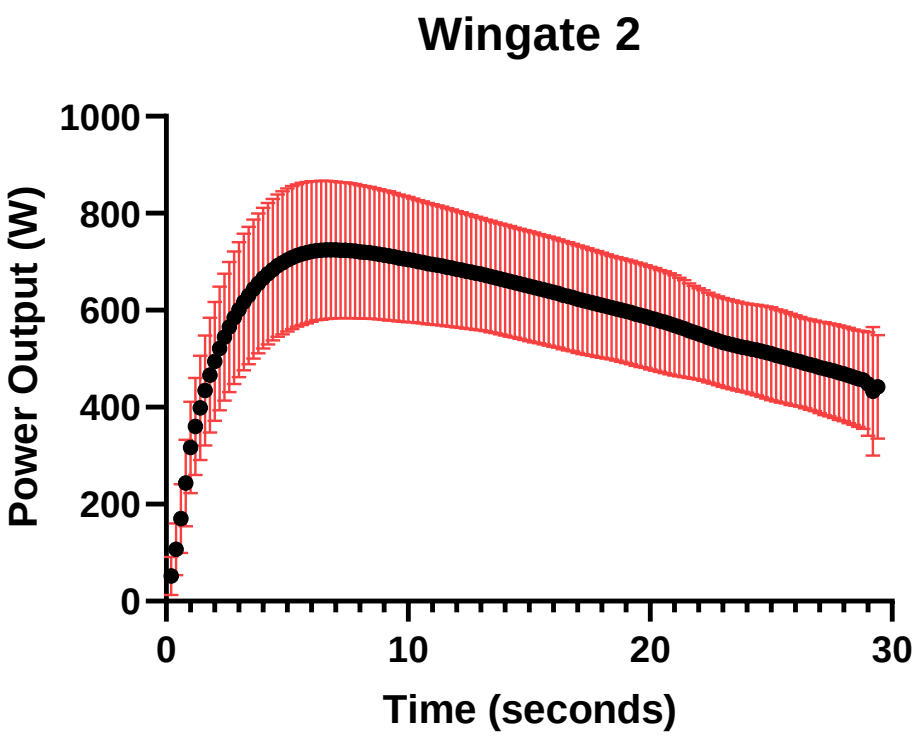


Figure 12 shows the mean power output profile of the second Wingate test. Mean power 627.27 ± 77.63, peak power 1192.55 ± 240.92 and fatigue index 38.72 ± 14.7.

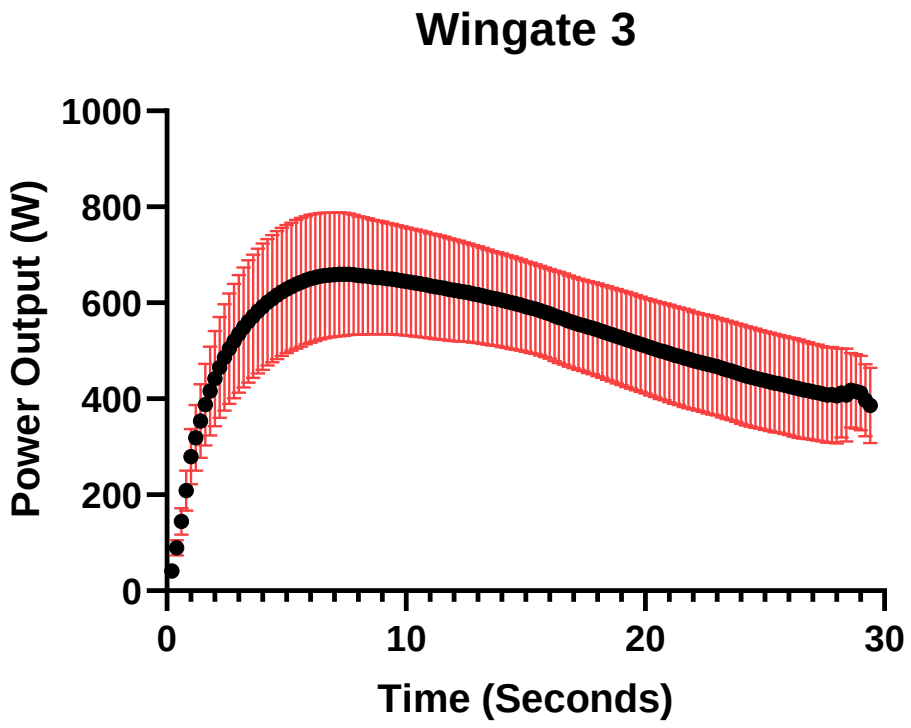


Figure 13 shows the mean power output profile of the third Wingate test. Mean power 544.09 ± 98.61, peak power 995.62 ± 218.25 and fatigue index 42.86 ± 15.15.

