21-3-2018

The Fatigue Response Following a Team-Sport Match Simulation

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The Fatigue Response Following a Team-Sport Match Simulation

By

Paul Tofari

In Total Fulfilment of the Degree of

Doctor of Philosophy

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Statement of Authorship and Sources

This thesis contains no material published elsewhere or extracted in whole or in part from a thesis by which I have qualified for or been awarded another degree or diploma. No parts of this thesis have been submitted towards the award of any other degree or diploma in any other tertiary institution.

No other person’s work has been used without due acknowledgment in the main text of the thesis. All research procedures reported in the thesis received the approval of the relevant Ethics/Safety Committees.

Study 1 in this thesis, titled: A Self-Paced Intermittent Protocol on a Non-Motorised Treadmill: A Reliable Alternative to Assessing Team-Sport Running Performance, was contributed to equally by Paul Tofari and Blake McLean (45% each). A statement to this effect appears on the publication in the Journal of Sports Science and Medicine. This manuscript represents a chapter in both Paul Tofari’s and Blake McLean’s theses, as approved by the Dean of Research at Australian Catholic University.

Paul Tofari          Date: 21/03/2018
Acknowledgements

To my Yiayia Georgia: you made an everlasting impression on me. This is for you.

A big thank you to my principal supervisor, Assoc Prof Stuart Cormack. Stu, we got together a few years ago and had some ideas about a potential research project, and this thesis is the result. Any time I had a question or wanted to chat, you've made yourself available without hesitation. I appreciate your support and our open dialogue over the years. I'm looking forward to continuing our work in the future, and hopefully having a glass of wine or two together to celebrate this work. Thanks for everything, Stu, I've learned a lot.

I have been extremely lucky to have Prof Justin Kemp as a friend, mentor and associate supervisor for this PhD. Justin, I can safely say that I wouldn't be where I am today without your guidance and assistance. Your ability to manage your time between your growing family, work and rocking out while supervising me is amazing. This will likely be the only part of the thesis that you haven't proof read. I hope you're able to read the words and not just search for grammatical errors.

Special thanks to Ryan Timmins, David Opar, Jack Hickey, Jill Leckey, Brett O'Connell, Blake McLean and all my other friends and colleagues in the School of Exercise Science at Australian Catholic University in Melbourne. I think we're all very lucky to be a part of this department, it's a great place to work.

To all my friends and family, thanks for understanding why I haven’t been able to attend all the activities I’d like to. I’m sure I’ll have a lot more time now with a 6-month old at home… To Will Morgan, it’s great always having you around. You may drink all my beer and tell me my squats are ugly, but I wouldn’t change a thing.

I took up weightlifting near the start of my PhD. Little did I know I'd become addicted to the sport and make such strong friendships. To my coach, Robert Kabbas, and training partners Nicodemus Scheelings, Matthew Pit and Geordie Biss, thank you for providing me with a much-needed distraction throughout my candidature.

This work could not have been completed without the volunteers who participated in my studies. I appreciate every one of you giving up your time to be a part of this project.
To my Mum, Dad and Sisters, Antoinette and Lisa, I'm not sure you know exactly what I've been doing the past few years, but you're always supportive and I appreciate that. Thanks also to my sisters for marrying Ziggy and Enrico, I'm yet to hear either of them say no to a beer, which definitely comes in handy. It's fun watching our family grow with Katinaki and Baby DG on the way. Thanks to you all for checking in on me every now and then, and sorry for missing a few family functions along the way.

Lastly to Emma, what a big few years it has been. We've achieved so much together during this PhD, including starting our beautiful little family. You're already an amazing mother to our daughter Georgia and I'm looking forward to what the future has in store for us. I wouldn't have started this PhD without your encouragement and sacrifice. I couldn't have finished it without your love and support.
# Table of Contents

Statement of Authorship and Sources .................................................................ii
Acknowledgements ...............................................................................................iii
Table of Contents .....................................................................................................v
List of Publications Related to This Thesis .............................................................ix
Manuscript Currently In Review .............................................................................ix
List of Figures ..........................................................................................................x
List of Tables ..........................................................................................................xii
Abstract ..................................................................................................................xiii

1. Chapter 1: Introduction and Overview ...............................................................1

2. Chapter 2: Literature Review .............................................................................4
   2.1. Team Sport Competition .............................................................................4
   2.2. Time-Motion Analysis ..............................................................................4
   2.3. Match Simulations ....................................................................................5
   2.4. Fatigue and Exercise ................................................................................6
   2.5. Models of Exercise Induced Fatigue and Intensity Regulation ....................7
      2.5.1. Cardiovascular/Anaerobic Model .........................................................7
      2.5.2. Energy Supply Model .........................................................................7
      2.5.3. Biomechanical Model ........................................................................8
      2.5.4. Central and Peripheral Models ............................................................8
   2.6. Fatigue and Pacing During Team-Sport Exercise .........................................11
   2.7. Biological Responses to Team-Sport Exercise ..........................................13
      2.7.1. Neuromuscular fatigue ......................................................................13
      2.7.2. Biochemical responses .....................................................................20
   2.8. Perceptual Responses to Team-Sport Exercise ..........................................26
   2.9. Conclusions ...............................................................................................27

3. Chapter 3: Methodology and Design ................................................................28
   3.1. Study One - A Self-Paced Intermittent Protocol on a Non-Motorised Treadmill: A Reliable Alternative to Assessing Team-Sport Running Performance .........................28
      3.1.1. Non-Motorised Treadmill Model .........................................................28
      3.1.2. Protocol Development ........................................................................29
      3.1.3. Testing Sessions ..................................................................................29
      3.1.4. Data Analysis .....................................................................................30
   3.2. Study Two - Reliability of Measures of Quadriceps Muscle Function Using Magnetic Stimulation .........................................................................................31
      3.2.1. Study Design ........................................................................................31
      3.2.2. Participants ........................................................................................31
3.2.3. Surface EMG...................................................................................................... 31
3.2.4. Peripheral Magnetic Stimulation ....................................................................... 32
3.2.5. Rate of Torque Development ............................................................................. 33
3.2.6. Data Analysis ..................................................................................................... 33

3.3. Study Three - A Self-Paced Team Sport Match Simulation Results In Reductions in Voluntary Activation and Modifications to Biological, Perceptual and Performance Measures at Half-Time, and for up to 96 Hours Post-Match............................................... 34
   3.3.1. Experimental Approach to the Problem............................................................ 34
   3.3.2. Familiarization 1 and 2 .................................................................................. 34
   3.3.3. Main Testing Session ...................................................................................... 35
   3.3.4. Subjects ........................................................................................................... 35
   3.3.5. Procedures ....................................................................................................... 36

3.4. Study 4 - Biological and Perceptual Responses to Simulated Fixture Congestion in Soccer................................................................................................................................... 40
   3.4.1. Experimental Approach to the Problem............................................................ 40
   3.4.2. Familiarization 1 and 2 .................................................................................. 40
   3.4.3. Simulated Match Sessions ............................................................................. 40
   3.4.4. Subjects ........................................................................................................... 41
   3.4.5. Procedures ....................................................................................................... 41

4. Chapter 4: Study One - A Self-Paced Intermittent Protocol on a Non-Motorised Treadmill: A Reliable Alternative to Assessing Team-Sport Running Performance ............. 46
   4.1. Linking Paragraph ............................................................................................ 47
   4.2. Abstract ............................................................................................................. 48
   4.3. Introduction ....................................................................................................... 49
   4.4. Methods ............................................................................................................ 50
       4.4.1. Non-Motorised Treadmill Model ................................................................. 50
       4.4.2. Protocol Development ............................................................................... 51
       4.4.3. Testing Sessions ......................................................................................... 54
       4.4.4. Data Analysis ............................................................................................ 54
   4.5. Results ............................................................................................................... 55
       4.5.1. Speed and Distance Reliability .................................................................. 55
       4.5.2. Power Reliability ...................................................................................... 55
   4.6. Discussion ......................................................................................................... 58
   4.7. Conclusion ....................................................................................................... 60

5. Chapter 5: Study Two - Reliability of Measures of Quadriceps Muscle Function Using Magnetic Stimulation ................................................................. 62
   5.1. Linking Paragraph ............................................................................................ 63
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.2.</td>
<td>Abstract</td>
<td>108</td>
</tr>
<tr>
<td>7.3.</td>
<td>Introduction</td>
<td>109</td>
</tr>
<tr>
<td>7.4.</td>
<td>Method</td>
<td>111</td>
</tr>
<tr>
<td>7.4.1.</td>
<td>Experimental Approach to the Problem</td>
<td>111</td>
</tr>
<tr>
<td>7.4.2.</td>
<td>Familiarization 1 and 2</td>
<td>111</td>
</tr>
<tr>
<td>7.4.3.</td>
<td>Simulated Match Sessions</td>
<td>111</td>
</tr>
<tr>
<td>7.4.4.</td>
<td>Subjects</td>
<td>112</td>
</tr>
<tr>
<td>7.4.5.</td>
<td>Procedures</td>
<td>112</td>
</tr>
<tr>
<td>7.5.</td>
<td>Results</td>
<td>117</td>
</tr>
<tr>
<td>7.5.1.</td>
<td>Match running variables</td>
<td>117</td>
</tr>
<tr>
<td>7.6.</td>
<td>Discussion</td>
<td>130</td>
</tr>
<tr>
<td>7.6.1.</td>
<td>Match Running Variables</td>
<td>130</td>
</tr>
<tr>
<td>7.6.2.</td>
<td>Neuromuscular Function</td>
<td>131</td>
</tr>
<tr>
<td>7.6.3.</td>
<td>Performance Tests</td>
<td>133</td>
</tr>
<tr>
<td>7.6.4.</td>
<td>Biochemical Responses</td>
<td>134</td>
</tr>
<tr>
<td>7.6.5.</td>
<td>Perceptual Responses</td>
<td>135</td>
</tr>
<tr>
<td>7.6.6.</td>
<td>Influence of Physical Qualities</td>
<td>135</td>
</tr>
<tr>
<td>7.6.7.</td>
<td>Implications</td>
<td>136</td>
</tr>
<tr>
<td>8.</td>
<td>Chapter 8: Discussion and Conclusions</td>
<td>138</td>
</tr>
<tr>
<td>8.1.</td>
<td>Limitations</td>
<td>141</td>
</tr>
<tr>
<td>8.2.</td>
<td>Practical applications</td>
<td>141</td>
</tr>
<tr>
<td>8.3.</td>
<td>Future Research Directions</td>
<td>142</td>
</tr>
<tr>
<td>9.</td>
<td>Chapter 9: References</td>
<td>144</td>
</tr>
<tr>
<td>9.1.</td>
<td>Appendix I – Research Portfolio</td>
<td>163</td>
</tr>
<tr>
<td>9.2.</td>
<td>Appendix II – Published paper which forms the basis of Chapter 4</td>
<td>168</td>
</tr>
<tr>
<td>9.3.</td>
<td>Appendix III – Published paper which forms the basis of Chapter 5</td>
<td>169</td>
</tr>
<tr>
<td>9.4.</td>
<td>Appendix IV – Published paper which forms the basis of Chapter 6</td>
<td>170</td>
</tr>
<tr>
<td>9.5.</td>
<td>Appendix V – Ethics approvals, letters to participants and consent forms</td>
<td>171</td>
</tr>
<tr>
<td>9.6.</td>
<td>Study 1: Letter to participants and consent forms</td>
<td>172</td>
</tr>
<tr>
<td>9.7.</td>
<td>Study 2: Letter to participants and consent forms</td>
<td>176</td>
</tr>
<tr>
<td>9.8.</td>
<td>Study 3 and 4: Letter to participants and consent forms</td>
<td>181</td>
</tr>
</tbody>
</table>
List of Publications Related to This Thesis

   *Authors made an equal contribution to the manuscript*


3. **Tofari P,** Kemp J, Cormack S. A Self-Paced Team Sport Match Simulation Results in Reductions in Voluntary Activation and Modifications to Biological, Perceptual and Performance Measures at Half-Time, and for up to 96 Hours Post-Match. *Journal of Strength and Conditioning Research.* 2017: **Publish Ahead of Print.**

Manuscript Currently In Review

List of Figures

Figure 4.1: A ten-minute portion of the self-paced match-simulation protocol. This 10-min period was repeated three times to make up the complete 30-min protocol. Participants self-selected their chosen running speeds. The area high-lighted in grey depicts a period of ‘low’ activity, simulating a rest period (interchange) common in Australian Football. Participants were permitted to consume water during this period. .............................................................. 52

Figure 5.1: Vastus lateralis M-Wave amplitude (A, top) and quadriceps twitch torque (B, bottom) in response to magnetic stimulation. As part of a ramp protocol, participants were administered 2 non-potentiated stimulations to the femoral nerve every 30 s at increasing intensities (50, 60, 70, 80, 90, 95, 100% of stimulator output) to assess a plateau in M-Wave and torque outputs (i.e., whether a maximal stimulation has occurred). Data presented are means, error bars represent ± SD. ............................................................................................ 69

Figure 5.2 (A) Representative output from the twitch interpolation method. The participants perform a maximal voluntary contraction, from which peak torque is obtained (a). Once a plateau in torque is observed, a superimposed twitch (depicted by the green line) is administered using a magnetic stimulator (b: maximal voluntary contraction + superimposed twitch; c: superimposed twitch). Approximately 4 seconds post-maximal voluntary contraction, a potentiated twitch is administered (d). These variables are input into a number of equations to calculate levels of voluntary activation. (B) Representative output from a resting twitch during the ramp stimulation protocol. The participants are given a series of incremental twitches at rest from 50 – 100% of the magnetic stimulator output to assess whether the twitch is maximal (100% stimulator output shown here). The magnetic stimulation creates an artifact in the EMG signal (e). However, this does not affect the interpretation of the M-wave variables (f: peak-to-peak duration; g: peak-to-peak amplitude) .................................................................................................................................................. 70

Figure 6.1: A) Maximal voluntary contraction, B) Voluntary activation, C) RMSPPA\Mwave and D) Potentiated twitch Pre, half time (HT) and up to 4-days post a self-paced, match simulation protocol. Data are mean ± SD. ................................................................................................................................. 95

Figure 6.2: Performance response Pre, half time (HT), and up to 4-days post in A) Countermovement jump (CMJ) height, B) Squat jump (SJ) height, and C) Isometric mid-thigh pull (IMTP) relative force. Data are mean ± SD................................................................................................................................. 97
Figure 6.3: Biochemical response Pre, and up to 4-days post for A) Creatine Kinase, B) Uric Acid, C) Testosterone, D) Cortisol and E) T:C Ratio. Data are mean ± SD. ................................. 98

Figure 6.4: Correlation (r) between changes in creatine kinase (CK) and maximal voluntary contraction (MVC), squat jump height (SJ height), isometric mid-thigh pull relative force (IMTP N.kg⁻¹) and soreness between Pre and 4-days post......................................................... 100

Figure 7.1: Effect size changes ± 90%CL for within match variables across halves within matches, across halves between matches and overall between matches. ACC = acceleration, LSA = low-speed activity, HSR = high-speed running, VHSR = very high-speed running, TD = total distance, HR = heart rate, RPE = rate of perceived exertion. .............................................. 121

Figure 7.2: Changes in variables associated with the quadriceps femoris assessed via the interpolated twitch technique after two matches played within 72 hours. MVC = maximal voluntary contraction, %VA = voluntary activation. ................................................................. 122

Figure 7.3: Response in performance tests assessed using a force plate following two matches played in 72 hours. IMTP = isometric mid-thigh pull, SJ = squat jump, CMJ = countermovement jump. ........................................................................................................ 123

Figure 7.4: Biochemical response to two match simulations separated by 72 hours. .......... 125

Figure 7.5: Correlations (r) between pre-match physical qualities and changes in perceptual, neuromuscular and biochemical markers. IMTP = isometric mid-thigh pull, GMS = general muscle soreness, Pot = potentiated twitch torque................................................................. 129
List of Tables

Table 4.1: The speed commands (W = walk, St = stand, R = run, J = jog, Sp = sprint) and time spent (s) in each speed band for a ten-minute portion of the self-paced team-sport running simulation. This 10-min period was repeated three times, comprising the complete 30-min protocol. ................................................................................................................................... 53

Table 4.2: Reliability of distance (and speeds) across team-sport simulation trials and activity blocks within trials. .................................................................................................................. 56

Table 4.3: Reliability of mean power output across team-sport simulation trials and activity blocks within trials. .................................................................................................................. 56

Table 5.1: The inter-day reliability for measures collected when magnetic stimulation was applied to the femoral nerve during a maximal voluntary contraction of the knee extensors and a control, potentiated twitch to the resting muscle. ........................................................... 73

Table 5.2: The intra-day reliability for measures collected when magnetic stimulation was applied to the femoral nerve during a maximal voluntary contraction of the knee extensors and during a control, potentiated twitch to the resting muscle. .................................................... 74

Table 5.3: The inter-day reliability for the ramp protocol performed prior to testing on each day, including torque and EMG derived variables. The ramp protocol incorporated magnetic stimulation of the femoral nerve at increasing stimulator intensities. ............................................. 76

Table 6.1: First and second half activity profile, heart rate, body mass (mean ± SD) and change from first to second half (ES ± 90% CI). .................................................................................................................... 94

Table 6.2: Effect Size (± 90% CL) changes at half time to Post96 compared to Pre. ............... 96

Table 7.1: Match variables recorded during the two match simulations separated by 72 h. . 118

Table 7.2: Effect size ± CL at all time points compared to match 1 pre-test values. .......... 119

Table 7.3: Relationship between pre-match wellness scores and activity profile variables .. 127

Table 7.4: Relationship between physical qualities and activity profile variables .......... 127

Table 7.5: Relationship between physical qualities and between-half changes in running variables ........................................................................................................................................ 128
Abstract

The activity profiles of team sports such as soccer incorporate high-intensity, intermittent running patterns combined with match specific actions. Performing these activities during competition results in acute and longer-term disruptions to homeostasis, which may be exacerbated during periods of fixture congestion. Better understanding of these responses may provide greater insight into the mechanisms and potential mitigating factors of team-sport fatigue. In turn, this could benefit athletes and practitioners by optimising player management and informing the training process. Therefore, the overall aim of this work was to describe the biological and perceptual responses to a single match, and to multiple team-sport matches within a given week. Assessing the within- and post-match responses to team-sport exercise is ideally completed following competitive matches. However, the proximity to laboratories and match contextual factors can limit experimental control. Match simulations aim to overcome these issues, but their ecological validity is questionable in current protocols. This issue stems from externally pacing participant effort, and from using motorised treadmills or tethered non-motorised treadmills (NMT) which can result in unrepresentative accelerations and decelerations, and limit maximal speed.

The aim of Study 1 was to create a reliable, self-paced running protocol that simulated the activity profile of team-sport while overcoming the limitations of current simulation protocols. A curved NMT is now available that allows untethered running, permitting more representative accelerations and decelerations when compared with previous tethered NMT models. Ten male team-sport athletes completed a 30-min match simulation protocol once a week for five weeks, including a familiarisation session. The 30-min protocol consisted of three identical ten-minute blocks, with participants self-selecting their locomotor speeds based on visual and audible commands, specifically: “stand still”, “walk”, “jog”, “run” and “sprint”. The inter-trial reliability of the protocol, as assessed via the coefficient of variation (CV), was < 6% for all locomotor speeds, which is comparable to other externally paced simulations. The self-paced design of this protocol provides a reliable approach to simulating team-sport activity profiles in a laboratory. Given the protocol is self-paced with respect to running speeds and accelerations and decelerations, it likely provides a more ecologically-valid match simulation than externally-paced alternatives. Therefore, this protocol provides the opportunity to directly measure fatigue during and following team-sport running using time-sensitive, laboratory-based techniques.
A principal method to directly determine central and peripheral fatigue following exercise is the interpolated twitch technique, which has been reported sparingly with respect to team-sport activity. Magnetic or electrical stimulation can be used for this assessment by calculating voluntary activation (i.e., central fatigue) using a superimposed stimulus during maximal voluntary contractions (MVC) and control twitches to potentiated muscle (i.e., peripheral fatigue). Given magnetic stimulation provides a less painful alternative to electrical stimulation, it may be more suitable for assessing central and peripheral fatigue with certain populations, or during periods where repeat trials are necessary (like post-exercise fatigue monitoring). For the latter, the test-retest reliability of the technique needs to be determined. Therefore, Study 2 assessed the reliability of magnetic stimulation when using the interpolated twitch technique to determine central and peripheral fatigue of the quadriceps femoris muscle group. Fifteen men completed two familiarisations and three reliability trials to assess muscle function. Within- and between-day reliability of torque and electromyographic (EMG) variables were estimated using typical error ± 90% confidence limits expressed as a percentage (CV) and the intraclass correlation coefficient. Within- and between-day torque variables for MVC were reliable (CV<4%, ICC 0.98, and <5%, 0.99, respectively). EMG variables were less reliable than torque variables, with CVs ranging from 7–18%. These data established magnetic stimulation of the femoral nerve as a suitable method for assessing quadriceps femoris muscle function within and between days.

With the reliability established for critical methods of the study design, Study 3 aimed to assess the biological, perceptual, and performance responses to a self-paced, simulated soccer match protocol using a curved NMT. Twelve male team-sport athletes performed the 90-min match simulation. Match activity, quadriceps twitch interpolation [voluntary activation (%VA) and potentiated twitch (POT)], biochemical markers, strength and power performance, rating of perceived exertion (RPE) and self-report wellness were collected pre-, half-time, post-, and 2, 24, 48, 72 and 96 h post-match. Change compared to pre-match was calculated using the effect size (ES) ±90% confidence limits, and relationships were assessed using regression analysis. Reductions in %VA and POT were present at half-time (-0.38 ± 0.46 and -0.79 ± 0.30, respectively), potentially contributing to reduced second-half running volume and intensity observed in the study. These reductions in %VA and POT persisted post-match, but the magnitude did not increase. Squat jump height decreased at half-time (-0.42 ± 0.31) and remained decreased until 96-h post-match. Perceived fatigue and soreness (-0.92 ± 0.88 and -1.49 ± 0.76, respectively) peaked at Post24, identified as reduced rating scores, and circulating creatine kinase (CK: 1.11 ± 0.43) peaked at Post24. Pre-test strength (N.kg⁻¹) was inversely
related to changes in CK ($r = -0.58$ to $-0.81$), while peak oxygen consumption ($VO_{2peak}$) correlated with higher ratings of perceived wellness at Post24 ($r = 0.44$ to 0.58) and lower RPE post-match ($r = -0.71 \pm 0.28$). The activity profiles and heart rate responses, as well as the magnitude and duration of the post-match responses, to the match simulation were similar to a competitive soccer match, providing support for the ecological validity of our NMT protocol. Therefore, the outcomes of this work likely have implications for competitive on-field performance. The associations observed between physical capacities (i.e., lower-body strength and aerobic capacity) and a reduction in the magnitude of post-match perturbations suggests a training focus should be placed on developing lower-body strength and lower-body strength for team-sport athletes.

To simulate fixture congestion that is common in professional soccer, Study 4 assessed the within- and post-match responses to two match simulations performed in a 72-hour period. In agreement with Study 3, reduced %VA (ES ± 90% CL: $-1.52 \pm 1.41$ and $-0.50 \pm 0.58$) and POT ($-0.50 \pm 0.37$ and $-0.31 \pm 0.37$) were observed at half time in the first and second matches, respectively, which may have influenced the reduced second-half running volume and intensity evident in both matches. However, differences in the activity profiles between matches were unclear. Both match simulations resulted in acute neuromuscular, biochemical, perceptual and performance decrements, with the magnitude and duration of these responses similar to competitive soccer matches. Also, consistent with Study 3, greater lower-body strength was associated with less perceived general muscle soreness and fatigue (range: $r = 0.27$ to 0.69), as well as less peripheral fatigue (i.e., change in POT). Additionally, greater aerobic fitness resulted in less of an increase in CK concentrations ($r$ range = $-0.28$ to $-0.70$). This further supports the notion that lower-body strength and aerobic capacity are important for both improving match running performance and for reducing disruptions to homeostasis caused by match play.

In summary, this thesis outlines the biological and perceptual responses within and following a reliable, self-paced match simulation. The findings support the ecological validity of the match simulation, given the activity profiles performed during the protocol and the associated post-match responses are similar to competitive soccer. An important observation in both Studies 3 and 4 was the previously unreported finding that central and peripheral fatigue exist as early as half-time. This half-time fatigue may be responsible for reduced running volume and intensity in the second half of matches. Therefore, strategies to mitigate this half-time fatigue might assist in maintaining second-half activity. The physical qualities of lower-body strength and aerobic
capacity were associated with greater running volume and intensity, and a smaller magnitude of post-match perturbations. Due to the performance benefit and protective effect, development of these physical qualities should be prioritised in training for team-sport athletes.
1. Chapter 1: Introduction and Overview

Team sports such as soccer, Australian football and hockey incorporate intermittent, high-intensity running bouts interspersed with directional changes, rapid accelerations and decelerations [1-4]. Performing these activities during competitive matches is concomitant with acute and longer-term exercise-induced fatigue [5, 6]. This fatigue response might be present from as early as half-time as, seen by modified second-half activity profiles, and remain for multiple days post-exercise with reports of incomplete recovery even at 72 h post-match [3, 4, 7-11]. As players are often required to participate in multiple matches per week and continue training, their performance can be compromised and their risk of injury increased [12, 13]. Therefore, understanding and assessing how players respond to these activities is imperative for optimising player management [14, 15].

Monitoring team-sport athletes can be performed by assessing their external load during training sessions and matches, and measuring their responses to these activities [8, 9, 16-19]. The magnitude and duration of the response to exercise can be measured using a variety of objective and subjective variables, monitored during and after the activity [9, 18]. A combination of biological and perceptual alterations is commonly investigated to ascertain the extent of the post-match response [10, 16, 20-22]. These measures can include: twitch interpolation for the assessment of neuromuscular fatigue via changes in voluntary activation (%VA) [8, 21, 23, 24]; biochemical analysis measuring hormonal changes, inflammatory and oxidative stress markers [25-28]; performance measures such as countermovement jump (CMJ) height and sprinting speed [6, 16, 29]; and perceptual responses to exercise (e.g., ratings of perceived exertion [RPE]) [18, 19, 30-32]. However, these markers are often collected in isolation and, therefore, the interactions between different variables have not been comprehensively assessed, presenting a topic of interest for further research.

The post-match responses to team-sport exercise may be exacerbated by the requirement to play multiple matches in a short period of time. Research assessing post-match responses shows incomplete recovery in many variables, even at 72-hour post-match [10, 11, 33]. Furthermore, athletes participating in multiple matches within a week are more susceptible to injury [13, 34]. However, few post-match responses have been assessed after multiple matches in a week [12, 35, 36], with changes in neuromuscular, biochemical, physical performance and perceptual
responses in combination following multiple team-sport matches in a short period of time having not been explored.

Competitive team-sport matches represent the most ecologically-valid modality for assessing the response within and following team-sport performance. However, experimental control is compromised due to variability in the match situations (e.g., scoring) and tactics, which in turn can influence the activity profile of athletes [9]. The competitive environment also introduces logistical time delays when assessing the post-match response, which may result in an underestimation of the observed response (e.g., neuromuscular function) [37]. To overcome this limitation, match simulation protocols incorporating overground shuttle-running or motorised/non-motorised treadmills have been used [9]. However, these protocols have been externally paced, dictating running speeds to the athletes [34]. In contrast, competitive team-sport matches require athletes to self-select their locomotor speeds based on the match situation, in conjunction with their psychological motivation and physical capacity to do so [38]. Therefore, to more appropriately assess biological and perceptual responses to team-sport matches, the creation of a reliable, self-paced running protocol based on team-sport activity profiles is required. Using a non-motorised treadmill allows participants to choose their locomotor speed in response to audible or visual commands, ultimately self-selecting their running speed in a similar manner to if they were responding to contextual cues within a match, to better simulate the activity profile of a competitive match. Additionally, conducting such a protocol in the laboratory environment could allow the assessment of a variety of parameters at half-time (such as interpolated twitch) which has not been previously described.

An athlete’s physical capacity can influence the activity profile performed and the biological and perceptual responses to this activity [39-41]. Athletes with greater lower-body strength and power are able to sprint faster and jump higher, and may have a reduced injury risk [41-44]. Furthermore, athletes with a higher aerobic capacity can recover more rapidly from periods of high-intensity within matches [42]. Importantly, greater strength and aerobic capacity might be key mitigating factors of exercise-induced fatigue, but little is known about the protective effect of these physical qualities on post-match responses [45, 46].

This work aimed to comprehensively describe the biological and perceptual responses to a single and to multiple team-sport matches in a week. The specific aims of this research were:
- Develop and determine the reliability of a self-paced match-simulation based on the activity profile and internal responses to team sport to allow for fatigue assessment in a laboratory environment (See: Study 1 – Chapter 4)

- Determine the reliability of magnetic stimulation of the femoral nerve to assess voluntary activation of the quadriceps muscle group within and across multiple days (See: Study 2 – Chapter 5)

- Assess the neuromuscular, biochemical, performance and perceptual responses to a self-paced match simulation by collecting this data set immediately at half- and full-time, 2 hours post-match and at daily intervals for 4 days (See: Study 3 – Chapter 6)

- Assess the neuromuscular, biochemical, performance and perceptual responses to two match simulations separated by two days, with data collected immediately at half-time, full-time and 2 hours after both matches, and at multiple days post-match. (See: Study 4 – Chapter 7).

Overall, this thesis comprises:

- Chapter 2 which contains a review of the current literature in the general area of fatigue and, more specifically, fatigue in relation to team-sport matches;

- Chapters 4-7 which contain published/under review experimental studies addressing the aims.

- Chapter 8 which encompasses an overall discussion, conclusion and limitations of this body of work.
2. **Chapter 2: Literature Review**

2.1. **Team Sport Competition**

Team-sport athletes endure high physical and psychological demands during training and competition [1, 4]. Generally, team-sports combine skill-based activities with high-intensity intermittent running patterns and sports-specific actions (e.g., jumping, kicking) [47]. While athletes routinely compete on a weekly basis in-season, they are often exposed to periods of higher-frequency match play. Soccer teams can be required to play up to three matches in a 7-day period [48]. And, at the international level of team-sport competition, tournaments often consist of a highly congested round-robin format, with reports of up to 6 matches in 9 days being played in field hockey [49]. Athletes are seemingly able to cope with these periods of fixture congestion, as evidenced by similar activity profiles performed by the athletes across two matches separated by 72-96 hours [13]. However, it is unclear whether the physiological and perceptual response to the subsequent match is more pronounced than the first match.

The successful preparation of these athletes requires careful management of training and competition loads [50]. As a result, the training process facilitating athletic performance is essentially a trade-off between their fitness and fatigue levels. To accomplish a balance between training stress and recovery, extensive resources are directed to monitoring the internal and external load of team-sport activity profiles, and the athletes’ biological and perceptual response to this stimulus [51].

2.2. **Time-Motion Analysis**

In team-sport environments, the volume and intensity of training and matches are routinely monitored via micro-technology sensors (e.g., global positioning systems [GPS] and accelerometers) and video analysis software (e.g., ProZone) [3, 52, 53]. The main outcome measures from this time-motion analysis are various metrics of running speed and distance [1, 2, 54, 55]. For example, mean running distances of approximately 13 km have been measured during elite Australian football matches, incorporating around 4 km of high-speed running [1]. Midfielders in other team sports, such as hockey and soccer, complete ~10 and ~12 km during a match, respectively, with approximately 3 km of high-speed running (~ > 14 km.h⁻¹) [52, 56]. Another important metric assessed is the acceleration and deceleration activities performed during a match [57]. This is because athletes can achieve maximal acceleration and not reach peak speed, which could potentially underestimate the energy cost of the performance if not
taken into account \[58, 59\]. Combined with information gathered regarding skill-based activities (e.g., passing, shooting,) \[60\] and body contacts during matches \[61\], the activity profiles of many team sports are now well defined. However, while monitoring the external load performed by team-sport athletes in competition is becoming more widespread, there are limitations to assessing the biological and perceptual responses to competitive match-play. For example, variability of team-sport activity profiles exists between playing positions and matches, making comparative and repeat measures difficult (such as during periods of fixture congestion). Furthermore, half-time and immediately post-match measurements are difficult to achieve due to the proximity of playing fields to laboratories, where some measures are required to be collected.

2.3. Match Simulations

Competitive team-sport matches provide the most ecologically-valid approach for the assessment of post-match responses. However, the assessment of some responses, such as neuromuscular fatigue, can be limited in a ‘field’ setting \[8\]. Often these variables require laboratory-based equipment, thereby presenting logistical problems such as time delays. For instance, research measuring central and peripheral fatigue following a soccer match resulted in a 40-min delay in initial testing \[8\]. Such delays potentially underestimate the actual fatigue response, as recovery of some factors (e.g., peripheral fatigue) can occur as quickly as 2 min post-exercise \[37\].

To overcome these logistical problems, extensive research detailing activity profiles in team-sports has informed the development of match simulation protocols for use in a controlled environment. Examples of these include shuttle-running based simulations like the ball-sport endurance and sprint test (BEAST90) \[62\], soccer-specific aerobic fitness test (SAFT90) \[63\], and the Loughborough intermittent shuttle test (LIST) \[64\]. These tests allow for various running patterns, including backwards running and cutting, with the BEAST90 incorporating jumping and kicking actions. However, the tests require a large area (over 20-m long), which limits their use in a laboratory setting. Match simulations performed on a motorised treadmill allow the whole testing battery to be completed in the laboratory \[38, 65\], but, motorised treadmills are often limited by their maximal speed (~25 km.h\(^{-1}\)) and their inability to change speed rapidly. Due to these limitations, non-motorised treadmills have been utilised more recently for laboratory-based match simulations \[32, 66, 67\]. Non-motorised treadmills allow for rapid changes in speed, and their maximal speed is limited only by the participant’s ability. However, early non-motorised treadmills required the athletes to wear a tether around their waist and anchored
behind them to allow them to overcome the inertial load of the treadmill belt (Woodway Force, Woodway, USA). Furthermore, this style of treadmill may disadvantage lighter athletes, as the force required to constantly accelerate the treadmill belt might be a high proportion of their overall mass [68]. However, newer, untethered non-motorised treadmills might be advantageous for match simulations. These newer models (Woodway Curve, Woodway, USA) incorporate a curved treadmill surface, allowing the individual to accelerate and decelerate without the aid of a tether.

Importantly, the majority of previous treadmill-based protocols have used external pacing – that is, the speeds the participants are required to achieve are pre-set [66, 67, 69]. However, numerous factors influence pacing strategies in team-sports, with evidence of fitness levels, playing standard and match importance all contributing to modified activity profiles [70, 71]. Furthermore, in actual team-sport match-play, athletes also pace themselves based on external cues (e.g., opposition, score line) [9]. Thus, while the reliability of an externally paced protocol on a tethered, non-motorised treadmill has been previously determined, this protocol may lack ecological validity [66]. Therefore, the development of a match-simulation protocol that allows the athlete to choose their running speed should be given consideration. With the advent of the curved non-motorised treadmills, an opportunity for the development of a more ecologically-valid, self-paced protocol exists over what is currently available in the laboratory setting.

2.4. Fatigue and Exercise

While numerous definitions of fatigue exist (see: [46, 72, 73]), it is commonly referred to as an acute or prolonged (i.e., minutes to days) transient reduction in muscle force and increased perception of effort in response to a stimulus [74, 75]. As a result, a contemporary framework regarding fatigue describes it as the interplay of perceived and performance fatigability [76]. Thus, perceptual influences prior to and during an event, coupled with physiological responses, are responsible for the intensity regulation of exercise. Research regarding fatigue dates back over a century ago [77] and continues to progress with the introduction of newer technologies and methods (e.g., transcranial magnetic stimulation) [24, 78-80]. The source of fatigue is multifaceted, and can originate from central (i.e., central nervous system) or peripheral (i.e., muscular and supporting cellular structures) components, or a combination of both [37, 81]. Furthermore, the source of fatigue is task dependent, as it is influenced by the specific nature of the exercise bout performed [82]. Therefore, to appropriately assess fatigue, the specific exercise undertaken must be considered. In turn, this can influence the method of assessment [72, 82].
2.5. Models of Exercise Induced Fatigue and Intensity Regulation

Numerous attempts have been made to explain fatigue and the mechanisms responsible for diminished force production and increased perceived exertion in response to exercise [see reviews: [73, 83, 84]]. This has resulted in the development of various models to explain fatigue, including; the cardiovascular/anaerobic model, energy supply and depletion model, neuromuscular model and the biomechanical model [46]. Additionally, various models exist proposing that perceptual factors are responsible for the regulation of exercise intensity, including the central governor theory and the psychobiological/motivational model [73, 85].