9.4 Fatigue Profile and Peak Power

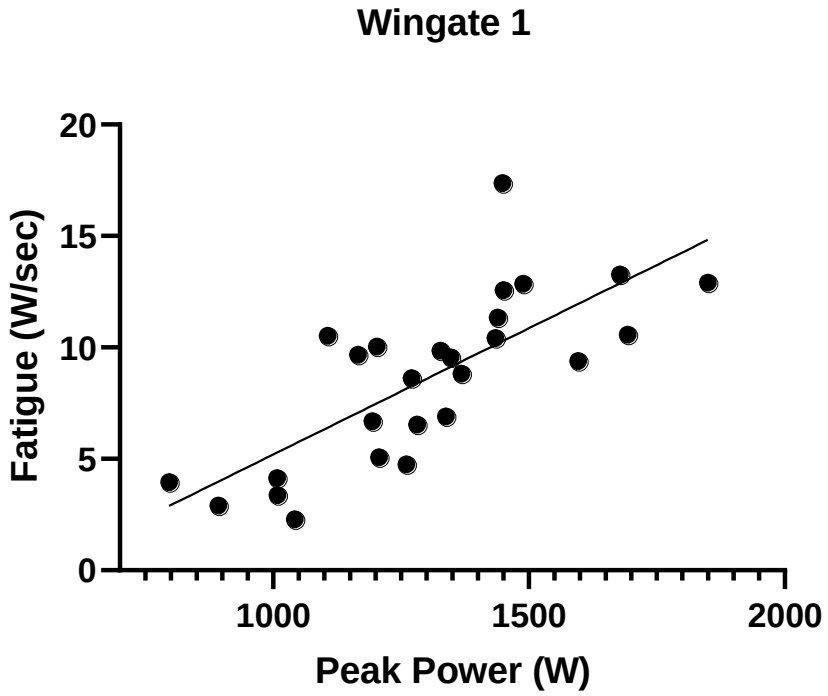


Figure 14 shows a significant and positive and significant correlation (r= 0.75; p=< 0.0001) between the fatigue profile from the first Wingate (30 seconds) and peak power.

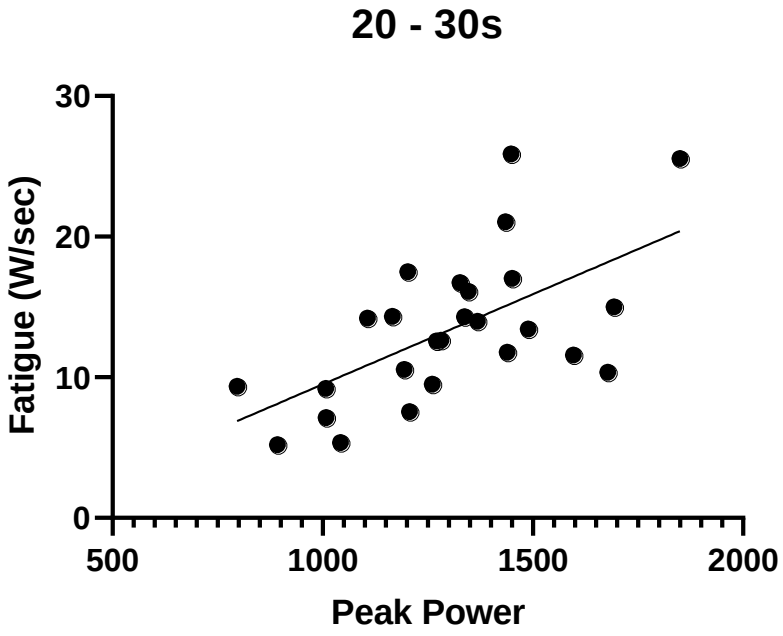


Figure 15 shows a significant and positive and significant correlation (r= 0.61; p= 0.001) between the fatigue profile from the last 10 seconds of the first Wingate and peak power.

10.0 Conference Abstracts

10.1 ASCA International Conference Abstract

The Relationship between Muscle Fibre Composition and Fatigues During Maximal Anaerobic Exercise?

Rick A. **Dann1**, Vincent G **Kelly2**, David G **Jenkins1**

University1 of Queensland Queensland2 University of Technology

Email: Rick.dann@uqconnect.edu.au

BLUF: Individuals with predominantly type II muscle fibres may show greater reductions in mean power and a greater degree of fatigue in the latter stages of maximal anaerobic exercise.

INTRODUCTION: Muscle Fibre Composition (MFC) is an important characteristic in determining how a muscle performs and fatigues. It's well documented that at a cellular level; type I muscle fibres produce less force and fatigue less whilst type II fibres produce greater force and fatigue more quickly. However, the significance of possessing different proportions of these fibres and its effect on maximal anaerobic performance and fatigue is still uncertain. The current literature is limited by the use of single Wingate protocols to induce fatigue. Therefore the aim of this study was to examine the relationships between muscle biopsy determined MFC and various performance and fatigue measures derived from three repeated Wingate bouts.

METHODS: Twenty-six healthy males (age: 25 ± 4 y; weight 74.0 ± 9.8kgs; height: 1.75 ± 0.11m) were recruited for this study. Each participant had a muscle biopsy from the vastus lateralis muscle and completed three Wingate (30 second sprints) on a LODE cycle ergometer, with 3 minutes of passive recovery between trials. Mean Power (MP), anaerobic capacity (AC), peak power (PP), total work (TW) and a fatigue index (FI) was calculated for

each Wingate. The analysis included standard descriptive statistics, Pearson correlation and linear regressions.