2.5.1. Cardiovascular/Aerobic Model

The cardiovascular/anaerobic fatigue model proposes that exercise intensity is reduced or ceases due to an inadequate supply of oxygenated blood to the working muscles, and an inability to remove waste metabolites from the energy production process [46, 73, 86, 87]. Evidence for this model originated over 100 years ago, when researchers observed a relationship between increases in lactic acid and a reduction in force output (and eventually muscle rigor) in amphibian leg muscles, *ex vivo* [88]. Further work reported increases in oxygen consumption and metabolites (e.g., lactic acid) as exercise intensity increased in humans, with the latter potentially responsible for the reduced physical exertion observed [89]. The production of waste metabolites is due to anaerobic glycolysis, occurring when the requirement for oxygenated blood at the working muscles is not being met by cardiac output [90]. And, while lactic acid is no longer suggested to be responsible for reduced exercise intensity [91], an inability to clear other metabolites (such as inorganic phosphate, hydrogen ions and potassium) during intense exercise may be responsible for the reduction in force production during high-intensity exercise [91-93].

2.5.2. Energy Supply Model

The energy supply model, which is associated with the cardiovascular/anaerobic model, suggests that fatigue is related to an inability to produce adequate adenosine triphosphate (ATP) to continue exercise [46, 94]. This is contradictory to research suggesting that ATP supply is rarely reduced by more than 30% during intense exercise [95]. Additionally, short duration (i.e., 30 s), maximal exercise in normoxic and hypoxic conditions results in identical performance
outcomes, due to an increase in ATP production during hypoxic exercise [96]. Hence, ATP availability is not a limiting factor in exercise performance [96]. In contrast, the energy depletion model of fatigue stipulates that reductions in fuel substrates (i.e., phosphocreatine, blood glucose and glycogen) during exercise lead to fatigue [97, 98]. The specific substrate utilisation is determined by the intensity and duration of exercise. For example, high-intensity and short-duration exercise can be limited by phosphocreatine availability [99], while glycogen depletion can be a factor in reduced exercise performance in activities lasting 90-min or more [100, 101].

2.5.3. Biomechanical Model

As exercise intensity increases, the requirement for energy production and utilisation also rises. The biomechanical model of fatigue proposes that greater efficiency of movement results in more economic fuel substrate usage [102]. The muscles and tendons (i.e., musculotendinous unit) of the lower limbs during running can be considered as springs, as they absorb and produce force during the eccentric and concentric (i.e., landing and take-off, respectively) portions of running gait [103]. The efficiency of this musculotendinous unit is an important consideration in both running performance and fatigue, and can be related to the stiffness of that unit [103, 104]. A stiffer musculotendinous unit may be more resistant to eccentrically-induced muscle damage post-exercise [105]. Additionally, a more efficient musculotendinous unit may result in improved running economy, a key factor in endurance running performance [106, 107]. This improved running economy is responsible for a reduced oxygen consumption at a given intensity, reduced accumulation of metabolites, and less heat accumulation during exercise [46].

2.5.4. Central and Peripheral Models

Other models of fatigue have been proposed, originating from central (spinal or supraspinal) alterations and/or modifications to peripheral contractile function, as described by the neuromuscular fatigue model [81, 108, 109]. Central fatigue can generally be defined as occurring proximal to the neuromuscular junction, while peripheral fatigue occurs at or distal to this region [81]. To assess the extent of central fatigue post-exercise, a peripheral nerve or transcranial magnetic stimulation technique can be utilised [8, 110-115]. The technique, termed twitch interpolation, assesses voluntary activation (%VA) of specific muscle groups by measuring maximal voluntary isometric contractions (MVC) with a superimposed stimulation [116-118]. Reductions in %VA suggest alterations in central motor drive (known as central activation failure), and have been observed following various exercise modalities [119-122].
Changes in central motor drive may also be linked with modifications in the balance of neurotransmitters in the brain, such as dopamine, serotonin and noradrenaline, as they have been associated with feelings of fatigue, lethargy and mood state [81, 108, 123, 124]. For example, increased noradrenaline (in response to a reuptake inhibitor) has been found to decrease time-trial performance, as well as increasing central fatigue [111]. However, administration of bupropion (a dopamine reuptake inhibitor) has resulted in improvements in time-trial performance in the heat, but not temperate conditions [125]. A combination of neurotransmitters may provide a better explanation of the fatigued state than any individual marker, such as the serotonin to dopamine ratio – with a high ratio relating more to fatigue, and a low ratio being associated with improved performance [124].

Neuromuscular fatigue can encompass both central and peripheral alterations, with considerable crossover possible between the two sources. For example, muscle afferent fibres (specifically group III/IV) are responsible for providing feedback from the working muscles to the central nervous system, reducing central motor drive to prevent additional localised muscular fatigue [80, 121, 126]. Furthermore, reducing group III/IV afferent feedback using lumbar epidural anaesthesia caused increased exercise intensity, as well as greater metabolite accumulation and peripheral fatigue post-exercise [80]. An additional peripheral mechanism of neuromuscular fatigue is a reduction in the muscle’s ability to respond to an electrical signal to produce force (termed neuromuscular propagation failure) [73]. This may be due to modifications in Ca\(^{2+}\) distribution and uptake during exercise, which can deleteriously impact excitation-contraction coupling during exercise [90, 127]. Furthermore, exercise-induced interruptions to Na\(^+\)/K\(^+\) pumps, instrumental in the generation of action potentials, can contribute to reductions in force production during exercise [127-129].

Although numerous models defining various physiological mechanisms exist to explain fatigue, there may be psychological processes that also play a role, and it is likely that these physiological and psychological processes are not independent of one another. For example, research exploring the impact of mentally-fatiguing tasks performed pre-exercise have been shown to reduce exercise performance [32, 123]. Hence, the motivation to complete a task and the perceptual response to a fatiguing task could play an important role in the regulation of exercise intensity and exhaustion. Brehm’s theory of motivation intensity proposed that an individual’s task engagement was merely based on their willingness to perform a certain task, and their perceived ability to do so [130]. A lack of motivation during an activity can, therefore, be responsible for task dissociation and result in the cessation of exercise [79, 131]. This motivational
theory is the basis for a more contemporary, psychological model of fatigue, known as the psychobiological model [132]. This model suggests that an individual’s rating of perceived exertion (RPE [133]) is a key factor in the regulation of exercise duration and intensity [132, 134]. For example, while mental exertion tasks have shown to result in reductions in exercise performance (i.e., reduced time to exhaustion), neuromuscular function assessed after exhaustion was not linked to neuromuscular fatigue, but a higher perception of effort [79, 135]. Overall, the psychobiological model suggests a conscious decision regulates exercise intensity based on the perception of the effort required being higher than can be willingly achieved, or believing a maximal effort has been produced.

An alternate theory to the psychobiological model suggests that exercise intensity is subconsciously regulated by the brain [97, 136]. Known as the central governor model, this theory suggests that the central nervous system maintains homeostasis during exercise by altering the amount of motor units recruited to avoid catastrophic physiological failure (myocardial ischaemia, rigor mortis, etc.) [97, 136]. While the central governor model includes feedback and feedforward mechanisms [137], the subconscious anticipatory system proposed in it has been criticised for its unnecessary complexity [132]. Furthermore, recent work suggests that a major flaw in the central governor model is that the initiation of exercise would not occur if the maintenance of homeostasis was the goal [138]. As a result, an integrative governor theory has been suggested, where psychological and physiological negative feedback loops constantly oscillate throughout an activity to maintain homeostasis based on metabolic set points [138].

The above models highlight the breadth of work undertaken to explain exercise-induced fatigue. Although the propositions are vast and complex, it remains unclear as to whether an individual theory is responsible for exercise-induced reductions in force production, or if a combination of theories are necessary. Furthermore, the response to exercise is related to the specific mode, intensity and duration of the activity. As a result, recent work has defined fatigue as a combination of performance fatigability (central and peripheral factors) and perceived fatigability (psychological and homeostasis related factors) [76]. Therefore, complex interactions of various systems are likely responsible to modulate exercise intensity, and in turn maintain physiological homeostasis [83].
2.6. Fatigue and Pacing During Team-Sport Exercise

The activity profiles in team sports such as Australian football, soccer and hockey incorporate intermittent, high-intensity running interspersed with periods of low-speed activity or complete rest [47, 49]. Variations in locomotor activity can occur from match to match, generally as a result of players responding to external cues and events occurring during competition [9, 139]. For example, a player able to more accurately interpret on-field cues (e.g., opposition ball movement) may complete more efficient running patterns [139], or the score line may influence the amount of physical activity performed during the match [9]. There is evidence to suggest that running volume and intensity performed throughout a match is linked to performance and overall team success [40, 140]. Specifically, more high-intensity running performed within an Australian football or soccer match has been linked with positive match outcomes and increased skill involvements [39, 40, 141]. Furthermore, the volume of high-speed running within matches has been linked with intermittent running ability in professional soccer players [142]. Even so, reduced running output is evident in team sports between first and second halves [47]. This can include a reduced total distance, and less high- and low-speed running [1, 8, 143, 144]. In addition, passing and shooting accuracy in elite soccer declines as the match progresses, and this reduction in performance is larger in players with lower aerobic fitness [145, 146].

The modification of activity profiles during team-sport match play may be a result of acute fatigue in response to the high-intensity, intermittent running patterns performed. For example, this mode of running may be responsible for the accumulation of various metabolites causing short-term reductions in running performance [147, 148]. Moreover, there is evidence to suggest that the periods of the highest-intensity running in team-sports are subsequently followed by periods of very low intensities, possibly due to these short-term metabolic responses [1, 144]. Alternatively, reductions in team-sport running intensity as the match progresses may be due to an impaired energy supply [9]. As the duration of most team-sports exceeds 80 min, the decrease in total distance covered by athletes between the first and second half of games may be related to glycogen depletion [1, 9, 144]. In support of this theory, carbohydrate supplementation has been shown to maintain cognitive ability and physical capacity in a variety of team-sport specific tasks [149]. This may be due to a reduced perception of effort following carbohydrate ingestion, or an increase in the availability of carbohydrate which is related to muscle glycogen sparing [150, 151].
Team-sport exercise might also result in neuromuscular fatigue. However, studies assessing neuromuscular fatigue during matches (i.e., at half time) are limited [152, 153]. Moreover, these results are conflicting due to the assessment of different muscle groups (i.e., hamstrings or quadriceps) [152, 153]. Furthermore, the interpretation of data in these studies utilised traditional methods of statistical analysis (i.e., significance testing), which may substantially underestimate the practical importance of observed decrements and result in incomplete conclusions regarding the presence of central and peripheral fatigue [153]. The paucity of research regarding neuromuscular responses during team sports is likely due to the technology and environment required for data collection; generally, a laboratory with a dynamometer and an electrical or magnetic stimulator [113]. To overcome this, match-simulations are often performed indoors (e.g., treadmill; shuttle running on a sprung floor) [62, 66, 69] [see discussion in section 2.3]. However, these simulations (as used in [152, 153]) are limited by their use of external pacing, where running speeds are dictated to athletes. In turn, this might reduce the ecological validity of the protocol and subsequent outcomes, as externally-paced exercise has been shown to increase physiological strain over self-paced exercise, even when matched for intensity and duration [154].

While causative links between physiological-based models of fatigue and modified activity profiles can be drawn, consideration should also be given to psychological factors. For example, pacing strategies may exist in sport, with the premise being that, although fatigue is inevitable during competition, athletes vary their running patterns to preserve energy and optimise their performance [155-157]. As a result, pacing strategies undertaken during a team-sport match might be responsible for the adjustments in running intensity [155]. This interaction between physical fitness and optimal pacing can be observed in fitter athletes who pace themselves at higher relative intensities during tournament competitions than their less fit counterparts [71]. Evidence of pacing in team sports may be demonstrated by temporal reductions in low-to-moderate intensity running, while high-intensity running is maintained [1, 158]. Athletes competing in warm climates have adopted similar pacing strategies, maintaining very-high intensity running while lower intensities are reduced and core temperature stabilised [159, 160]. However, unlike typical closed-loop endurance sports (e.g., running, triathlon) whereby the athlete is aware of the endpoint [157], team sports consist of constant situational changes known to influence performance [161]. For example, the importance of the match, match location, quality and activity profile of the opposition, score line and substitutions have all been shown to affect team-sport activity profiles [70, 161, 162]. Further, the duration of the match can vary based on extra time in various sports, meaning that there is no fixed endpoint for athletes to base their pacing strategy.
As a result, the most common pacing profile observed during team-sports is a general decrease in overall distance and intensity as the match progresses (termed “slow-positive”), without an “end spurt” often seen in endurance sports with pre-determined distances [155, 163].

The pacing of activity profiles during team-sport matches could also indicate the occurrence of fatigue as a conscious response to perceived exertion, or a subconscious response based on afferent feedback to maintain homeostasis [79, 138, 164]. Furthermore, suggestions of complex, subconscious processes regulating exercise intensity have been described in both individual endurance and team-sport events [165, 166]. This response is augmented when a mentally fatiguing task is performed prior to exercise [123, 167]. For example, the impact of mental fatigue on intermittent running concomitant with team-sport matches results in reductions of low-intensity running during a soccer simulation, increased RPE, and diminished performance in fitness testing relating to soccer performance [32, 167].

2.7. Biological Responses to Team-Sport Exercise

The activity profiles undertaken by athletes during team sports result in acute (i.e., minutes) and longer-term (i.e., multiple days) post-match fatigue [7, 9]. Generally, team-sport athletes are required to participate in large volumes of training, as well as multiple matches in a short time frame (e.g., within 3 days) [168]. As some post-match responses are yet to recover at 72 hours post-exercise, it is likely that athletes compete in subsequent matches while still recovering from a previous match [7, 9, 34]. Hence, a clear understanding of the impact that a single match has on the days following and during upcoming matches is imperative for appropriate athlete management [14]. The response of specific variables can be assessed at various stages during and following a team-sport match to assess the influence of the activity profile on the athletes’ biological state [78, 169, 170]. Several of these key variables are outlined in the ensuing sections.

2.7.1. Neuromuscular fatigue

Neuromuscular fatigue encompasses any transient reductions in voluntary force production following a stimulus (e.g., exercise) that is a result of alterations to the central nervous system and/or working muscles [75, 113]. It is possible to assess neuromuscular fatigue using a variety of field- or laboratory-based assessments. For example, field-based performance tests (such as a countermovement jump [CMJ]) have revealed neuromuscular fatigue following team-sport matches in elite Australian football players [6]. However, neuromuscular fatigue can comprise
changes to both central and peripheral structures. Therefore, while performance testing is sensitive enough to detect neuromuscular fatigue, it is unable to determine the contribution of changes stemming from central drive and peripheral contractile function. In contrast laboratory-based testing such as the interpolated twitch technique uses electrical or magnetic stimulation delivered to maximally contracting or resting muscles to determine contributions of central and peripheral fatigue [171, 172]. However, laboratory-based testing is impractical in many field settings, due to the equipment required (e.g., dynamometer). A more detailed understanding of the relationship between field- and laboratory-based measures of neuromuscular fatigue may provide a more suitable assessment tool in a practical setting. Furthermore, understanding the central and peripheral contributions to fatigue following team-sport exercises might be beneficial when prescribing recovery interventions after a game [173].

2.7.1.1. Central Fatigue

Central fatigue is defined as a reduced ability to voluntarily activate a particular muscle following a bout of exercise [81]. Alterations at the spinal and/or supraspinal level are responsible for these reductions (i.e., occurring above the neuromuscular junction) [81]. Various techniques have been employed to assess the presence and magnitude of central fatigue following exercise, such as near-infrared spectroscopy and transcranial magnetic stimulation [24, 174]. These techniques measure central fatigue at the brain by providing insight into altered cerebral oxygenation and reductions in neural drive from the motor cortex, respectively [81, 174]. However, these particular techniques can be invasive and difficult to administer following team-sport exercise [172]. As a result, surrogate measures of central fatigue, such as electromyography (EMG) and the assessment of %VA via muscle or peripheral nerve stimulation, are often incorporated in studies of fatigue [21, 112, 175]. Surface EMG measured during maximal contractions alone does not provide adequate information to determine central fatigue, as modifications in sarcolemmal excitability may also be present [176]. As a result, the ratio of the EMG signal (root mean square [RMS]) obtained during a voluntary contraction to the maximal motor unit action potential (i.e., M-Wave) obtained during a maximal twitch is assessed [177, 178]. Greater reductions in the EMG signal than the M-Wave peak-to-peak amplitude (PPA) can be interpreted as diminished central activation, assessed as the RMS/PPA ratio [176]. However, central fatigue following team-sport exercise, is more commonly assessed using the interpolated twitch technique to assess %VA [8, 21, 152, 179]. This technique generally involves superimposing an electrical or magnetic stimulation to a peripheral nerve innervating
a muscle group (e.g., quadriceps) during and soon after (~5 s) a maximal isometric contraction in order to calculate %VA (see equation 1) [180].

Equation 1: Voluntary activation (%VA) calculation using data obtained during the interpolated twitch technique [181, 182]

\[
\text{Voluntary Activation (\%VA)} = \left(1 - \frac{\text{superimposed twitch} \times \text{force level at stimulation}}{\text{MVC force} \times \text{potentiated twitch}}\right) \times 100
\]

Currently, electrical stimulation is most commonly used for assessments of %VA following exercise [8, 21, 115]. However, electrical stimulation is often considered painful by participants, which may limit its use with certain populations [183]. A valid alternative to electrical stimulation is magnetic stimulation [184-186]. Generally, magnetic stimulation is considered painless, which may make it a more viable technique for a range of participants [183, 187]. However, there is limited evidence describing the reliability of magnetic stimulation of the femoral nerve to assess %VA [23, 188]. Furthermore, the reliability of magnetic stimulation to assess interpolated twitch responses across multiple days (as may be required following a sporting match) has not been established; hence, this investigation is warranted and should be undertaken prior to any assessments of this nature.