RESULTS: The percentage of type II fibres was significantly correlated with a decrease in MP throughout the last 15 seconds of the 2nd (r=-0.4201, p=0.0366) and 3rd (r=-0.4388; p=0.0282) Wingate bouts. These correlations were also found within each 5 second period (15-20, 20-25, 25-30sec) from both of the last two Wingate test. There was also a significant correlation (r=0.4075; p=0.0432) found between the percentage type II fibres and the fatigue profile from the 3rd Wingate bout. No significance was found between type II percentage and MP, AC, PP, TW or FI in the first Wingate.

DISCUSSION: The available evidence suggests the rate at which H+ ions are produced in muscles during high intensity exercise is related to the type of muscle fibres contributing towards the movement. It's also commonly accepted that individuals who have the capacity to regulate H+ ions (increased buffer capacity) and/or produce less of them during intense exercise may be able to delay fatigue and potentially improve sprint and high intensity exercise performance. Therefore the decrease in MP and increase in FI with type II dominant individuals may be explained by the increased production of H+ ions and the inability to regulate them.

PRACTICAL APPLICATIONS: The use of a single Wingate test may not provide an accurate understanding on how an individual performs and fatigues during maximal anaerobic exercise. Based off performance in the latter stages of this protocol, practitioners can estimate whether an individual may possess an increased percentage of type I or II fibres. From this information, subtle changes in rest periods, training volume and intensities could be made to individualise strength and conditioning programs.

10.1.1 ASCA International Conference Poster

The Relationship Between Muscle Fibre Composition and Fatigue During Maximal Anaerobic Exercise

The University Of Queensland AUSTRALIA

Rick A. Dann[1], Vincent G. Kelly[2], David G. Jenkins[1]

[1] The University of Queensland, St Lucia, QLD, Australia

[2] Queensland University of Technology, Kelvin Grove, QLD, Australia

INTRODUCTION

- Muscle Fibre Composition (MFC) is an important characteristic in the function of skeletal muscle
- It's well documented that at a cellular level, type I muscle fibres are somewhat resistant to fatigue whilst type II fibres fatigue very quickly and easily
- However, the significance of possessing different proportions of these fibres and its effect on fatigue induced by maximal anaerobic exercise is still uncertain.

STUDY AIM

- **The aim** of this study was to examine the relationships between muscle biopsy determined **MFC** and **fatigue measures** derived from a repeated Wingate protocol
- **It was hypothesised** that type II dominant individuals would show greater signs of fatigue in each Wingate

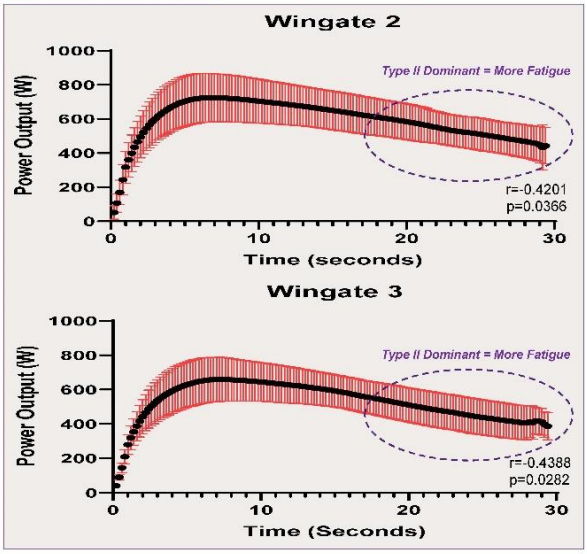
METHODS

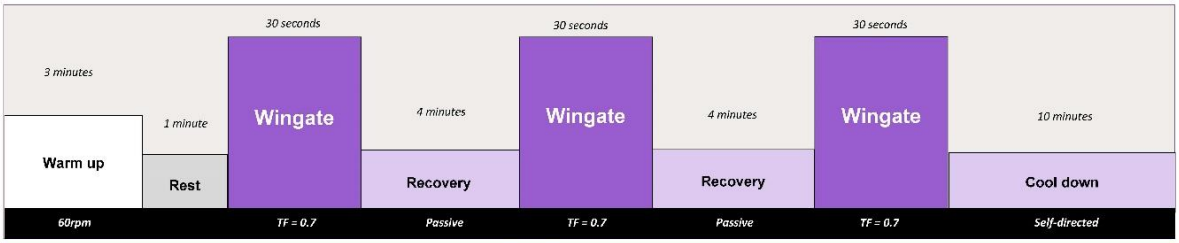
- **Twenty-six** healthy males (age: 25 ± 4 years) had a **muscle biopsy** from the Vastus Lateralis muscle to determine their **MFC**
- Each participant completed **three Wingate tests** (30 second sprints) on a LODE cycle ergometer, with four minutes of passive recovery between trials.
- Mean Power (**MP**), Anaerobic Capacity (**AC**), Peak Power (**PP**), Total Work (**TW**) and a Fatigue Index (**FI**) were calculated for each Wingate.

RESULTS

Table 1. Raw Muscle Biopsy Data

Analysis	Mean	SD +/-	Range
Type II (%)	60.57	11.94	29.11 – 80.44
Type II CSA (%)	62.82	11.28	36.35 – 82.95
$\frac{Average\ Type\ II\ CSA}{Average\ Type\ I\ CSA}$	1.25	0.14	0.89 – 1.56





CONCLUSIONS

- **Type II fibres** were positively correlated with a **decrease in MP** during last 15 s of the **2nd and 3rd Wingate** tests
- **Type II fibres** were positively correlated (r=0.4075; p=0.0432) with the **fatigue profile** from the **3rd Wingate**
- **Type II fibres** were not correlated with any **performance** or **fatigue** measure in the **1st Wingate**

PRACTICAL APPLICATIONS

- Individuals with **more type II fibres** may show greater signs of **fatigue** in the **latter stages** of **anaerobic exercise**.

→ This may be due to the **increase in H+ ion production** and/or the inability to regulate them

- One **single Wingate** test may not provide an accurate understanding on **anaerobic performance** and **fatigue**
- Performance in the **latter stages** of this protocol may provide practitioners with **estimation** of individuals' **MFC**
- Subtle changes in **rest periods**, **training volume** and **intensities** could be made to **individualise training programs**

Rick.dann@uqconnect.edu.au

@RickyDann7

10.2 UQ HMNS 2019 Conference Abstract

Does Muscle Fibre Composition effect Performance and Fatigue during Maximal Anaerobic Exercise?

Rick A. **Dann1**, Vincent G **Kelly2**, David G **Jenkins1**

University1 of Queensland Queensland2 University of Technology

Presenter: Ricky Dann

Email: Rick.dann@uqconnect.edu.au

Title: Does Muscle Fibre Composition effect Performance and Fatigue during Maximal Anaerobic Exercise?

INTRODUCTION: Muscle Fibre Composition (MFC) is commonly accepted as an important factor in determining how a muscle performs and fatigues. However, the significance of possessing different proportions of type I and II fibres and its effect on maximal anaerobic performance and fatigue is still uncertain. Therefore the aim of this study was to examine the relationships between MFC and various performance and fatigue measures derived from three repeated Wingate bouts. METHODS: Twenty-six healthy males (age: 25 ± 4 years) were recruited for this study. Each participant had a muscle biopsy from the vastus lateralis muscle and completed three Wingates on a LODE cycle ergometer, with 3 minutes of passive recovery between trials. Mean Power (MP), anaerobic capacity (AC), peak power (PP), total work (TW) and a fatigue index (FI) was calculated for each Wingate. RESULTS: The percentage of type II fibres was significantly correlated with a decrease in MP throughout the last 15 seconds of the 2nd ($r=-0.4201$, $p=0.0366$) and 3rd ($r=-0.4388$; $p=0.0282$) Wingate bouts. There was also a significant correlation ($r=0.4075$; $p=0.0432$) between the percentage of type II fibres and fatigue profile from the 3rd Wingate bout. CONCLUSION: This suggests that individuals with predominantly type II fibres may produce more H+ ions resulting in an accumulation of lactate in the latter stages of anaerobic exercise. From this information, general MFC estimations can be made, whilst also subtle changes to strength and conditioning programs for optimising performance and recovery.

10.3 UQ HMNS 2018 Conference Abstract

An Innovate Insight into Muscle Fibre Composition Prediction

Rick A. **Dann1**, Vincent G **Kelly2**, David G **Jenkins1**

University1 of Queensland Queensland2 University of Technology

Email: Rick.dann@uqconnect.edu.au

Through the knowledge of an athletes muscle fibre composition (MFC), high performance staff can implement more effective training programs, individualise training stimuli and possibly assist in reducing the likelihood of musculoskeletal injury. The current gold standard and most accepted method however (muscle biopsy), is invasive and not practical for elite athletes. The aim of this thesis is to i) address and critique alternate (non-invasive) methods which can predict muscle fibre composition ii) explore the validity of a new non-invasive method (MRI) which has shown promising results in previous research. A literature review titled 'Determining Muscle Fibre Type: An Updated Review' explores the current attempts in the literature to predict muscle fibre composition and include; MRI, Tensiomyography (TMG), strength testing and exercise protocols. Part two of this investigation will examine the validity of the MRI method through a comparison study with the traditional biopsy method. The updated review found that MRI is the most valid method whilst strength testing and repetition max based protocols proved less reliable but more cost effective. Based off previous research, it's expected that the second study in this thesis will show an agreement between the two methods (MRI and traditional biopsy) when predicting muscle fibre composition. The analysis for each MF prediction method will serve as an important tool when taking an innovative approach to developing athletes' exercise programs. The validation of the MRI method will promote the integration of an evidence based, non-invasive and practical approach to MF prediction within high performance sport.