As the interpolated twitch technique stimulates peripheral nerves, it is unable to define the location of fatigue at the spinal or supraspinal level. Regardless, since the original research incorporating interpolated twitch was performed by Merton in 1954 [118], numerous studies have utilised this technique to understand central fatigue [112, 120, 189, 190]. As a result, evidence of reduced voluntary activation has been demonstrated following numerous exercise modalities, including prolonged and intermittent cycling, running, and resistance training [112, 115, 191-193]. However, the assessment of central fatigue following team-sport competition is somewhat limited. Significant reductions in %VA and RMS/PPA have been observed following a soccer match with professional players, recovering by 48 h post-match [8]. Reductions in these variables were significantly related to the amount of running (i.e., total distance) covered in the match [8]. In contrast, research measuring changes in %VA following competitive rugby matches has shown no significant differences between pre- and post-match results [21, 194, 195]. Players involved in the rugby matches completed considerably less total distance than the abovementioned soccer match (5585 – 6221 m vs 11,764 m, respectively) [8, 21, 195]. As total distance has demonstrated a significant relationship to reductions in %VA, this may explain the lack of reduction in %VA following rugby matches [8].
As has been discussed, the most ecologically-valid assessment of central fatigue would be performed immediately after a competitive match. However, the equipment required to perform these assessments (e.g., dynamometer and stimulation unit) is not often near playing fields. As a result, assessments of %VA after matches have been performed at 15-40 min post-exercise \[8, 195\]. Previous research has shown that recovery in %VA can occur in as little as 2-min after exercise completion; therefore, important changes following competitive match play may be missed with such delays \[120, 196\]. To circumvent this issue, intermittent sprint and simulated team-sport running protocols have been performed in the field (e.g., running track, indoor court) or on motorised or non-motorised treadmills \[62, 65, 66, 153, 197\]. One commonly used intermittent sprint protocol consisting of 2 x 30 min of exercise, with 10-min recovery between bouts, has demonstrated reductions in %VA immediately following and up to 24-h post-protocol \[175, 197-199\]. However, while the total distance covered during the intermittent sprint activity is short (i.e., ~ 4 km), approximately 43-85% of this running is completed at a high intensity \[198, 199\]. This is in contrast to activity profiles in soccer and Australian football, which demonstrate high-intensity running volumes of ~ 25-30% of total match-distance \[1, 56\]. Therefore, the response to intermittent sprint exercise as performed in the aforementioned protocol may not be a valid indicator of responses occurring during team sport match play.

Team-sport simulations attempt to provide a more accurate representation of actual match demands in a controlled environment (e.g., laboratory, indoor court) \[66, 200\]. Reductions in %VA and/or RMS/PPA have been demonstrated in response to match simulations in various studies \[78, 152, 201\]. Specifically, half-time reductions in %VA of the quadriceps \[201\] and decreased RMS/PPA in the hamstrings \[152\] muscle groups have been observed, persisting until post-match. However, no further assessments of post-match responses were performed following the immediate post-match measures in these examples. One study has measured responses in %VA post-match simulation (but not at half-time), and up to 72-h post, displaying a return to baseline at the final measurement (i.e., 72 h) \[78\]. Therefore, these match simulations result in similar (or slightly prolonged \[78\]) reductions in %VA as observed in a soccer match \[8\]. However, the activity profiles performed during actual matches are dictated by the athlete in response to the match situation (i.e., self-paced, see section 2.3) \[9\]. In contrast, the majority of match-simulation protocols (including those mentioned above \[64, 202\]) are externally paced. Hence, the running speeds required are pre-set and dictated to the athletes during the simulations. In addition, while the overall activity profiles may be similar, self-paced exercise is less physiologically demanding than externally-paced exercise, even when matched for intensity and duration \[154\]. This may potentially explain the prolonged reduction in %VA during the
match simulation\textsuperscript{[78]} compared to the soccer match\textsuperscript{[8]}. As eluded to in section 2.3, overcoming the methodological and ecological shortfalls presented by competitive matches and existing match simulations might be possible with the development of a self-paced match simulation protocol performed on a non-motorised treadmill.

2.7.1.2. Peripheral Fatigue Assessment

A reduced ability to produce force following exercise is also associated with modifications to peripheral mechanisms; that is, changes occurring at or distal to the neuromuscular junction\textsuperscript{[81]}. As previously discussed, these peripheral responses can be caused by exercise-induced modifications in excitation-contraction coupling, ionic changes, neuromuscular propagation failure, etc.\textsuperscript{[73, 95]}. Peripheral fatigue can be assessed post-exercise by delivering a stimulation to a nerve innervating a resting, potentiated muscle group (e.g., femoral nerve innervating quadriceps femoris)\textsuperscript{[8, 37]}. This potentiated stimulation is most often performed during the interpolated twitch technique. As such, numerous investigations have assessed exercise-induced peripheral alterations in muscle function via peripheral nerve stimulation\textsuperscript{[37, 109, 121, 190, 203]}. Reduced twitch torque has been reported following cycling exercise using both magnetic and electrical stimulation\textsuperscript{[112, 120, 191]}. Furthermore, short- to moderate-duration, high-intensity running resulted in reduced twitch torque immediately post-exercise\textsuperscript{[204, 205]}. However, prolonged, low-intensity running exercise (i.e., 5 to 8.5 h) has demonstrated an increase in the potentiated twitch response\textsuperscript{[206, 207]}. While this finding is unexpected due to the muscle damage associated with prolonged stretch-shortening cycle exercise (i.e., running), the authors suggest that the increased musculotendinous stiffness that can present following such long-duration running could be responsible for the potentiated result post-exercise\textsuperscript{[207]}. Consistent with work assessing central fatigue in team sports, measurements of peripheral fatigue via nerve stimulation are hindered by issues of protocol design, proximity of match locations to laboratories, as well as their choice of statistical procedures. For example, reduced twitch torque lasting 48 h was observed following a soccer match, and this was related to perceived soreness post-match\textsuperscript{[8]}. Additionally, reduced twitch responses were measured following rugby matches, persisting up to 2-h post (when data collection ceased)\textsuperscript{[21, 194]}. Yet, the earliest assessments occur between 5 to 40 min after the matches ended\textsuperscript{[8, 21]}. As significant recovery in potentiated twitch responses can occur within 2-min following exercise, these data may underestimate the initial post-match peripheral fatigue experienced following competitive matches\textsuperscript{[37]}.
While match simulations have been performed to overcome the abovementioned delays in data collection, few research projects have assessed peripheral fatigue via stimulation following a match-simulation protocol \[78, 152, 153\]. Furthermore, for those that have, the match simulations were externally-paced which can increase the physical demand on the participant (see discussion in 2.7.1.1). Previous work has displayed significant reductions in twitch torque from the quadriceps femoris for up to 72 h post soccer match simulation \[78\]. In contrast, no statistically significant changes were observed in twitch torque at half- or full-time following a soccer match simulation \[153\]. However, when re-calculating these data \[153\] using effect-size statistics, a moderate difference was demonstrated at half-time, with a trivial difference at match end. While reductions in quadriceps twitch torque have been detected following a soccer match-simulation, similar protocols have resulted in no change to hamstring twitch torque \[152\]. This might suggest the role of the hamstrings during the specific soccer simulation was not of a high enough intensity to induce peripheral fatigue \[152\]. In turn, this highlights the importance of assessing the prime movers (e.g., quadriceps femoris) during team-sport exercise when examining fatigue responses.

2.7.1.3. Performance Testing

In order to more accurately determine the origin of neuromuscular fatigue (i.e., from central or peripheral regions), processes such as the interpolated twitch technique are required \[81\]. However, it might be impractical to perform such assessments following competitive match play. Therefore, tests other than the interpolated twitch technique can be performed to assess the neuromuscular responses to exercise, including: countermovement and squat jumps \[6, 19, 153, 208\], maximal voluntary contractions \[18, 204\], sprints and repeat sprint running \[10, 209, 210\], and cycling ergometer sprints \[211\]. While these assessments provide a more ‘global’ measure of neuromuscular fatigue, they may still provide a practical and effective measure of neuromuscular status which can be useful for athlete monitoring practices \[14, 16\].

Countermovement and squat jumps are predominantly performed on a force platform to assess variables that may be indicative of neuromuscular fatigue, including: peak and relative force and power, peak and average velocity, jump height, flight time, contraction time, eccentric time, concentric time, etc. \[212, 213\]. Reductions in countermovement jump height and power output have been identified following competitive match play in a variety of team sports, such as soccer, Australian football, rugby league and rugby union \[5, 6, 19, 27, 208\]. Generally, this reduction lasts between 24 to 48 h post-match, with similar outcomes observed from match-simulation...
protocols performed in a laboratory or other controlled environment (e.g., indoor court) [67, 78, 153, 179]. However, due to the relatively brief timeline of post-match reductions in jump height, other countermovement jump variables might be of more interest. For example, modifications to the flight time:contraction time ratio persist longer than jump height, providing a more sensitive measure of neuromuscular fatigue [6, 214]. This suggests that, although the performance outcome of the task is preserved, the movement strategy undertaken by the athlete is altered to achieve this [213]. Overall, countermovement jump performance and its associated variables are a useful tool to assess neuromuscular status, supporting the contention that tasks incorporating the stretch-shortening cycle are of value for measuring muscle function [215].

Various lower-body strength tasks have been used to assess neuromuscular function following team-sport matches and simulations, including isometric and isokinetic knee extension [8, 10, 179] and isometric squat tests [67]. Reductions in strength are commonly observed following match play; however, the magnitude and duration of the response can vary from 24 to 72 h [8, 78, 201]. Furthermore, the majority of isometric knee extension assessments are performed in conjunction with the interpolated twitch technique, providing more detailed information regarding the origin of the fatigue response. Although the quadriceps are commonly assessed in these tests as they are considered a prime-mover in team-sport exercise [152], the isolation of a single muscle group may not be representative of all tasks performed during team sports. As a result, attention has been given to short sprint tests (e.g., 20 – 40 m) as a more relevant performance indicator of fatigue relating to team-sports [5, 8, 11, 67, 78, 153]. While no changes in 6-s sprint power output on a non-motorised treadmill were observed following a treadmill-based match simulation [67], others have reported declines in sprint performance immediately post [78, 153], and up to 72-h post-match [5]. However, a maximal sprinting test might be too strenuous and impractical for regular athlete monitoring [216]. Furthermore, while athletes might be able to recover sprinting performance readily (e.g., within 24 h), their running technique might be modified to compensate for any neuromuscular fatigue they are experiencing [29]. As a result, sprint testing may not be a useful monitoring tool for assessing neuromuscular fatigue.

While performance testing may provide an effective and practical assessment of neuromuscular fatigue following exercise in some situations, a key limitation is the non-specific nature of the fatigue assessments. A clearer understanding of the source of neuromuscular fatigue (i.e., central or peripheral) could be beneficial in directing specific recovery modalities to more readily recover fatigue-related symptoms [172]. Limited research has assessed the relationship between central and peripheral responses compared to performance testing following team-
sport exercise [8]. This information would be valuable to practitioners, potentially improving the interpretation of performance testing and, in turn, directing more specific recovery modalities for better athlete preparation.

2.7.2. **Biochemical responses**

The acute and chronic fluctuations in biochemical variables such as creatine kinases (CK), uric acid (UA), hormones (e.g., testosterone [T] and cortisol [C]), cytokines, amongst others; following team-sport exercise are the topic of numerous investigations [6, 19, 22, 217-219]. These variables are of interest to practitioners, as they might provide insight into muscle damage, oxidative stress and hormonal responses of athletes to a given stimulus (e.g., training or match play) [16, 220, 221]. Furthermore, the longer-term tracking of these variables might be useful for assessing the impact of intensified training periods, as well as determining any overtraining risk in athletes due to prolonged fluctuations in some measures [222, 223].

2.7.2.1. **Muscle Damage**

Exercise-induced muscle damage can occur in response to eccentric and long-length isometric contractions [224], but not necessarily concentric exercise [225]. Muscle damage can be characterised by disruptions to the muscle fibres (including sarcomeres, myofibrils and Z-disks) [226], as well as an increase in inflammatory processes and alterations to the excitation-contraction coupling mechanism [227]. Furthermore, muscle damage can result in a reduced ability to produce force, as well as an increase in muscle soreness, typically lasting 24 to 48-h post-exercise (i.e., delayed-onset muscle soreness) [9, 227]. As previously mentioned, the activity profiles of team-sport exercise contain actions likely to induce muscle damage [48]. While the assessment of force production has been established as a valid and reliable indirect marker of exercise-induced muscle damage [220, 228, 229], blood-borne markers present an alternative measure that is also responsive to exercise-induced muscle damage [230]. Various blood-borne markers of skeletal muscle damage exist. For example, cytokines such as interleukin-6 (IL-6), interleukin-1 beta (IL-1β) and tumour necrosis factor-alpha (TNF-α), are secreted by cells at the site of muscle tissue injury (e.g., exercise-induced muscle damage) [11, 35, 86, 231]. Additionally, myoglobin (a myocellular protein) concentration increases significantly in response to muscle damage [232]. Increases in cytokines [11, 35] and myoglobin [10] have been observed following soccer matches, indicating muscle damage and inflammation may be present following this activity.
The most common blood-borne measure of muscle damage following exercise is creatine kinase. Exercise resulting in skeletal muscle damage can cause large increases in serum creatine kinase concentrations, generally peaking at ~48 h post-exercise \[11, 233\]. However, there is some conjecture regarding the presence of creatine kinase being directly related to muscle damage due to a variety of factors \[227\]. For example, as creatine kinase is released from damaged muscle tissue into the blood stream, the release rate and the clearance rate can influence the total measured creatine kinase \[227\]. Furthermore, both the release rate and clearance rate may be influenced by genetic factors or subsequent bouts of exercise \[227, 229\]. As a result, large inter-individual differences are often present when assessing the enzymatic responses to exercise \[229\]. Nonetheless, creatine kinase is widely used as a surrogate marker of muscle damage, most likely due to the large magnitude of change observed post-exercise compared to other blood-borne markers of muscle damage \[9, 227, 230\].

Significant positive relationships have been observed between high-intensity running variables (e.g., number of sprints, high-intensity running volume, etc.) and serum creatine kinase concentrations up to 48 h following a soccer match \[234\]. Additionally, large and very large correlations have been established in Australian football with high decelerations and accelerations performed during a match, respectively \[235\]. Similarly, physical contact resulting in impact-induced muscle damage, such as the number of rugby tackles performed, has been strongly related with the creatine kinase response post-exercise \[236, 237\]. The magnitude and duration of the creatine kinase response to exercise is mediated by a variety of factors, such as; training status (i.e., fitness and strength), standard of competition and physical contacts experienced during play \[9, 45\]. For example, fitter and stronger rugby league players, despite accumulating more internal and external load during a competitive match, exhibit a lower creatine kinase response post-exercise than less fit players \[45\]. In regards to playing standard, the magnitude of the creatine kinase response following a competitive soccer match is ~2 times greater in sub-elite competition \[10, 233\] compared to elite competition \[8, 238\], which may also be a reflection of the athletes’ training status.

Assessment of creatine kinase post-match is performed through a relatively simple test, requiring a small blood sample that can be obtained via finger prick or venepuncture. As a result, numerous studies have assessed the response following competitive match play in various sports (e.g., Australian football, rugby league, soccer) \[8, 10, 11, 45, 61, 238, 239\]. However,
due to limitations in collecting other indirect markers of muscle damage following actual match play (such as the timely collection of potentiated twitch; see sections 2.3 and 2.7.11), research comparing different markers with creatine kinase following actual match play is scarce. The creatine kinase response to simulated team-sport matches has been established though, with large increases detected following various shuttle running and treadmill-based soccer simulations \[5, 67, 78, 240\]. A comparison of a soccer simulation protocol with actual match play has reported similar post-match creatine kinase responses when assessing the same individuals \[240\]. This result may be expected, as the activity profiles between the matches were similar, and soccer matches tend to involve low amounts of physical contact which can be influential in the magnitude of the creatine kinase response \[61\]. However, as discussed, given various issues with current match simulations (see section 2.3), determining the creatine kinase response to a self-paced match simulation on a curved, non-motorised treadmill would be beneficial. This would enable the comparison of a more ecologically-valid, self-paced simulation with actual match play, and allow for the identification of any relationships between the simulation activity profile variables and the creatine kinase response. In addition, the laboratory-based nature of the protocol would allow for the assessment of interactions between other exercise-induced muscle damage variables with creatine kinase, including the impact of participant strength and fitness on the magnitude of the response.

2.7.2.2. Oxidative Stress

During normal cellular oxygen metabolism, reactive oxygen and nitrogen species (RONS) are produced \[233\]. Reactive oxygen and nitrogen species are involved in cellular signalling, and can be important for triggering the body’s adaptive response \[241\]. However, excessive RONS can be detrimental to cellular processes and, as a result, antioxidants are produced to maintain an appropriate redox balance \[241, 242\]. A chronic, excessive production of RONS has been linked with various diseases (including cardiovascular and rheumatoid arthritis) and impaired cellular function \[221, 241, 242\].

A shift in the redox balance, favouring RONS, is termed oxidative stress. This increase in RONS above the capacity of the antioxidant system commonly occurs during periods of increased mitochondrial oxygen consumption above basal levels and with muscle damage, stress and inflammation \[9, 10\]. Thus, acute oxidative stress commonly occurs following an exercise bout \[221, 242\]. However, it is difficult to assess oxidative stress in individuals \textit{in vivo} and, as a result, indirect markers of the redox process are commonly analysed \[241\]. One such
blood-borne marker is uric acid, which acts as a scavenger for free radicals – accounting for up to two thirds of all free-radical scavenging activity [11, 243]. Consequently, uric acid has been measured in numerous investigations to assess oxidative stress following acute bouts of exercise [219, 243-245].

Oxidative stress has been quantified following numerous forms of exercise, including resistance training, running, wrestling, triathlon, basketball and soccer matches [210, 233, 244, 246, 247]. Transient increases in uric acid concentrations following an exercise bout have been observed for up to 96-h post-exercise [11]. In relation to team-sport exercise, contrasting evidence exists regarding the duration of the uric acid response to soccer matches and simulations. For example, professional soccer players exhibited no increase in uric acid following a competitive match, while second division players had an increase in uric acid at 30-min post-exercise, only [210, 240]. However, elite and sub-elite players in other investigations have shown increased uric acid levels, persistent for 48-96 h [10, 11, 233]. Inter-individual variation in oxidative stress can exist depending on the intensity, duration, population and type of exercise performed [242]. For example, soccer players performing a soccer simulation (Loughborough intermittent shuttle test) and a friendly match on different days had a lower heart rate and oxidative stress response following the simulation [240].

While acute oxidative stress post-exercise is a normal outcome that can stimulate the body’s adaptive response, prolonged oxidative stress may result in a blunting of the adaptive response and a prolonged state of fatigue [241]. Consequently, exogenous antioxidant supplements are frequently ingested to combat this oxidative stress [242, 244]. However, excessive consumption of exogenous antioxidants may also blunt the adaptive response [241]. Therefore, assessing variables indicative of the oxidative stress response (i.e., uric acid) immediately following, and for subsequent days post-exercise, might be a useful tool for athlete monitoring [233]. Assessing the oxidative stress response following an ecologically-valid team-sport match simulation, in conjunction with detailed activity profiling and other key post-match responses, may help establish which components of the activity profile are more related to the oxidative stress response. In addition, a clearer understanding of persistent (e.g., 72 hours) oxidative stress on subsequent activity profiles and recovery following multiple matches with short inter-match recovery (e.g., 2-3 days) would be of interest to researchers and practitioners alike.
The acute and chronic endogenous hormone response to exercise bouts and prolonged training might provide information regarding acute training stress and training status (e.g., overreaching) \cite{28, 248, 249}. Various hormones (such as adrenocorticotropic hormone [ACTH], testosterone, cortisol, epinephrine and norepinephrine) have been assessed following acute exercise bouts (i.e., a match) or profiled over a longer period (e.g., competitive season) \cite{6, 16}. Understanding the acute responses may provide insight into the effectiveness of a training stimulus, while prolonged alterations may highlight the various phases of overreaching and overtraining syndrome.

Measurements of hormonal status can be influenced by various factors, including the method of collection, analysis, and timing of the sample collection \cite{250}. For example, cortisol concentrations can be quantified via salivary or blood samples, with the two methods displaying a strong, significant correlation \cite{251-253}. However, it may be beneficial to collect blood samples when multiple variables of interest are required from an identical time point in order to standardise the collection method \cite{250, 254}. Another key factor influencing hormonal status is diurnal variation, with statistically significant variations in hormonal status established in participants during rest days and throughout normal daily activities \cite{249, 250, 255-257}. As a result, standardising the method and timing of sample collection is imperative to obtaining meaningful results \cite{250, 258, 259}.

Due to the relative simplicity of assessing hormonal status via saliva or blood samples, numerous studies have assessed the endocrine response to team-sport exercise \cite{4, 6, 16, 19, 22, 27, 249, 256, 260-262}. Two hormones that have been heavily researched following acute or prolonged bouts of training and match play are testosterone and cortisol \cite{6, 16, 217, 248, 261-263}. Testosterone is an anabolic hormone, required for protein and glycogen synthesis \cite{6, 28, 264}. In response to stress (in this case, exercise), testosterone is released by the testes via the hypothalamic-pituitary-gonadal (HPG) axis. The secretion of testosterone (and other endocrine hormones) is dependent on the type of exercise undertaken \cite{264}. For example, serum testosterone concentrations have been shown to increase following resistance training exercise, which may be associated with the hypertrophic response to this training modality \cite{265}. However, more recent research suggests that post-exercise hormonal status may not be directly related to muscle hypertrophy \cite{266, 267}. Regardless, acute increases in anabolic hormones (such as testosterone) occur following resistance training exercise, and this may provide an optimal environment for muscle
hypertrophy \[^{266}\]. Conversely, the response of testosterone to endurance exercise is varied, with both increases and decreases post-exercise detected, specific to the endurance exercise intensity, duration and the athletes’ training status \[^{268, 269}\]. However, due to the individual variability in the hormonal response to exercise, the outcomes are often conflicting, unclear or not statistically significant. For example, reductions in testosterone compared to pre-match have been established following a rugby match \[^{27, 261}\]; however, other investigations in rugby and Australian football have shown an unclear response \[^{6, 19}\]. In contrast, a soccer match comprising a high proportion of sprinting activity resulted in a post-match increase in testosterone \[^{22}\].

Cortisol can be referred to as a catabolic hormone, secreted by the adrenal cortex in response to psychological or physiological stress via the hypothalamic-pituitary-adrenal (HPA) axis \[^{4, 28, 270}\]. The secretion of cortisol begins with the release of corticotropic-releasing hormone at the hypothalamus in relation to stress, which then stimulates the release of adrenocorticotropic hormone at the pituitary gland \[^{271}\]. The adrenocorticotropic hormone then stimulates the adrenal cortex to release cortisol \[^{270}\]. Cortisol has typically been shown to increase following an acute bout of resistance training exercise, as well as following endurance training modalities \[^{255, 269}\]. Furthermore, greater resting concentrations of cortisol can present as a chronic response to endurance training \[^{28, 264}\]. In relation to team-sport exercise, the response is also inconsistent, with increases in post-match cortisol observed following Australian Rules, soccer and rugby matches \[^{6, 11, 27}\], and a decrease observed following a soccer simulation \[^{263}\].

Due to the anabolic and catabolic association of testosterone and cortisol, respectively, changes in these hormones are often monitored to interpret athlete training status (i.e., anabolic or catabolic state) \[^{255}\]. The ratio of testosterone:cortisol has been suggested to be more sensitive to the athlete’s anabolic:catabolic status post-exercise as it considers the variation present in both individual measures \[^{28, 255, 264}\]. Acute decreases in the testosterone:cortisol ratio have been reported following an elite Australian football match \[^{6}\]. Furthermore, reductions in this ratio have been related to reductions in performance markers in soccer players \[^{272}\]. However, due to the variation in the individual markers, it is unsurprising that the testosterone:cortisol ratio is varied following team-sport exercise, with increases \[^{261}\] and unclear \[^{19}\] results also reported following various team-sport performances. From a chronic monitoring perspective, it has been proposed that a prolonged decrease in this ratio by more than 30% may be indicative of an overtraining state \[^{259}\]. However, conflicting evidence in the literature suggests that this ratio may be limited in determining overtraining \[^{273}\].
2.8. Perceptual Responses to Team-Sport Exercise

A simple technique to determine the internal response to a given exercise bout is to assess the athlete’s perceived exertion. In fact, ratio scales (such as the Rating of Perceived Exertion [RPE] scale) have been employed in one form or another for many years [133, 274]. The technique of multiplying the athletes’ response to the RPE scale (i.e., 0-10 score) by the duration of the activity can also provide insight into the internal training load experienced by the athlete, this is known as session RPE (or sRPE) [275]. Outcomes of validation studies comparing sRPE with other internal load measures (e.g., HR, TRIMP, etc.) suggest that it is an appropriate tool to determine internal load following team-sport exercise [18, 276, 277].

Due to the utility and cost-effectiveness of sRPE as an internal load measure, numerous studies have used it to quantify training and playing load [18, 276, 278, 279]. Moreover, important correlations have been identified between external load markers and sRPE following team-sport exercise, further highlighting the effectiveness of the simple perceptual measure [280, 281]. More recently, attention has been given to various mediators that may impact sRPE [278, 279]. These investigations emphasise the sensitivity of sRPE as a measure of internal load. For instance, aerobic fitness (i.e., VO_{2max}) has shown strong negative correlations to sRPE in elite Futsal players, while 2-km time-trial performance, playing position and experience level influence the relationship between external load and sRPE in elite Australian footballers [278, 282]. Furthermore, using motivational self-talk as a psychological intervention resulted in reductions in sRPE and improved performance during time-trial tasks [283]. However, perceptual ratings of sleep, stress, soreness and fatigue do not seem to influence sRPE in junior soccer players during sub-maximal training [284].

Consideration should be given to the appropriate collection of RPE from athletes following exercise. This includes providing verbal anchors to the athletes regarding the scale, and a simple, consistent question (e.g., “how was your workout?”) [18, 275]. Early research suggested that the timing of RPE collection should be at 30-min following the cessation of exercise, in order to negate any influence that the final performance activity may have on RPE response given by the athletes [275]. However, familiarised athletes can provide consistent responses at various time-points, from as early as 5 min and up to 24 h post exercise [285, 286].

Similar to RPE, profiling an athlete’s mood state using questionnaires can provide valuable insight into their perceptions of fatigue, soreness, stress, sleep quality and overall wellbeing [15].
While some established questionnaires exist (e.g., Profile Of Mood State), these have been criticised for their lack of practicality in everyday use for monitoring athletes, due mainly to their length \cite{170,282}. As a result, abbreviated questionnaires are suggested \cite{19,170}. There is evidence to suggest that these questionnaires are sensitive to modifications in training load, and provide a cost-effective monitoring tool that can influence training prescription \cite{19,30,287}. Furthermore, daily perceived wellness has shown strong correlations with modified activity profiles (i.e., high-intensity running distance) in elite soccer players \cite{288}. Similarly, reductions in pre-training wellness scores (i.e., worse scores) are strongly correlated with reduced player load in elite Australian football players during subsequent training sessions \cite{282}.

2.9. Conclusions

In summary, modifications to team-sport running performance appear related to a multifaceted array of physiological and psychological variables, both as an anticipatory response (i.e., perceived fatigability, such as motivation) or a causative response (i.e., performance fatigability, such as metabolites, perception of effort) to the exercise bout \cite{76}. Measurement of fatigue and pacing during team-sport matches (i.e., at half time) can be limited by the variation in match performance, the proximity of sporting arenas to laboratories, the lack of ecological validity in current externally paced match-simulation protocols, and the statistical interpretation of outcomes \cite{8,70,153,154}. As a result, a self-paced team-sport running simulation performed in a laboratory should allow researchers to elucidate further the aetiology of fatigue during team-sport matches. The ability to more reliably assess the impact of multiple matches on performance and fatigue in a short time-frame (e.g., 3-4 days) would be possible with this protocol, while reducing the inherent variability (e.g., score line, opposition) associated with match performance.
3. Chapter 3: Methodology and Design

As per university guidelines, the methods utilised within each study of this thesis are described in their entirety below. Subsequently chapters 4, 5, 6 and 7 contain the methods of each study presented according to guidelines provided by the respective journals.

3.1 Study One - A Self-Paced Intermittent Protocol on a Non-Motorised Treadmill: A Reliable Alternative to Assessing Team-Sport Running Performance.

Ten amateur team-sport athletes (20.3±1.2 y, 74.4±9.7 kg, $\dot{V}O_{2peak}$ 57.1±4.5 ml•kg$^{-1}$•min$^{-1}$) were recruited to participate in this study. All participants were required to have an aerobic capacity ($\dot{V}O_{2peak}$) ≥ 50 ml•kg$^{-1}$•min$^{-1}$ (tested during the initial laboratory visit) and be currently competing or training in team sports [e.g., soccer, Australian football, rugby, field hockey] at least three times per week. Participants were required to attend five testing sessions, involving an initial pre-test and familiarisation session, followed by four team-sport running simulations (trials 1-4), each separated by one week. Prior to each laboratory visit, participants completed a 48-h food diary and were asked to refrain from any strenuous physical activity preceding the testing day. Participants were asked to follow the same diet (as recorded in initial food diary) and exercise routine for 48 h prior to subsequent laboratory visits. Laboratory conditions were constant (21.4 ± 0.7 °C; 44.6 ± 2.9% relative humidity) and each individual was tested at the same time of day to limit diurnal fluctuations in performance.

3.1.1. Non-Motorised Treadmill Model

The treadmill used in the present study was a curved, non-motorised design (Woodway Curve 3.0, Woodway, USA). Unlike previous NMTs, the curved design allows for untethered running [66]. The static incline of the treadmill surface was set at 140 mm and 90 mm (distance from floor to the frame of the NMT) for the front and rear feet, respectively, per manufacturer specifications. The Curve 3.0 contains four load cells (on the left and right side at the front and rear of the treadmill belt) that measure vertical ground reaction force at 200 Hz, while treadmill belt speed is measured via photomicrosensors (Omron EE-SX670, Omron Corporation, Osaka, Japan) mounted on the running drum shaft. All data are collected and analysed through the manufacturer’s software (Pacer Performance System, Innervations, Australia). The aforementioned software package calculates horizontal force using the formula: horizontal force = acceleration * (body mass * belt friction), and power output was calculated via the product of horizontal force and horizontal displacement. Data were then exported to Microsoft Excel for detailed analysis of specific speed zones.
3.1.2. Protocol Development

Previous NMT-based team-sport running simulations have been developed to replicate time-motion profiles of a number of team sports (e.g., soccer, rugby league, rugby union, Australian football) [289]. These protocols achieve the desired activity profiles by prescribing running speeds based on percentage of maximal sprinting speed, requiring participants to match these speeds via visual feedback cues [66, 289].

In contrast, the protocol in the current study used visual and audible commands to direct participant locomotor speed (i.e., ‘Stand Still’, ‘Walk’, ‘Jog’, ‘Run’, ‘Sprint’), however, actual locomotor speeds were self-selected. Before commencing the protocol, participants were asked to follow visual and audible commands (as above) and were instructed that during ‘run’ periods they should be performing a ‘hard run, as if attempting to reach the next contest within a game’ and to ‘sprint maximally’ during ‘sprint’ periods. This initial guidance was provided to assist participants in differentiating between the discrete speed categories. During the sprint periods, standardised verbal encouragement was provided by the investigator. No other encouragement or feedback was provided. Our performance protocol was designed to achieve mean running velocities above the Australian football game mean (~125 m min⁻¹) [290], with the goal of creating significant physiological stress. Figure 4.1 shows a 10-min portion of the team-sport running protocol, which was repeated three times during each trial to form a 30-min performance test. Table 4.1 defines the time spent in each speed category for a 10-min block. Each 10-min block was made up of 8 min of simulated ‘on-field’ activity and a 2-min period of low activity, to mimic an Australian football interchange when the player is removed from the field of play. During these low activity periods, participants were permitted to consume water ad libitum. This duration of on-field activity and interchange period is typical of current Australian football practices [1]. The three identical 10-min blocks allow for the assessment of changes during specific time points of the activity. Furthermore, the 30-min duration of the performance test (approximately a quarter of an Australian football match, typically 4 x 30-min quarters) was deemed appropriate to assess changes in team-sport specific running performance, and has been utilised for a previous team-sport running protocol [66].

3.1.3. Testing Sessions

Visit 1 (pre-testing and familiarisation): Upon reporting to the laboratory, all participants underwent a standardised warm up, which involved 3 min of self-selected sub-maximal running
on a NMT (Woodway Curve 3.0, Woodway, USA) before completing a sequence of dynamic stretches of the major muscle groups of the lower limbs. Participants then completed an incremental motorised-treadmill (Pulsar 3p, HP Cosmos, Nussdorf-Traunstein, Germany) run to exhaustion while being monitored via open-circuit spirometry (TrueOne 2400, Parvo Medics, Utah, USA) for assessment of $\bar{V}O_2$peak. The incremental test involved two 3-min stages at 8 and 12 km•h$^{-1}$ with a grade of 0%. Thereafter, speed was increased by 1 km•h$^{-1}$ every min to 18 km•h$^{-1}$, at which point speed remained constant and grade was increased by 2% every minute until volitional exhaustion. After completing the run to exhaustion, participants rested for ~10 min before returning to the NMT to complete an initial familiarisation of the 30-min team-sport running simulation.

Visits 2-5 (reliability trials 1-4): Before completing trials 1-4, participants underwent the same standardised warm up as described above before performing a 3-min portion of the team-sport simulation, which included one sub-maximal sprint. Participants then rested for 5 min, towel dried and obtained body mass (PW-200KGL, A&D Weighing, Kensington, Australia) wearing shorts only, before commencing the 30-min team-sport running simulation.

3.1.4. Data Analysis

All variables were log transformed to reduce bias because of non-uniformity of error, and analysis was performed using a custom spreadsheet $^{[291]}$. Data were separated into locomotor zones for analysis of reliability, as defined by the speed commands described earlier, with designated standing periods removed from analysis. The inter-trial (e.g., Trial 1 v Trial 2) reliability of mean speed, mean/total distance and mean power output in all speed zones was estimated using the intra-class correlation coefficient and typical error ± 90% confidence limits (CL) expressed as a percentage [coefficient of variation (CV)]. The smallest worthwhile change (SWC), defined as the smallest change of practical importance, was calculated as $0.2 \times$ the between participant standard deviation (SD). Variables were considered capable of detecting the SWC if CV% $\leq$ SWC $^{[292]}$. Reliability was also calculated for total, maximum, and mean distance, speed, and power output per zone, and between 10-min blocks.
3.2. Study Two - Reliability of Measures of Quadriceps Muscle Function Using Magnetic Stimulation

3.2.1. Study Design

Participants attended the laboratory on 5 occasions across 2 weeks. The first 2 sessions were for familiarization and occurred during week 1 (48 hours apart). In the following week, the testing protocol was repeated for 3 consecutive days at the same time of day. Data collection was performed by the same researcher, who had approximately 300 hours of experience with the magnetic stimulation technique prior to study commencement.

3.2.2. Participants

Fifteen healthy and active men (23.5 ± 3.0 y, 181.8 ± 7.2 cm, and 80.7 ± 7.8 kg) were recruited. Participants were requested to abstain from caffeine and alcohol in the 24 hours prior to testing and throughout the testing period. Additionally, participants were asked to refrain from strenuous exercise on the day prior to initial testing and for the 3 consecutive testing days. All participants provided written informed consent, and the research was approved by the Australian Catholic University Human Research Ethics Committee in accordance with the Declaration of Helsinki.

3.2.3. Surface EMG

EMG was recorded from the vastus lateralis using surface electrodes (Duo-Trode, NAOL, New South Wales, Australia) connected directly to wireless probes that pre-amplified the signal (gain 400) and transmitted data in real-time to a wireless EMG system (Noraxon, Telemyo DTS, Arizona, USA). Data were collected at 1000 Hz through a custom software package (Labview 2013, National Instruments, Austin, TX). Prior to electrode placement, the measurement site was thoroughly prepared by shaving and abrading the skin, then cleaned with swabbing alcohol. A bipolar surface electrode was positioned on the vastus lateralis according to SENIAM guidelines, and the location was marked with indelible ink to ensure identical placement on subsequent sessions. The M-wave peak-to-peak amplitude (PPA) and duration (PPD) were measured during twitches, and the EMG signal during each maximal voluntary contraction (MVC) was quantified by using the root mean square (RMS) calculated over a 1-s period after the torque had reached a plateau (RMSMVC). During post-processing, the EMG signals were
rectified and filtered (bandwidth frequency: 10–500 Hz). The RMS\textsuperscript{MVC} was then normalized to the corresponding PPA by using the ratio RMS\textsuperscript{MVC}/PPA.

### 3.2.4. Peripheral Magnetic Stimulation

Participants lay supine on an isokinetic dynamometer (Humac Norm, Computer Sports Medicine, Inc., Massachusetts, USA) with the right thigh resting flat on the bench, the angle of the right knee set to 90° of flexion, and the right ankle fixed to the torque arm of the dynamometer just above the lateral malleolus. This knee angle lengthens the muscle, reducing the series elastic slackness that can influence control twitches.\cite{180} The participant’s hips and right leg were secured to the dynamometer using non-compliant straps to minimize body movement. A magnetic coil (D70\textsuperscript{2}, The Magstim Company Ltd, UK) powered by a magnetic stimulator (Magstim BiStim, The Magstim Company Ltd, UK) was positioned over the femoral triangle of each participant. Torque was collected at 1000 Hz via the dynamometer and synchronized with the EMG signal and timing of magnetic stimulation through the aforementioned custom software package. In order to locate the optimal stimulation position, twitches were administered at various locations in the vicinity of the femoral triangle with the stimulator intensity set to 50% of maximum output. The optimal position was defined as the location where the greatest torque and M-Wave PPA occurred from these aforementioned twitches. This coil position was marked on the participant with indelible ink for subsequent stimulations and test days.