11.0 Supporting Material

10.1 Participant Information Sheet

THE UNIVERSITY OF QUEENSLAND
AUSTRALIA

The University of Queensland
Brisbane Qld 4072 Australia
Telephone (07) 33656240
International +61 7 33656240
Facsimile (07) 33656877
Email enquiries@hms.uq.edu.au
Internet www.hmns.uq.edu.au
CRICOS PROVIDER NUMBER 00025B

PARTICIPANT INFORMATION SHEET

Establishing the Relationship between Repeated Wingate Testing Performance and Muscle Fibre Composition

Researchers: Dr Vince Kelly – Associate Lecturer (The School of Human Movement and Nutrition Sciences, The University of Queensland) and Performance Science Consultant (Brisbane Broncos) – Chief Investigator
Email: v.kelly2@uq.edu.au

Mr Rick Dann - MPhil Candidate (The School of Human Movement and Nutrition Sciences, The University of Queensland) – Co-Investigator
Phone: (07) 3365 6240
Email: rick.dann@uqconnect.edu.au

Associate Professor David Jenkins – (The School of Human Movement and Nutrition Sciences, The University of Queensland) – Co-investigator
Email: d.jenkins@uq.edu.au

Aims of the Study

The aim of this study is to identify any relationships between performance, fatigue and muscle fibre composition in healthy active males. This study also aims to establish the reliability of the Repeated Wingate Protocol as a predictor of muscle fibre composition. Although the biopsy procedure is currently accepted as the gold standard in the measurement of muscle fibre type composition, its invasive nature has limited research and knowledge surrounding athletic and elite sporting populations. Therefore having a valid, non-invasive alternative would open up many new areas for research which would result in a deeper understanding of muscle fibre characteristics of athletic and elite individuals.

The Experiment

For us to get develop a solid understanding of these three estimation techniques, we are aiming to recruit 30 healthy individuals to participate in the study. We are looking for volunteers who are i) recreationally active ii) elite athletes iii) ex-elite athletes who are between 18 – 80 years old who are not currently following a vegetarian diet or supplementing either beta-alanine.

As a participant, you will be required for a total of 2 sessions. The first session will be located at the University of Queensland and will simply consist of 3 x 30 second Wingate tests. You will perform a 5 min low intensity warm up on the cycle ergometer, before completing the first test. You will then be instructed to peddle as fast and as hard as you can for 30 seconds. Once this is complete, you will then receive 4 minutes of complete rest before repeating the test twice more. After the third test is complete, you will complete an active cool down. This session should not go for any longer than 15 minutes.

The second session is also conducted at UQ and will be the muscle biopsy procedure which will go for under an hour. This procedure is commonly employed within exercise science research and will be conducted by a doctor with the assistance from Ricky (the lead researcher). The procedure involves the removal of a small

piece of muscle tissue from your leg using a sterile needle. Local anaesthetic will be used before the needle is inserted into the muscle to reduce pain. Some pressure or discomfort may be experienced while the needle is collecting the tissue sample, but this will quickly pass and usually lasts no longer than 5 seconds. After the sample has been taken, the incision will be closed with sterile tape. After the conclusion of the procedure, there will be minimal discomfort and may resemble a bruise. A more in-depth description of this procedure can be found in the muscle biopsy information sheet. This includes how to prepare, what to do following and all potential risks associated with the procedure. This sheet needs to be read before commencement of the study.

Participation in this study is entirely voluntary. Even if you agree to participate, you can withdraw at any time, for any reason. If you choose to withdraw from the study your data and samples will be destroyed.

Safety, Risk and Discomfort

Local anaesthetic will be applied to your thigh before each biopsy to minimise any pain during the procedure. You may feel some slight discomfort or pain during the procedure, but this feeling will subside quickly. A compression bandage will be applied after the biopsy is collected to enhance recovery. The biopsy collection will not stop you from performing any activity after collection, whilst any discomfort felt after the procedure will subside within ~ 24 hrs. Please refer to the '**Biospy Information Sheet'** and '**Biopsy Care Form'** for more information regarding the procedure and all safety considerations.

The Wingate testing session will take approximately 30 minutes and will have limited risks associated with the protocol. Due to the 'maximal' nature of the Wingate test, you may feel light headed or nauseous during or post testing. This is completely normal and will be monitored throughout.

Confidentiality

All information collected and all results produced from this study will be kept completely confidential. This includes any information gathered throughout the study, data/ results from tests and your involvement in the study. All data once collected will be unidentified and kept safe and secure. The only time any details will be identifiable is at the request of a participant to receive their muscle fibre type composition. If the findings from the study are submitted for publication, individuals who participate will not be identifiable. If you chose to withdraw at any time during the study, all of your data will be destroyed. It should also be noted that the muscle samples collected for this study will be sent to collaborators overseas for analysis.

Financial Reimbursement

No financial reimbursement will be given for participation in this study.