To ensure that maximal twitches were delivered with the magnetic stimulator (i.e., plateau in twitch torque and M-wave PPA)\cite{294}, a ramp test was performed at the beginning of every session (see Figure 5.1). The ramp protocol consisted of 2 non-potentiated stimulations every 30 s at increasing intensities (50, 60, 70, 80, 90, 95, 100% of stimulator output). At completion of the ramp test, participants performed a warm-up comprising 3 isometric contractions at approximately 50, 80, and 100% MVC, separated by 60 s. At least 3 minutes after the warm-up, participants performed 2 isometric, maximal voluntary contractions (MVC) lasting 5 s, separated by 3 minutes. Participants were manually administered a single twitch (stimulator intensity of 100%) when the researcher visually identified a torque plateau during the MVC (see Figure 5.2 A and B). This twitch was used to assess the completeness of muscle activation, calculated using 4 methods (outlined below). At 5-seconds post-MVC, participants were administered a potentiated twitch.
Method 1: Central activation ratio (CAR) was calculated using the equation:[109]
\[ \text{CAR} = \frac{\text{MVC}}{\text{MVC} + \text{superimposed twitch}}. \]

Method 2: Voluntary activation was calculated using the equation: [295, 296]
\[ \%\text{VA} = (1 – \frac{\text{Superimposed Twitch}}{\text{Potentiated Twitch}}) \times 100. \]

Method 3: Voluntary activation was calculated by defining the superimposed twitch as the difference between the peak superimposed twitch force and the force averaged over 100 ms prior to that peak.[152]

Method 4: Voluntary activation was calculated when stimulation was applied prior to or following MVC; [181, 182].
\[ \%\text{VA} = (1 – \left[ \frac{\text{superimposed twitch} \times (\text{force level at stimulation}/\text{MVC force})}{\text{potentiated twitch}} \right] ) \times 100. \]

3.2.5. Rate of Torque Development

Using the aforementioned custom software package, rate of torque development (\(\Delta\text{torque}/\Delta\text{time}\)) was calculated for the potentiated stimulations, as well as during the ramp protocol. Onset of torque was defined as 5% of the peak twitch torque, and the rate was calculated from onset to peak torque output.

3.2.6. Data Analysis

The researcher was blinded to the data until data collection and analysis were completed. All raw data collected were analyzed through a custom software package (Labview 2013, National Instruments, Austin, TX) to provide all variables presented in Table 5.1, Table 5.2 and Table 5.3. All variables were log-transformed to reduce bias because of non-uniformity of error, and analysis was performed using a custom spreadsheet.[291] For clarity, all data are presented as raw values (mean ± SD) in the tables and text, unless otherwise stated. The peak of the best maximal voluntary contraction for each day was used to assess inter-day reliability. The intra-day (e.g., Trial 1 vs. Trial 2) and inter-day (e.g., Day 1 vs. Day 2) reliability of MVC, potentiated twitch, PPA, PPD, RMS_{MVC}/PPA, and twitch rate of torque development (RTD), and the various calculations of voluntary activation were estimated using the intra-class correlation coefficient [ICC (3,1)][297] and typical error ± 90% confidence limits (CL) expressed as a percentage [coefficient of variation (CV)].[297] The smallest worthwhile change (SWC), defined as the smallest change of practical importance, was calculated as \(0.2 \times \text{the between-participant standard deviation (SD)}\). Variables were considered capable of detecting the SWC if CV% ≤
Inter-day reliability was calculated for the ramp protocol at each stimulator intensity (70-100%) for torque, PPA, PPD and RTD. Confirmation of a plateau during the ramp protocol (i.e., to confirm maximal stimulation) was assumed when the change from the last 2 stimulator intensities (95-100%) of the ramp protocol were smaller than CV% for the resting twitch.

3.3. Study Three - A Self-Paced Team Sport Match Simulation Results In Reductions in Voluntary Activation and Modifications to Biological, Perceptual and Performance Measures at Half-Time, and for up to 96 Hours Post-Match

3.3.1. Experimental Approach to the Problem

Subjects were required to attend the laboratory on seven occasions over a two-week period. Week 1 consisted of two familiarization sessions separated by at least 48 hours. Week 2 consisted of a match-simulation protocol, followed by 4 consecutive days of follow-up testing. Subjects were requested to abstain from alcohol in the 24-hours prior to testing and caffeine 12-hours prior to the testing session and asked to consume a similar diet throughout the study.

3.3.2. Familiarization 1 and 2

Subjects performed a standardized warm-up including 3-min of self-paced running on the non-motorized treadmill (NMT) as well as dynamic lower-body stretches. During the first session, subjects undertook familiarization in the following order: magnetic stimulations and isometric knee extensions, countermovement jump (CMJ), squat jump (SJ), isometric mid-thigh pull (IMTP) and a 15-min portion of the non-motorized treadmill protocol. The second familiarization session began with the peak oxygen consumption ($\bar{V}O_2$peak) test (as described below). Ten minutes after the $\bar{V}O_2$peak test, subjects completed the same familiarization as session 1. Familiarization during the isometric knee extension was continued until consistent maximal contractions (i.e., plateaued torque curve) were performed. Two sessions have been shown to be suitable for this technique [299]. Equally, two sessions of running on the NMT were performed to ensure confidence on the apparatus. However, one session has been demonstrated to provide sufficient familiarization [300].
3.3.3. **Main Testing Session**

Subjects completed a self-report wellness questionnaire (as described below) prior to performing the standardized warm-up. Pre-testing included: magnetic stimulation ramp protocol, quadriceps twitch interpolation assessment, CMJ, SJ, IMTP and blood sampling. Following pre-testing, subjects towel-dried and body mass (PW-200KGL, A&D Weighing, Kensington, Australia) was obtained while wearing shorts only. Heart rate (HR) was monitored throughout the match simulation. Subjects then performed the first half (45 min) of the NMT match simulation. Within 2-min post the first half, subjects completed the quadriceps twitch interpolation assessment, followed by CMJ, SJ and IMTP (total time ~5 min). Once the half-time testing was complete, subjects had a 15-min break prior to the second half. With 5-min remaining in the half-time break, subjects completed a submaximal self-paced 3-min treadmill run as a re warm-up. Approximately 1-min prior to beginning the second half, subjects gave their RPE for the first half. At the completion of the second half, the same testing as half-time was repeated. In addition, a second blood sample was obtained. Participant RPE for the full match-simulation was obtained 15-min after completion of the second half. At 2-h post the match-simulation, subjects completed the standardized warm-up followed by the quadriceps twitch interpolation assessment, CMJ, SJ, IMTP and a blood sample.

Subjects presented to the laboratory at the same time of day to perform follow-up testing for four consecutive days following the match-simulation protocol. This included quadriceps twitch interpolation assessment, CMJ, SJ, IMTP, blood sample and self-report questionnaire.

3.3.4. **Subjects**

Twelve amateur male team-sport athletes (24.5 ± 3.9 y, 76.8 ± 5.1 kg, $\bar{V}O_{2peak}$ 52.3 ± 4.0 ml.kg$^{-1}$.min$^{-1}$) were recruited to participate in this study. These athletes had various sporting backgrounds (i.e., Australian Football, Soccer), and therefore, were experienced in completing intermittent team-sport activity. All subjects provided written informed consent and the research was approved by the University Research Ethics Committee.
3.3.5. Procedures

3.3.5.1. Peak oxygen consumption ($\dot{V}O_{2\text{peak}}$):

Subjects completed an incremental run to exhaustion on a treadmill (Pulsar 3p, HP Cosmos, Nussdorf-Traunstein, Germany) while monitored via open-circuit spirometry (TrueOne 2400, Parvo Medics, Utah, USA) for assessment of $\dot{V}O_{2\text{peak}}$ and for maximal HR using a HR monitor (Polar Team System, Polar Electro, Kempele, Finland). A run to exhaustion test was used as described previously [301], which involved initial speed being set to 10 km•h$^{-1}$ with a grade of 1% to more closely mimic overground running [302]. Thereafter, speed was increased by 1 km•h$^{-1}$ every min until volitional exhaustion.

3.3.5.2. Neuromuscular function

The method of neuromuscular function, comprising of a quadriceps twitch interpolation assessment, used in the current research has been described in detail previously and the variables associated with this method have acceptable reliability [299]. Subjects were secured in a supine position on an isokinetic dynamometer (Humac Norm, Computer Sports Medicine, Inc., Massachusetts, USA) with their right knee set to 90˚ of flexion and right ankle fixed to the torque arm of the dynamometer just above the lateral malleolus. A magnetic coil (D70, The Magstim Company Ltd, UK) powered by a magnetic stimulator (Magstim BiStim, The Magstim Company Ltd, UK) was positioned over the femoral triangle of each participant. The supine position allowed for proper placement of the magnetic coil in the femoral triangle. Torque, electromyography (EMG) signal and timing of magnetic stimulation was synchronized and collected through a custom software package (Labview 2013, National Instruments, Austin, TX).

To ensure the optimal magnetic stimulation location, twitches were administered on the femoral triangle at 50% of stimulator output to locate the highest torque and M-Wave peak-to-peak amplitude (PPA). This position was marked with indelible ink for subsequent stimulations. To confirm maximal twitches were delivered with the magnetic stimulator (i.e., plateau in twitch torque and M-wave PPA), a ramp test (consisting of two non-potentiated stimulations every 30 s at increasing intensities) was performed prior to the first testing session. Confirmation of a plateau during the ramp protocol (i.e., to confirm maximal stimulation) was assumed when the
change from the last 2 stimulator intensities (95-100%) of the ramp protocol were smaller than the CV% (4%) for the resting twitch [299].

EMG was recorded from the vastus lateralis using surface electrodes (Duo-Trode, NAOL, New South Wales, Australia) positioned according to SENIAM guidelines [303], connected directly to wireless probes that pre-amplified the signal (gain 400) and transmitted data in real-time to a wireless EMG system (Noraxon, Telemayo DTS, Arizona, USA). The measurement site was thoroughly prepared prior to collection by shaving and abrading the skin, and then cleaned with swabbing alcohol and the location was marked with indelible ink to ensure identical placement on subsequent sessions. The M-wave PPA was measured during twitches and the EMG signal during each maximal voluntary contraction (MVC) was quantified by using the root mean square (RMS) calculated over a 1-s period after the torque had reached a plateau (RMS\text{MVC}). During post-processing, the EMG signals were rectified and filtered (bandwidth frequency =10–500 Hz). The RMS\text{MVC} was then normalized to the corresponding PPA by using the ratio \text{RMS\text{MVC}}/\text{PPA}.

The assessment required subjects to perform an isometric MVC lasting 5 s. Subjects were manually administered a single twitch (stimulator intensity of 100%) at a visually identified torque plateau during the MVC. The twitch was used to assess voluntary activation (%VA). At five seconds post-MVC, subjects were administered a potentiated twitch (POT). The following equation was used to calculate %VA: %VA = (1 – Superimposed Twitch/Potentiated Twitch) x 100, where the superimposed twitch was defined as the difference between the peak superimposed twitch force and the force averaged over 100 ms prior to that peak [152].

3.3.5.3. Performance testing

Subjects performed a CMJ on a force plate sampling at 600 Hz (400 Series Force Plate, Fitness Technologies, South Australia). Subjects were instructed to maintain their hands on hips throughout the jump, and jump as high as possible [212]. Variables recorded included: jump height (cm), peak power relative to body mass (W\text{kg}^{-1}), peak force per kg (N\text{kg}^{-1}), flight time (s), and flight time: contraction time as per Cormack et al.[6]. Following the CMJ, a SJ was performed on the force plate to determine the subjects concentric-only jump performance. The subjects maintained their hands on their hips and were instructed to squat down to a self-selected depth and hold the position for three seconds prior to a maximal jump [212]. Variables
recorded included: jump height (cm), peak power relative to body mass (W·kg⁻¹), peak force per kg (N·kg⁻¹) and flight time (s).

Maximal isometric strength (N·kg⁻¹) was recorded using an isometric mid-thigh pull (IMTP) [304]. Subjects stood on the force plate and held an immovable barbell fixed at mid-thigh height. The height of the bar was adjusted for each participant to allow a hip angle of ~155-165˚ and a knee angle of 125-135˚ and kept constant throughout the testing period. Subjects wore wrist straps to assist their grip, and were instructed to pull up as hard and as fast as possible for approximately 5 s. All force-plate data was collected and analyzed using proprietary software (Ballistic Measurement System; Fitness Technology, Adelaide, Australia).

### 3.3.5.4. Biochemical analysis

An 8-mL sample of venous blood was drawn from an antecubital vein into serum-separating tubes on seven occasions (pre-, post-, 2h-post match simulation, and on days 1-4 following testing). The sample was left to clot at room temperature for 30 min before being centrifuged at 1500 G for 10 min at 4 °C. Serum samples were aliquoted into Eppendorf vials and frozen at -80 °C. The samples were returned to room temperature prior to analysis of testosterone, cortisol and uric acid (oxidative stress) using enzyme-linked immunosorbent assays (Abcam and Abnova corporations). Creatine kinase (CK) as a marker of muscle damage was determined using a Reflotron analyzer (Reflotron Plus, Bohringer-Mannheim, Indianapolis, IN, USA). The coefficient of variation (CV%) was calculated using 39 duplicate samples for creatine kinase, testosterone, cortisol and uric acid as 3.9, 4.8, 6.5 and 15.9%, respectively. The sensitivity of the testosterone assay was 0.05 ng/mL. The intra- and inter-assay reliability was <10% and <8.4% CV, respectively. The sensitivity of the cortisol assay was 1.5 ng/mL. The intra- and inter-assay reliability was <9.4% and <15% CV, respectively.

### 3.3.5.5. Perceptual response

Subjects provided a rating of perceived exertion (RPE) using Borg's category ratio 10-scale 15-min after the first half and following the match-simulation protocol [305]. Prior to the match-simulation and at the beginning of subsequent monitoring sessions, subjects were asked to complete a self-report questionnaire that assessed their fatigue, sleep quality, general muscle soreness, stress levels and mood on a five-point scale (scores of 1; poor to 5; very good), with overall well-being determined by summing the five scores [19].
3.3.5.6. **Match-simulation protocol**

Subjects performed a 90-min (two 45-min halves with 15-min rest between halves) match-simulation protocol on a non-motorized treadmill (Woodway Curve 3.0, Woodway, USA). This protocol was an extended version of a previously described, reliable protocol [300]. The protocol used visual and audible commands to direct participant locomotor speed (i.e., ‘Stand Still’, ‘Walk’, ‘Jog’, ‘Run’, ‘Sprint’), however, actual locomotor speeds were self-selected. This self-selected pacing allowed subjects to maintain their own pacing strategy throughout the match. Furthermore, the non-motorized treadmill allowed for maximal accelerations, decelerations and maximum speed running, not possible on a motorized treadmill. Modifications from the initial protocol [300] were to ensure the proportions of the match activity (e.g., run, sprint etc.) were equivalent to a soccer match. Before commencing the protocol, the visual cues and audible commands were explained to subjects and they were instructed that during ‘run’ periods they should perform a “hard run, as if attempting to reach the next contest within a game” and to “sprint maximally” during the defined sprint periods. This initial guidance was provided to assist subjects in differentiating between the discrete speed categories. Subjects were offered water on three occasions throughout each half. To allow for direct comparison with published literature, the following variables of the activity profile were assessed: total distance (TD), low-speed activity [under 14.4 km.h\(^{-1}\) (LSA)], high-speed running [over 14.4 km.h\(^{-1}\) (HSR)] and very-high speed running [over 20 km.h\(^{-1}\) (VHSR)] [306].

3.3.5.7. **Statistical Analyses**

All data were log-transformed to reduce bias because of non-uniformity of error and ES and % change ± 90% CL were calculated using a custom spreadsheet to assess the magnitude of change in all variables from initial pre-test values compared to other time points [307]. Effects of 0.2, 0.6 and > 1.2 were considered small, moderate and large, respectively. Effects of less than 0.2 were considered trivial and where the 90% CI overlapped the positive and the negative thresholds simultaneously the effect was deemed unclear [308]. Regression analysis was performed between match-simulation outcomes and performance variables. The magnitude of \( r \) ± 90% CL was classified as 0.1 to 0.3 small, 0.3 to 0.5 moderate, 0.5 to 0.7 large, 0.7 to 0.9 very large, and 0.9 to 0.99 nearly perfect [308], using an Excel spreadsheet [309].
3.4 Study 4 - Biological and Perceptual Responses to Simulated Fixture Congestion in Soccer

3.4.1. Experimental Approach to the Problem

Subjects were required to attend the laboratory on twelve occasions over a three-week period. The two initial sessions consisted of familiarisation separated by at least 48 hours. The experimental component consisted of ten consecutive days and began at least 4 days after familiarisation. This consisted of two match-simulation protocols separated by 72 h. Follow-up testing occurred between matches, and for four consecutive days after the second match. Subjects were requested to abstain from alcohol in the 24 hours and caffeine in the 12-hours prior to the testing session and asked to consume a similar diet throughout the study. No other exercise was permitted during the experimental period.

3.4.2. Familiarization 1 and 2

Subjects performed a standardized warm-up including 3-min of self-paced running on the non-motorized treadmill (NMT; Woodway Curve 3.0, Woodway, USA) as well as dynamic lower-body stretches. During the first session, subjects undertook familiarization in the following order: magnetic stimulations and isometric knee extensions, countermovement jump (CMJ), squat jump (SJ), isometric mid-thigh pull (IMTP) and a 15-min portion of the non-motorized treadmill protocol. The second familiarization session began with the peak oxygen consumption ($\dot{V}O_{2\text{peak}}$) test. At least ten minutes after the $\dot{V}O_{2\text{peak}}$ test, subjects completed the same familiarization as session 1. Familiarization during the isometric knee extension was continued until consistent maximal contractions (i.e., plateaued torque curve) were performed. Two sessions have been shown to be suitable for this technique [299]. Similarly, two sessions of running on the NMT were performed to ensure confidence on the apparatus, although it has been demonstrated that only one is required [300].