Access to your Results

Once the research has been complete, you will be provided with an overview of your results from the study. This overview will outline your results from both test and will provide you with your muscle fibre composition from the muscle biopsy, whilst also your results from the Wingate tests.

Ethical Clearance

This study adheres to the Guidelines of the ethical review process of The University of Queensland and the National Statement on Ethical Conduct in Human Research. Whilst you are free to discuss your participation in this study with project staff (contactable on 07 33656240 Mr Rick Dann), if you would like to speak to an officer of the University not involved in the study, you may contact the Ethics Coordinator on 3365 3924

11.2 Informed Consent Form

THE UNIVERSITY OF QUEENSLAND
AUSTRALIA

The University of Queensland
Brisbane Qld 4072 Australia
Telephone (07)33656240
International +61 7 33656240
Facsimile (07) 3365 6877
Email enquiries@hms.uq.edu.au
Internet www.hms.uq.edu.au
CRICOS PROVIDER NUMBER 00025B

PARTICIPANT INFORMED CONSENT FORM

Establishing the Relationship between Repeated Wingate Testing Performance and Muscle Fibre Composition

Researchers: Dr Vincent Kelly – Associate Lecturer (The School of Human Movement and Nutrition Sciences, The University of Queensland) and Performance Science Consultant (Brisbane Broncos) – Principle Investigator
Email: v.kelly2@uq.edu.au

Mr Rick Dann - MPhil Candidate (The School of Human Movement and Nutrition Sciences, The University of Queensland) – Co-Investigator
Phone: (07) 3365 6240
Email: rick.dann@uqconnect.edu.au

Associate Professor David Jenkins – (The School of Human Movement and Nutrition Sciences, The University of Queensland) – Co-investigator
Email: d.jenkins@uq.edu.au

This study adheres to the Guidelines of the ethical review process of The University of Queensland and the National Statement on Ethical Conduct in Human Research. Whilst you are free to discuss your participation in this study with project staff (contactable on 0448104671, Mr Rick Dann), if you would like to speak to an officer of the University not involved in the study, you may contact the Ethics Coordinator on 3365 3924.

1. I, the undersigned..... acknowledge that I have read the Participant Information Sheet, and that the specific sections of the document that are relevant to the present experiment have been drawn to my attention. I have been provided with a description of the study, including the aims, methods, demands, and possible risks, benefits or discomforts involved.

2. I am aware that I may withdraw from this research project at any time without penalty or justification, and that withdrawal will not lead to punishment, differential treatment or disrespect from the investigator/s or the University of Queensland. I am also aware that I am entitled to an explanation of any procedure in the study. I understand any information I provide will be treated confidentially, and that I may not obtain any benefit from participation other than what has been outlined in the participant information sheet. I am aware that any samples taken in the study will be sent to collaborators overseas for analysis. If you chose to withdraw at any time during the study, all of your data will be destroyed.

3. I hereby consent to being a research participant in this study.

(Signed)..... Date:.....
(Witnessed by)..... Date:.....

11.3 Muscle Biopsy Information Sheet

THE UNIVERSITY OF QUEENSLAND
AUSTRALIA

School of Human Movement Studies

The University of Queensland
Brisbane Qld 4072 Australia
Telephone (07)33656240
International +61 7 33656240
Facsimile (07) 3365 6877
Email enquiries@hms.uq.edu.au
Internet www.hms.uq.edu.au
CRICOS PROVIDER NUMBER 00025B

Muscle Biopsy Information Sheet

Muscle biopsies are commonly employed within the exercise sciences. They are usually employed in studies examining structure, metabolic and cellular state of a muscle in response to acute and chronic bouts of exercise. The following information is designed to inform individuals interested in participating in a muscle biopsy study of the specific procedures and their associated risks and discomfort. Muscle biopsies are always carried out by fully qualified and experienced practitioners.

The procedure of a muscle biopsy and possible associated discomfort

The muscle biopsy involves the removal of a small piece of muscle tissue from one of the muscles in your leg using a sterile hollow needle. The area over the outside of your lower thigh muscle (vastus lateralis muscle) will be carefully cleaned. A small amount of local anaesthetic will be injected into and under the skin. Then a small, ~5 mm incision will be made in your skin in order to create an opening for the biopsy needle. There is often a small amount of bleeding from the incision, but this is usually minimal. The biopsy needle will then be inserted through the incision into the thigh muscle and a small piece of muscle will be quickly removed and the needle taken out. During the time that the sample is being taken (about 5 seconds), you may feel sensations of pressure and/or discomfort. However, any discomfort will very quickly pass, and you are quite capable of performing exercise and daily activities. There may be some minimal bleeding when the needle is removed which may require application of pressure for a few minutes. Following the biopsy, the incision will be closed with sterile tape (steri-strips), and wrapped with a tensor bandage. Once the anaesthesia wears off, the biopsy site may feel a little tight, somewhat resembling a bruise.

What to do following a muscle biopsy

After the procedure, you may feel some mild discomfort and/or see some bruising. This often feels like the sensation of a mild 'dead leg'. This is perfectly normal and should not cause you any undue concern. The tightness in the muscle usually disappears within a day.

Potential risks associated with muscle biopsies

The local anaesthesia may cause a tingling feeling at the time of the injection. This will last only 5 – 10 seconds. There is an extremely low risk of allergic reaction to the local injection (1 in 1 million). The chance of a local skin infection is also less than 1 in 1000. Carefully cleaning the skin and keeping the area clean until the skin heals will minimize this. There is a very low risk of internal bleeding at the biopsy site which can result in more prolonged pain and stiffness in the leg. On rare occasions, a small lump of scar tissue may form under the site of the incision, but this should disappear within a couple of months at the most, or within a few weeks if massaged. A small visible scar may remain from the biopsy incision. There is a very low risk (estimated at less than 1/5000) of damage to a small nerve branch to the muscle. This would result in partial weakness of the muscle and would likely have no impact on day-to-day activities. Nerve injuries like this usually resolve in 8 – 12 months, but there is a theoretical risk of mild leg weakness.

Problems or concerns

Infection can be serious and if you therefore experience a lot of bleeding from the biopsy site, swelling or infection around the biopsy site, faintness, light headedness, heart pain, chest pain or increasing pain in your leg which is not relieved by Paracetamol, you must contact the practitioner who collected the biopsy *right away*. However, if for some reason, you are not able to contact this physician, you should contact your family doctor or go to the Accident and Emergency Department.

Thanks for taking time to read this information sheet.
If you're still interested in participating in the biopsy components of the study, please complete the muscle biopsy screening questionnaire.

11.4 Muscle Biopsy Screening Form

THE UNIVERSITY OF QUEENSLAND
AUSTRALIA

School of Human Movement and Nutrition Sciences

The University of Queensland
Brisbane Qld 4072 Australia
Telephone (07)33656240
International +61 7 33656240
Facsimile (07) 3365 6877
Email enquiries@hms.uq.edu.au
Internet www.hms.uq.edu.au
CRICOS PROVIDER NUMBER 00025B

Muscle biopsy screening form

To help us ensure your safety and well-being prior to the biopsy, and suitability for a biopsy, please answer the following questions, and read the advice on the following page.

1. Have you ever had a negative or allergic reaction to local anaesthetic (e.g. during dental procedures)?
   No ☐ Yes ☐

2. Do you have any tendency toward easy bleeding or bruising (e.g with minor cuts or shaving)?
   No ☐ Yes ☐

3. Are you currently taking any medications that may increase the chance of bleeding or bruising (e.g. Aspirin, Anti-inflammatories (ibuprofen, nurofen), fish oil, warfarin or heparin), or do you suffer from hemophilia?
   No ☐ Yes ☐

4. Have you ever fainted or do you have a tendency to faint when undergoing or watching medical procedures?
   No ☐ Yes ☐

5. Will you contact the physician who did the biopsy and member of the research team if you have any concerns about the biopsy site including: excessive redness, swelling, infection, pain or stiffness of the leg?
   No ☐ Yes ☐

6. Are you willing to visit the physician who did the biopsy 7 – 14 days following the biopsy for an assessment of the biopsy site if deemed necessary?
   No ☐ Yes ☐

11.5 Post-Biopsy Care Form

THE UNIVERSITY OF QUEENSLAND
AUSTRALIA

School of Human Movement and Nutrition Sciences

The University of Queensland
Brisbane Qld 4072 Australia
Telephone (07)33656240
International +61 7 33656240
Facsimile (07) 3365 6877
Email enquiries@hms.uq.edu.au
Internet www.hms.uq.edu.au
CRICOS PROVIDER NUMBER 00025B

Post-biopsy care

Although a muscle biopsy is a minor procedure, and the amount of muscle collected is not going to affect your muscle's ability to function in any way, please adhere to the guidelines detailed below, and sign overleaf to acknowledge that you are willing to comply. You will be given a copy of this page to keep.

1. Avoid strenuous activity for 72 hours following the biopsy (unless this is a part of the research investigation, or otherwise advised by the study team). If you have an exercise session/activity planned within the 72 hours after the biopsy, talk to a member of the research team to check suitability.

2. The compression bandage applied to the leg is designed to minimise discomfort and swelling. Please leave the bandage on for ~12 hours after the biopsy. For example, if your biopsy was collected in the morning, remove the bandage prior to going to bed. This will in the majority of cases aid in the leg feeling close to 100% within 14 days after the biopsy, at the latest.

3. The clear dressing with absorptive pad applied to the biopsy site is waterproof under most circumstances e.g. showering. Therefore, showering after the biopsy for as long as the dressing is in place is OK. However, if the pad of the dressing does get wet, remove the dressing yourself and replace it with a new one, or contact a member of the research team to do this on your behalf. If the strips beneath the pad also come off, feel free to also replace these yourself, or contact a member of the research team to do this on your behalf.

4. Prolonged immersion e.g. during a bath may cause water to seep beneath the dressing. Therefore, if possible, bathing should be avoided for 7 days following the biopsy. Procedures can be put in place if you will require the biopsy site to be submerged for a prolonged period of time e.g. swimming; however, talk to a member of the research team to allow this procedure to be put in to action.