3.4.3. Simulated Match Sessions

Subjects completed a self-report wellness questionnaire (as described below) prior to performing the standardized warm-up. Pre-testing included: blood sampling, magnetic stimulation ramp protocol (match 1 only), quadriceps interpolated twitch assessment, CMJ, SJ and IMTP. Following pre-testing, subjects towel-dried and body mass (PW-200KGL, A&D
Weighing, Kensington, Australia) was obtained while wearing shorts only. Heart rate (HR) was monitored throughout the match simulation. Subjects then performed the first half (45 min) of the NMT match simulation. Within 2-min post first half, subjects completed the quadriceps twitch interpolation assessment, followed by CMJ, SJ and IMTP (total time ~5 min). Once the half-time testing was complete, subjects had a 15-min break prior to the second half. With 5 min remaining in the half-time break, subjects completed a submaximal self-paced 3-min treadmill run as a re-warm-up. Approximately 1-min prior to beginning the second half, subjects gave their RPE for the first half. At the completion of the second half, the same testing as half-time was repeated, with the addition of a blood sample. Rating of perceived exertion specific to the second half and to the full match-simulation was obtained 15-min after completion of the second half. Participants were provided with a standardized carbohydrate (1.2 g of CHO per kg of body mass) and protein (30 g) beverage to control their initial post-match meal. At 2-h post-match simulation a blood sample was taken, then subjects completed the standardized warm-up followed by the quadriceps interpolated twitch assessment, CMJ, SJ, IMTP.

Subjects presented to the laboratory at the same time of day as the start of the match simulation to perform follow-up testing for the two days between matches, and for four consecutive days following the second match-simulation protocol. This testing included a self-report questionnaire (see 3.5.5 below), blood sample, quadriceps interpolated twitch assessment, CMJ, SJ and IMTP.

3.4.4. Subjects

Eleven amateur male team-sport athletes (24.4 ± 3.8 y, 82.4 ± 8.4 kg, \( \dot{V}O_{2}\text{peak} \) 53.8 ± 3.5 ml.kg\(^{-1}\).min\(^{-1}\)) were recruited to participate in this study. These athletes had various team sport backgrounds (i.e., Australian Football, Soccer) and, therefore, were experienced in completing intermittent team-sport activity. All subjects provided written informed consent, and the research was approved by the University Research Ethics Committee.

3.4.5. Procedures

3.4.5.1. Peak oxygen consumption (\( \dot{V}O_{2}\text{peak} \)):

Subjects completed an incremental run to exhaustion on a treadmill (Pulsar 3p, HP Cosmos, Nussdorf-Traunstein, Germany) while monitored via open-circuit spirometry (TrueOne 2400,
Parvo Medics, Utah, USA) for assessment of $\dot{V}O_{2\text{peak}}$ and for maximal HR using a HR monitor (Polar Team System, Polar Electro, Kempele, Finland). A run to exhaustion test was used as described previously [301], which involved initial speed being set to 10 km•h$^{-1}$ with a grade of 1% to more closely mimic overground running [302]. Thereafter, speed was increased by 1 km•h$^{-1}$ every min until volitional exhaustion.

3.4.5.2. Neuromuscular function

The method of neuromuscular function, comprising of a quadriceps interpolated twitch interpolation assessment, used in the current research has been described in detail previously and the variables associated with this method have acceptable reliability [299]. Subjects were secured in a supine position on an isokinetic dynamometer (Biodex System 4 Quick Set, Biodex Medical Systems Inc, New York, USA) with their right knee set to 90˚ of flexion and right ankle fixed to the torque arm of the dynamometer just above the lateral malleolus. A magnetic coil (D702, The Magstim Company Ltd, UK) powered by a magnetic stimulator (Magstim BiStim, The Magstim Company Ltd, UK) was positioned over the femoral triangle of each participant. The supine position allowed for proper placement of the magnetic coil in the femoral triangle. Torque, electromyography (EMG; Noraxon, Telemyo DTS, Arizona, USA) signal and timing of magnetic stimulation was synchronized and collected through a custom software package (Labview 2013, National Instruments, Austin, TX).

To ensure the optimal magnetic stimulation location, twitches were administered on the femoral triangle at 50% of stimulator output to locate the highest torque and M-Wave peak-to-peak amplitude (PPA). This position was marked with indelible ink for subsequent stimulations. To confirm maximal twitches were delivered with the magnetic stimulator (i.e., plateau in twitch torque and M-wave PPA), a ramp test (consisting of two non-potentiated stimulations every 30 s at increasing intensities) was performed prior to the first testing session. Confirmation of a plateau during the ramp protocol (i.e., to confirm maximal stimulation) was assumed when the change from the last 2 stimulator intensities (95-100%) of the ramp protocol were smaller than the CV% (4%) for the resting twitch [299].

EMG was recorded from the vastus lateralis using surface electrodes (Duo-Trode, NAOL, New South Wales, Australia) positioned according to SENIAM guidelines [303], connected directly to wireless probes that pre-amplified the signal (gain 400) and transmitted data in real-time to a wireless EMG system (Noraxon, Telemyo DTS, Arizona, USA). The measurement site was
thoroughly prepared prior to collection by shaving and abrading the skin, and then cleaned with swabbing alcohol, and the location was marked with indelible ink to ensure identical placement on subsequent sessions. The M-wave PPA was measured during twitches, and the EMG signal during each maximal voluntary contraction (MVC) was quantified by using the root mean square (RMS) calculated over a 1-s period after the torque had reached a plateau (RMS_{MVC}). During post-processing, the EMG signals were rectified and filtered (bandwidth frequency =10–500 Hz). The RMS_{MVC} was then normalized to the corresponding PPA by using the ratio RMS_{MVC}/PPA.

The assessment required subjects to perform an isometric MVC lasting 5 s. Subjects were manually administered a single twitch (stimulator intensity of 100%) at a visually identified torque plateau during the MVC. The twitch was used to assess voluntary activation (%VA). At five seconds post-MVC, subjects were administered a potentiated twitch (POT). The following equation was used to calculate %VA: %VA = (1 – Superimposed Twitch/Potentiated Twitch) x 100, where the superimposed twitch was defined as the difference between the peak superimposed twitch force and the force averaged over 100 ms prior to that peak [152]. If the twitch was administered prior to or following the plateau in MVC, a correction was applied to the equation as per Strojnik and Komi [182].

3.4.5.3. Performance testing

Subjects performed a CMJ on a force plate sampling at 600 Hz (400 Series Force Plate, Fitness Technologies, South Australia). Subjects were instructed to maintain their hands on hips throughout the jump, and jump as high as possible [212]. Variables recorded included: jump height (cm), peak power relative to body mass (W·kg⁻¹), peak force per kg (N·kg⁻¹), flight time (s), and flight time:contraction time [6]. Following the CMJ, a SJ was performed on the force plate to determine concentric-only jump performance. The subjects maintained their hands on their hips and were instructed to squat down to a self-selected depth and hold the position for three seconds prior to a maximal jump [212]. Variables recorded included: jump height (cm), peak power relative to body mass (W·kg⁻¹), peak force per kg (N·kg⁻¹) and flight time (s).

Maximal isometric strength (N·kg⁻¹) was recorded using an IMTP [304]. Subjects stood on the force plate and held an immovable barbell fixed at mid-thigh height. The height of the bar was adjusted for each participant to allow a hip angle of 155-165° and a knee angle of 125-135° and kept constant throughout the testing period. Subjects wore wrist straps to assist their grip, and
were instructed to pull up as hard and as fast as possible for approximately 5 s. All force-plate
data were collected and analyzed using proprietary software (Ballistic Measurement System;
Fitness Technology, Adelaide, Australia).

3.4.5.4. Biochemical analysis

An 8-mL sample of venous blood was drawn from an antecubital vein into serum-separating
tubes on ten occasions (pre-, post-, 2h-post match simulations, every other day during the
experimental period). The sample was left to clot at room temperature for 30 min before being
centrifuged at 1500 G for 10 min at 4 °C. Serum samples were aliquoted into Eppendorf vials
and frozen at -80 °C. The samples were returned to room temperature prior to analysis of uric
acid and creatine kinase via enzymatic assay, and testosterone and cortisol via immunoassay
(Siemens Healthcare Diagnostics). The coefficient of variation (CV%) was < 1.3%, < 4.2%, <
20% and < 6% for uric acid, creatine kinase, testosterone and cortisol, respectively.

3.4.5.5. Perceptual response

Participants provided a rating of perceived exertion (RPE) using Borg's category ratio 10-scale
at 15-min after the first half and 15 min following the match-simulation protocol [305]. Post-
match, participants were asked to independently rate their RPE for the second half and for the
whole match.

Prior to the match-simulation and at the beginning of subsequent monitoring sessions, subjects
were asked to complete a self-report questionnaire that assessed their fatigue, sleep quality,
general muscle soreness, stress levels and mood on a five-point scale (scores of 1; poor to 5;
very good), with overall well-being determined by summing the five scores [19].

3.4.5.6. Match-simulation protocol

Subjects performed a 90-min (two 45-min halves with 15-min rest between halves) match-
simulation protocol on a curved non-motorized treadmill (Woodway Curve 3.0, Woodway,
USA). This protocol was an extended version of a previously described, reliable protocol [300].
The protocol used visual and audible commands to direct participant locomotor speed (i.e.,
‘Stand Still’, ‘Walk’, ‘Jog’, ‘Run’, ‘Sprint’), however, actual locomotor speeds were self-
selected. This self-selected pacing allowed subjects to maintain their own pacing strategy throughout the match. Furthermore, the non-motorized treadmill allowed for maximal accelerations, decelerations and maximum speed running, not possible on a motorized treadmill. Modifications from the initial protocol [300] were to ensure the proportions of the match activity (e.g., run, sprint, etc.) were equivalent to a soccer match. Before commencing the protocol, the visual cues and audible commands were explained to subjects and they were instructed that during ‘run’ periods they should perform a “hard run, as if attempting to reach the next contest within a game” and to “sprint maximally” during the defined sprint periods. This initial guidance was provided to assist subjects in differentiating between the discrete speed categories. Subjects were offered water on three occasions throughout each half. To allow for direct comparison with published literature, the following variables of the activity profile were assessed: total distance (TD), low-speed activity [under 14.4 km.h\(^{-1}\) (LSA)], high-speed running [14.4 km.h\(^{-1}\) to 20 km.h\(^{-1}\) (HSR)] and very-high speed running [over 20 km.h\(^{-1}\) (VHSR)] [306].

3.4.5.7. Statistical Analyses

All data were log-transformed to reduce bias because of non-uniformity of error and effect size (ES) and % change ± 90% confidence limits (CL) were calculated using a custom spreadsheet to assess the magnitude of change in all variables from initial pre-test values compared to other time points [307]. Effects of 0.2, 0.6 and > 1.2 were considered small, moderate and large, respectively. Effects of less than 0.2 were considered trivial, and where the 90% CL overlapped the positive and the negative thresholds simultaneously the effect was deemed unclear [308]. Regression analysis was performed between match-simulation outcomes and physical qualities. The magnitude of \( r \pm 90\% \) CL was classified as 0.1 to 0.3 small, 0.3 to 0.5 moderate, 0.5 to 0.7 large, 0.7 to 0.9 very large, and 0.9 to 0.99 nearly perfect [308], using an Excel spreadsheet [309].

Publication statement:

This chapter is comprised of the following paper published in the *Journal of Sports Science and Medicine*.

4.1. Linking Paragraph

Competitive team sport matches provide the most ecologically-valid method for assessing within- and post-match responses to match play. However, contextual variation in competitive matches can limit experimental control. Hence, match simulations are used to overcome this issue, whilst also allowing more mechanistic, laboratory-based measures to be collected. As outlined in section 2.3, the externally-paced nature of current match-simulation protocols, as well as the apparatus they are performed on, may limit their ability to accurately simulate team-sport running. Recently, manufacturers have developed a curved, non-motorised treadmill that allows untethered running. Unlike previous flat, tethered versions, this curved treadmill permits performance of more realistic accelerations and decelerations. Chapter 4 aimed to develop and assess the reliability of a completely self-paced match simulation on a curved non-motorised treadmill.
4.2. Abstract

This study assessed the reliability of a ‘self-paced’ 30-min, team-sport running protocol on a Woodway Curve 3.0 non-motorised treadmill (NMT). Ten male team-sport athletes (20.3±1.2 y, 74.4±9.7 kg, $\dot{V}O_{2\text{peak}}$ 57.1±4.5 ml•kg⁻¹•min⁻¹) attended five sessions ($\dot{V}O_{2\text{peak}}$ testing + familiarisation; four reliability trials). The 30-min protocol consisted of three identical 10-min activity blocks, with visual and audible commands directing locomotor activity; however, actual speeds were self-selected by participants. Reliability of variables was estimated using typical error ± 90% confidence limits expressed as a percentage [coefficient of variation (CV)] and intraclass correlation coefficient. The smallest worthwhile change (SWC) was calculated as $0.2 \times$ between participant standard deviation. Peak/mean speed and distance variables assessed across the 30-min protocol exhibited a CV < 5%, and < 6% for each 10-min activity block. All power variables exhibited a CV < 7.5%, except walking (CV 8.3-10.1%). The most reliable variables were maximum and mean sprint speed (CV < 2%). All variables produced a CV% greater than the SWC. A self-paced, team-sport running protocol performed on a NMT produces reliable speed/distance and power data. Importantly, a single familiarisation session allowed for adequate test-retest reliability. The self-paced design provides an ecologically-valid alternative to externally-paced team-sport running simulations.

Keywords: Exercise test; Athletic Performance; Running; Reproducibility of Results
4.3. Introduction

Running performance in team sports has been shown to influence overall team success\cite{39, 140, 141}. The activity profile within team sports consists of periods of high intensity running, interspersed with lower intensity activity and/or complete rest\cite{143}. Therefore, the physiological determinants of team-sport running performance differ somewhat from traditional endurance exercise. As an alternative to more traditional endurance tests, a number of high-intensity intermittent performance tests have been developed to assess running performance specific to team sports\cite{310}. While these tests provide greater specificity when testing team-sport athletes, most do not incorporate the wide range of locomotor activities experienced in team-sport competition (i.e., walking to sprinting). Furthermore, the majority of current high-intensity, intermittent running performance tests are externally paced (e.g. shuttle speeds guided by sound, running speeds guided by visual feedback), whereas locomotor speeds during team-sport competition are determined by the individual athlete, dependent on game situations.

The use of non-motorised treadmills (NMT)\cite{68} has allowed for the development of simulated team-sport running protocols that mimic team-sport running (i.e., rapid speed changes) in a controlled environment in which different performance variables (e.g., speed, distance, power) can be systematically measured\cite{311}. Assessing the reliability of these protocols is an important consideration for researchers and practitioners in determining the smallest practically important change that may be detected following training interventions\cite{66, 292}. Original NMT models (e.g., Woodway Force, Woodway, USA) require runners to wear a tether belt around the waist and be anchored behind, allowing them to overcome the inertia of the treadmill belt to perform locomotor activities. Recently, a curved NMT has been manufactured (Woodway Curve 3.0., Woodway, USA) allowing participants to complete locomotor tasks without being anchored via a waist tether. Additionally, the curved NMT has shown good reliability and validity during short duration (30 s) sprint testing\cite{312, 313}.

While this technology provides a promising tool to assess team-sport specific running performance, the reliability of these measures collected on a Woodway Curve 3.0 NMT has not been reported. To date, all published, treadmill-based team-sport running simulation protocols\cite{66, 289} use externally-paced movement velocities (e.g., percentage of maximal sprinting speed), or a very small portion of self-selected velocity (2.7% of total activity)\cite{69}, in order to assess team-sport specific running performance. As the self-paced nature of team-sports may have a significant impact on movement strategies adopted throughout a game\cite{163}, internally paced
performance tests may provide a more ecologically valid assessment tool than externally paced alternatives. Although some partial or completely self-paced, field-based team-sport running tests exist \[62, 314\], these do not allow for the detailed measurement of variables such as power output. Therefore, the purpose of this study was to assess the reliability of a self-paced team-sport running protocol on the Woodway Curve 3.0 NMT. A secondary purpose was to assess the number of familiarisation sessions needed to produce reliable data.

**4.4. Methods**

Ten amateur team-sport athletes (20.3±1.2 y, 74.4±9.7 kg, \(\dot{V}O_{2\text{peak}} 57.1±4.5\ \text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}\)) were recruited to participate in this study. All participants were required to have an aerobic capacity (\(\dot{V}O_{2\text{peak}}\)) ≥ 50 ml\cdot kg^{-1}\cdot min^{-1} (tested during the initial laboratory visit) and be currently competing or training in team sports [e.g., soccer, Australian football, rugby, field hockey] at least three times per week. Participants were required to attend five testing sessions, involving an initial pre-test and familiarisation session, followed by four team-sport running simulations (trials 1-4), each separated by one week. Prior to each laboratory visit, participants completed a 48-h food diary and were asked to refrain from any strenuous physical activity preceding the testing day. Participants were asked to follow the same diet (as recorded in initial food diary) and exercise routine for 48 h prior to subsequent laboratory visits. Laboratory conditions were constant (21.4 ± 0.7 °C; 44.6 ± 2.9% relative humidity) and each individual was tested at the same time of day to limit diurnal fluctuations in performance.

**4.4.1. Non-Motorised Treadmill Model**

The treadmill used in the present study was a curved, non-motorised design (Woodway Curve 3.0, Woodway, USA). Unlike previous NMTs, the curved design allows for untethered running \[66\]. The static incline of the treadmill surface was set at 140 mm and 90 mm (distance from floor to the frame of the NMT) for the front and rear feet, respectively, per manufacturer specifications. The Curve 3.0 contains four load cells (on the left and right side at the front and rear of the treadmill belt) that measure vertical ground reaction force at 200 Hz, while treadmill belt speed is measured via photomicrosensors (Omron EE-SX670, Omron Corporation, Osaka, Japan) mounted on the running drum shaft. All data are collected and analysed through the manufacturer’s software (Pacer Performance System, Innervations, Australia). The aforementioned software package calculates horizontal force using the formula: horizontal force = acceleration * (body mass * belt friction), and power output was calculated via the
product of horizontal force and horizontal displacement. Data were then exported to Microsoft Excel for detailed analysis of specific speed zones.

4.4.2. Protocol Development

Previous NMT-based team-sport running simulations have been developed to replicate time-motion profiles of a number of team sports (e.g., soccer, rugby league, rugby union, Australian football) [289]. These protocols achieve the desired activity profiles by prescribing running speeds based on percentage of maximal sprinting speed, requiring participants to match these speeds via visual feedback cues [66, 289].