5. The steri strips applied to the biopsy site should be kept dry, and left in place until they come off naturally, or for 7 days to allow the site to heal as best as it can naturally.

6. Do not remove the steri strips yourself, unless e.g. they become wet as detailed in point 3. If you do replace them yourself, apply the strips in the same manner in which they were applied, keeping the incision tightly closed to minimise any scarring.

7. If any other symptoms appear e.g. localised swelling, excessive bruising of the site, bruising in areas other then where the biopsy was collected, or signs of infection e.g. excess warmth to the touch and/or weeping of the site. Contact a member of the research team immediately. Contact details for the research team are detailed in the participant information sheet.

Subject Name (print): _____

Subject Signature: _____

Date: _____

Signature of Person Conducting Assessment: _____

11.6 Recruitment Poster

THE UNIVERSITY OF QUEENSLAND
AUSTRALIA

The University of Queensland
Brisbane Qld 4072 Australia
Telephone (07) 33656240
International +61 7 33656240
Facsimile (07) 33656877
Email enquiries@hms.uq.edu.au
Internet www.hmns.uq.edu.au
CRICOS PROVIDER NUMBER 0002513

PARTICIPANTS WANTED

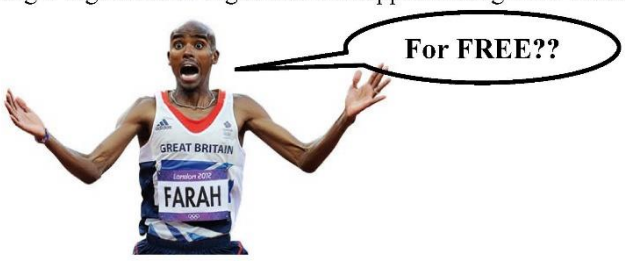
Find out your Muscle Fibre Composition for FREE!

The aim of this study is to establish the reliability of Wingate Testing as a predictor of Muscle Fibre Composition

This study will give coaches and researchers a deeper understanding of muscle fibre types in athletic and elite individuals.

Can I be a participant?

This study is recruiting healthy and active ***MALES*** between ***18 – 80 years old***. Recreational and elite athletes are especially encouraged due to the benefits you will receive from knowing your MFC. Participants must ***NOT*** be following a vegetarian or vegan diet OR supplementing Beta- Alanine (currently or in last 3 months).



What does it involve?

As a participant, you will be required for a ***total of 2 sessions***. **1)** At **UQ** – ***Wingate test (3 x 30 second sprints)*** on a stationary bike **2)** ***Muscle biopsy procedure*** (from quad muscle) BOTH sessions will be conducted at UQ (Human Movement Building 26B)

Your benefit!

You will receive your Muscle fibre composition and a fatigue profile for free! With this information we can discuss how you can make your training programs and exercise performance more effective and efficient! We can also discuss how to use your MFC and fatigue profile to personalise your current exercise routines!

More Information

Contact Ricky Dann (researcher)

rick.dann@uqconnect.edu.au

Participant Information sheets and consent forms can be sent for your perusal.

This study adheres to the Guidelines of the ethical review process of The University of Queensland and the National Statement on Ethical Conduct in Human Research.

11.7 Ethics Approval Letter

THE UNIVERSITY OF QUEENSLAND

Institutional Human Research Ethics Approval

Project Title:	Establishing the Relationship between Muscle Fibre Composition and fatigue profiles from Wingate and Squat testing
Chief Investigator:	Dr Vincent Kelly
Supervisor:	Dr Vincent Kelly, A/Prof David Jenkins
Co-Investigator(s):	A/Prof David Jenkins, Mr Rick Dann
School(s):	Human Movement and Nutrition Sciences
Approval Number:	2019001335
Granting Agency/Degree:	2018 Faculty of Health and Behavioural Sciences Research Collaboration Seeding Grant
Duration:	27th February 2020

Comments/Conditions:

- Response to committee, 27/08/2019
- Responses to feedback,16/07/2019
- HREA Application, 01/06/2019
- Project Description, 04/06/2019
- Participant Information Sheet, 17/07/2019
- Informed Consent Form, 04/06/2019
- Portion Guide Sheet, 17/07/2019
- APSS Screen Tool v1.1, 04/06/2019
- Food Diary, 04/06/2019

Note: If this approval is for amendments to an already approved protocol for which a UQ Clinical Trials Protection/Insurance Form was originally submitted, then the researchers must directly notify the UQ Insurance Office of any changes to that Form and Participant Information Sheets & Consent Forms as a result of the amendments, before action.

Name of responsible Committee:
University of Queensland Human Research Ethics Committee A
This project complies with the provisions contained in the *National Statement on Ethical Conduct in Human Research* and complies with the regulations governing experimentation on humans.

Name of Ethics Committee representative:
Dr Gordon McGurk
Chairperson
University of Queensland Human Research Ethics Committee A
Registration: EC00456

Signature _____ Date 27/08/2019