In contrast, the protocol in the current study used visual and audible commands to direct participant locomotor speed (i.e., ‘Stand Still’, ‘Walk’, ‘Jog’, ‘Run’, ‘Sprint’), however, actual locomotor speeds were self-selected. Before commencing the protocol, participants were asked to follow visual and audible commands (as above) and were instructed that during ‘run’ periods they should be performing a ‘hard run, as if attempting to reach the next contest within a game’ and to ‘sprint maximally’ during ‘sprint’ periods. This initial guidance was provided to assist participants in differentiating between the discrete speed categories. During the sprint periods, standardised verbal encouragement was provided by the investigator. No other encouragement or feedback was provided. Our performance protocol was designed to achieve mean running velocities above the Australian football game mean (~125 m-min⁻¹) [290], with the goal of creating significant physiological stress. Figure 4.1 shows a 10-min portion of the team-sport running protocol, which was repeated three times during each trial to form a 30-min performance test. Table 4.1 defines the time spent in each speed category for a 10-min block. Each 10-min block was made up of 8 min of simulated ‘on-field’ activity and a 2-min period of low activity, to mimic an Australian football interchange when the player is removed from the field of play. During these low activity periods, participants were permitted to consume water ad libitum. This duration of on-field activity and interchange period is typical of current Australian football practices [1]. The three identical 10-min blocks allow for the assessment of changes during specific time points of the activity. Furthermore, the 30-min duration of the performance test (approximately a quarter of an Australian football match, typically 4 x 30-min quarters) was deemed appropriate to assess changes in team-sport specific running performance, and has been utilised for a previous team-sport running protocol [66].
Figure 4.1: A ten-minute portion of the self-paced match-simulation protocol. This 10-min period was repeated three times to make up the complete 30-min protocol. Participants self-selected their chosen running speeds. The area high-lighted in grey depicts a period of ‘low’ activity, simulating a rest period (interchange) common in Australian Football. Participants were permitted to consume water during this period.
Table 4.1: The speed commands (W = walk, St = stand, R = run, J = jog, Sp = sprint) and time spent (s) in each speed band for a ten-minute portion of the self-paced team-sport running simulation. This 10-min period was repeated three times, comprising the complete 30-min protocol.

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53
4.4.3. Testing Sessions

Visit 1 (pre-testing and familiarisation): Upon reporting to the laboratory, all participants underwent a standardised warm up, which involved 3 min of self-selected sub-maximal running on a NMT (Woodway Curve 3.0, Woodway, USA) before completing a sequence of dynamic stretches of the major muscle groups of the lower limbs. Participants then completed an incremental motorised-treadmill (Pulsar 3p, HP Cosmos, Nussdorf-Traunstein, Germany) run to exhaustion while being monitored via open-circuit spirometry (TrueOne 2400, Parvo Medics, Utah, USA) for assessment of \( \dot{V}O_{2\text{peak}} \). The incremental test involved two 3-min stages at 8 and 12 km•h\(^{-1}\) with a grade of 0%. Thereafter, speed was increased by 1 km•h\(^{-1}\) every min to 18 km•h\(^{-1}\), at which point speed remained constant and grade was increased by 2% every minute until volitional exhaustion. After completing the run to exhaustion, participants rested for ~10 min before returning to the NMT to complete an initial familiarisation of the 30-min team-sport running simulation.

Visits 2-5 (reliability trials 1-4): Before completing trials 1-4, participants underwent the same standardised warm up as described above before performing a 3-min portion of the team-sport simulation, which included one sub-maximal sprint. Participants then rested for 5 min, towel dried and obtained body mass (PW-200KGL, A&D Weighing, Kensington, Australia) wearing shorts only, before commencing the 30-min team-sport running simulation.

4.4.4. Data Analysis

All variables were log transformed to reduce bias because of non-uniformity of error, and analysis was performed using a custom spreadsheet \[291\]. Data were separated into locomotor zones for analysis of reliability, as defined by the speed commands described earlier, with designated standing periods removed from analysis. The inter-trial (e.g., Trial 1 v Trial 2) reliability of mean speed, mean/total distance and mean power output in all speed zones was estimated using the intra-class correlation coefficient and typical error ± 90% confidence limits (CL) expressed as a percentage [coefficient of variation (CV)]. The smallest worthwhile change (SWC), defined as the smallest change of practical importance, was calculated as 0.2 × the between participant standard deviation (SD). Variables were considered capable of detecting the SWC if CV% ≤ SWC \[292\]. Reliability was also calculated for total, maximum, and mean distance, speed, and power output per zone, and between 10-min blocks.
4.5. **Results**

Table 4.1 and Table 4.2 display mean ±SD, SWC, CV% ± 90% CL, and percentage change in mean for distance and speed covered across each trial (Trials 1-4) and separated for 10-min blocks, respectively. All variables produced a CV% greater than the SWC.

4.5.1. **Speed and Distance Reliability**

The most reliable variables were maximum speed and mean sprint speed for the entire trial (CV 1.8% and 1.9%, respectively). The least reliable of all variables was the inter-trial jogging distance/mean speed of Block 3 (CV 5.7%). The range of CV% for all variables between trials 2-1 was 1.8 to 6.8%, similar to trials 3-2 (CV 1.8 to 4.9%) and 4-3 (CV 2.1 to 5.7%).

4.5.2. **Power Reliability**

Overall, mean power output during sprint periods was the most reliable power measure (CV 2.7%). Mean and between block power output during walking were the least reliable measures (range CV 8.3 – 10.1%). All other power output variables displayed a CV% < 7.5%.
Table 4.2: Reliability of distance (and speeds) across team-sport simulation trials and activity blocks within trials.

<table>
<thead>
<tr>
<th>Block</th>
<th>Sprint Distance (m)</th>
<th>Running Distance (m)</th>
<th>Jogging Distance (m)</th>
<th>Walk Distance (m)</th>
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<td>Mean Distance (m)</td>
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<td></td>
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<td>Avg. % Change in Mean</td>
<td>Avg. SWC (%)</td>
<td>Avg. ICC [%] 90 CL</td>
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<td>0.31 1.2</td>
<td>0.31 1.2</td>
<td>0.31 1.2</td>
<td>0.30 1.2</td>
</tr>
</tbody>
</table>

Data presented are mean ± SD for all variables, CV (90% CL) for both distance and speed, average percent change in mean, average SWC, and average ICC (90% CL). Also presented are mean ± SD speeds for each block. CV: coefficient of variation; CL: confidence limit; SWC: smallest worthwhile change; ICC: intraclass correlation coefficient.
Table 4.3: Reliability of mean power output across team-sport simulation trials and activity blocks within trials.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Mean Sprint Power (W)</th>
<th>Mean Power (W)</th>
<th>CV [%] 90 CL</th>
<th>Avg. % Change in Mean</th>
<th>Avg. SWC (%)</th>
<th>Avg. ICC [%] 90 CL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>297 ± 18</td>
<td>301 ± 20</td>
<td>303 ± 14</td>
<td>3.6 (2.6-6.0)</td>
<td>5.1 (4.1-6.9)</td>
<td>0.15 (0.61-1.02)</td>
</tr>
<tr>
<td></td>
<td>269 ± 19</td>
<td>299 ± 19</td>
<td>5.9 (4-3.9-9.9)</td>
<td>4.6 (3.9-9.1)</td>
<td>5.4 (3.9-9.1)</td>
<td>0.13 (0.60-1.00)</td>
</tr>
<tr>
<td>2</td>
<td>290 ± 16</td>
<td>289 ± 24</td>
<td>288 ± 17</td>
<td>4.7 (3.4-7.8)</td>
<td>4.6 (3.7-6.3)</td>
<td>0.06 (0.56-0.94)</td>
</tr>
<tr>
<td></td>
<td>291 ± 17</td>
<td>290 ± 19</td>
<td>4.6 (3.3-7.7)</td>
<td>4.6 (3.4-7.7)</td>
<td>4.6 (3.4-7.7)</td>
<td>0.06 (0.56-0.94)</td>
</tr>
<tr>
<td>3</td>
<td>285 ± 17</td>
<td>278 ± 15</td>
<td>2865 ± 14</td>
<td>4.3 (3.2-7.3)</td>
<td>4.2 (3.3-5.7)</td>
<td>0.22 (0.52-0.87)</td>
</tr>
<tr>
<td></td>
<td>288 ± 24</td>
<td>284 ± 18</td>
<td>4.8 (3.5-8.0)</td>
<td>3.2 (2.3-5.3)</td>
<td>4.2 (3.3-5.7)</td>
<td>0.22 (0.52-0.87)</td>
</tr>
<tr>
<td>Mean Sprint Power (W)</td>
<td>297 ± 18</td>
<td>301 ± 20</td>
<td>303 ± 14</td>
<td>3.6 (2.6-6.0)</td>
<td>5.1 (4.1-6.9)</td>
<td>0.15 (0.61-1.02)</td>
</tr>
<tr>
<td></td>
<td>269 ± 19</td>
<td>299 ± 19</td>
<td>5.9 (4-3.9-9.9)</td>
<td>4.6 (3.9-9.1)</td>
<td>5.4 (3.9-9.1)</td>
<td>0.13 (0.60-1.00)</td>
</tr>
<tr>
<td>Mean Running Power (W)</td>
<td>146 ± 25</td>
<td>137 ± 23</td>
<td>133 ± 24</td>
<td>4.4 (3.2-7.3)</td>
<td>4.9 (3.5-8.1)</td>
<td>0.31 (0.30-0.50)</td>
</tr>
<tr>
<td></td>
<td>137 ± 24</td>
<td>133 ± 24</td>
<td>133 ± 24</td>
<td>4.4 (3.2-7.3)</td>
<td>4.9 (3.5-8.1)</td>
<td>0.31 (0.30-0.50)</td>
</tr>
<tr>
<td></td>
<td>128 ± 25</td>
<td>128 ± 25</td>
<td>128 ± 25</td>
<td>4.4 (3.2-7.3)</td>
<td>4.9 (3.5-8.1)</td>
<td>0.31 (0.30-0.50)</td>
</tr>
<tr>
<td></td>
<td>126 ± 23</td>
<td>127 ± 24</td>
<td>127 ± 24</td>
<td>4.4 (3.2-7.3)</td>
<td>4.9 (3.5-8.1)</td>
<td>0.31 (0.30-0.50)</td>
</tr>
<tr>
<td>Mean Jogging Power (W)</td>
<td>112 ±17</td>
<td>111 ±17</td>
<td>116 ±18</td>
<td>5.7 (4.1-9.5)</td>
<td>7.1 (4.9-8.4)</td>
<td>0.02 (0.32-0.54)</td>
</tr>
<tr>
<td></td>
<td>110 ±17</td>
<td>110 ±17</td>
<td>110 ±17</td>
<td>5.7 (4.1-9.5)</td>
<td>7.1 (4.9-8.4)</td>
<td>0.02 (0.32-0.54)</td>
</tr>
<tr>
<td></td>
<td>108 ±15</td>
<td>108 ±18</td>
<td>108 ±18</td>
<td>5.7 (4.1-9.5)</td>
<td>7.1 (4.9-8.4)</td>
<td>0.02 (0.32-0.54)</td>
</tr>
<tr>
<td>Mean Walk Power (W)</td>
<td>41 ± 7</td>
<td>40 ± 7</td>
<td>39 ± 6</td>
<td>6.8 (4.9-11.5)</td>
<td>6.5 (5.8-9.9)</td>
<td>0.03 (0.34-0.57)</td>
</tr>
<tr>
<td></td>
<td>40 ± 7</td>
<td>40 ± 7</td>
<td>39 ± 6</td>
<td>6.8 (4.9-11.5)</td>
<td>6.5 (5.8-9.9)</td>
<td>0.03 (0.34-0.57)</td>
</tr>
<tr>
<td></td>
<td>38 ± 6</td>
<td>37 ± 6</td>
<td>37 ± 6</td>
<td>6.8 (4.9-11.5)</td>
<td>6.5 (5.8-9.9)</td>
<td>0.03 (0.34-0.57)</td>
</tr>
</tbody>
</table>

Data presented are mean ± SD for all variables, CV (90% CL) for both distance and speed, average percent change in mean, average SWC, and average ICC (90% CL). CV: coefficient of variation; CL: confidence limit; SWC: smallest worthwhile change; ICC: intraclass correlation coefficient.
4.6. Discussion

To our knowledge, this is the first study to assess the reliability of entirely self-paced team-sport running, incorporating a spectrum of running intensities, on a Woodway Curve 3.0 NMT. Previous treadmill-based team-sport running protocols utilise external pacing, by asking participants to achieve a prescribed percentage of maximal sprinting speed\(^\text{[66, 67, 69]}\) or a speed relating to a percentage of VO\(_{2\text{max}}\)\(^{[64]}\). Some externally-paced team-sport running simulations have been performed on motorised treadmills and, thus, are limited by the maximal speed of the treadmill (generally 25 km\(\cdot\)h\(^{-1}\)) and the inability for the treadmill to change speed quickly\(^{[38, 65]}\). The use of NMTs allows for more rapid speed changes and a maximal speed limited only by the athlete’s ability. For this reason, research using NMTs has gained popularity to better emulate team-sport running\(^{[5, 55, 69, 315]}\). Previous investigations have shown good reliability for distance covered in all speed bands (CV ~2-5\%)\(^{[55, 69]}\) during NMT team-sport running protocols. However, all speeds were externally paced; therefore, good reliability for distance covered is not unexpected. In the present work with a self-paced running protocol, we report similar reliability for the distance variables (see Table 4.3, mean CV < 6\%), highlighting the ability for athletes to repeatedly ‘self-select’ a consistent locomotor pace based on simple instruction. A recent study which incorporated periods of variable running distance (i.e., self-paced) during a soccer-specific NMT simulation\(^{[69]}\) reported better reliability (CV 1.4\%) in comparison to the ‘running’ periods of our study (mean CV 4.4\%). However, the variable running distance accounted for only 2.7\% of the entire protocol, while the entire team-sport running simulation in the present study was self-paced.

Although previous research using team-sport running simulation protocols on a NMT recommends a minimum of two familiarisation sessions\(^{[55, 67, 69]}\), our data indicate that participants were familiarised following trial 1, with CV < 5\% across all speed/distance variables (Table 4.2) between trials 1 and 2. Mean CV\% for maximal and mean sprint speed, potentially the most difficult movement speed to complete on the NMT, was the lowest for any variable measured (CV 1.8\% and 1.9\%, respectively). This compares well with other externally paced team-sport running simulations performed on a NMT, which present maximal sprinting speed reliability of CV ~1.3\%\(^{[55]}\), and CV 4.5\%\(^{[69]}\). Furthermore, the reliability obtained in a specific repeat sprint test ranged from CV 0.8 to 1.5\%\(^{[316]}\), which also compares well to the present work.
All speed/distance variables assessed in this study demonstrated high reliability, exhibiting CVs < 6%. All power output variables, except walking, returned CVs < 7.5%. However, all CV% were greater than the SWC, and therefore were not capable of detecting the SWC. Our analysis also shows high reliability for total distance (CV 2.7%). In comparison, a 60-min self-paced test on a motorised treadmill with trained runners presented similar reliability for total distance (CV 2.7%) \(^{[317]}\). Similarly, trained female cyclists performing a 60-min cycle-ergometer test demonstrated a CV of 2.7% for mean power output across the whole test \(^{[318]}\). As speed is not generally measured during ergometer cycling, power output in this instance provides a surrogate for speed, as the two are very closely related in a controlled environment \(^{[319]}\). Importantly, these two comparative studies did not require changes in speed as demanded in the present study. This indicates that, even with changes in speed during a self-paced team-sport running simulation protocol, athletes are able to consistently repeat their performance across testing sessions.

The CV for mean power output (2.7%) across the 6-s sprints within the team-sport running protocol was the most reliable power measure, while peak power output, and mean running/jogging and peak sprint power were all similar (CV ~6%). Previous research assessing peak power reliability on an NMT has reported CVs of 7.9% \(^{[315]}\) and 9.0% \(^{[66]}\). However, the latter study analysed sprinting reliability via a separate peak sprint test, while the former, as in the present study, assessed sprinting reliability throughout the entire protocol. The CV%, coupled with the SWC, can be used to estimate sample sizes required for prospective studies using the equation proposed by Hopkins: \(^{[297]}\) \(N \approx 8 \times CV^2/d^2\) where \(d = SWC\). For example, to detect a SWC of 2% in total sprint distance requires a sample size of 23, while peak power output (SWC = 2.27%) would require 60 participants. Previous research using an externally paced protocol on an NMT \(^{[315]}\) calculated required sample sizes of 13 and 56 for the above variables, respectively, using the same methods.

The intraclass correlation coefficient (ICC), (see Table 4.2 and Table 4.3) was high (greater than 0.8) at all speeds and distances. This is similar to externally paced team-sport running simulations on a flat NMT \(^{[66, 69]}\). Power output displayed lower ICCs (0.37 to 0.76) compared to other NMT literature \(^{[66, 313]}\). However, as previously mentioned, these studies assessed reliability from sprinting in isolation, not during a long intermittent team-sport simulation. Furthermore, these lower ICCs may be a reflection of the homogeneity of the participant group rather than error in the measurement \(^{[320]}\).
This curved NMT belt differs from the flat belt, tethered version in previous team-sport running simulations (Woodway Force, Woodway, USA) [66, 67, 69], and may alter running ergonomics when compared to overground running. However, to date, no research has assessed potential changes in running ergonomics on the Woodway Curve 3.0 NMT. A further limitation to the current protocol is the lack of team-sport specific actions (i.e., jumping, changing direction, kicking, etc.) [5, 240].

4.7. Conclusion

This work shows that a team-sport running simulation protocol that is entirely self-paced presents reliability similar to that of externally-paced team-sport running simulations. Moreover, with as little as one familiarisation session on the Woodway Curve NMT, team-sport athletes can reliably reproduce self-selected distances/speeds across a range of locomotor commands. Given the self-paced nature of the protocol in the present study, this and similar self-paced curved NMT protocols may provide a more ecologically valid, laboratory-based performance test than externally-paced alternatives. However, as the CV% exceeds the SWC, small but meaningful changes may not be detected with this test. As a result, practitioners should ensure changes exceed the CV% to declare a meaningful change.

Key Points:

- Self-paced team-sport running protocols on a curved NMT that closely match the locomotor demands of competition deliver reliable test-retest measures of speed, distance and power.
- Such protocols may be sensitive to changes in running profile following an intervention that may not be detectable during externally-paced protocols.
- One familiarisation session is adequate to ensure test-retest reliability.
4.8. Addendums Following Review

- **Results:** The following data represents the reliability of peak sprinting speed and total distance covered during the match simulation protocol.

Table 4.2: Reliability of distance (and speeds) across team-sport simulation trials and activity blocks within trials.

<table>
<thead>
<tr>
<th>Trial</th>
<th>CV [%] 90 CL</th>
<th>Avg. % Change in Mean</th>
<th>Avg. SWC [%] 90 CL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2-1</td>
<td>2-1</td>
<td>0.19</td>
</tr>
<tr>
<td>2</td>
<td>3-2</td>
<td>1.1</td>
<td>0.91</td>
</tr>
<tr>
<td>3</td>
<td>4-3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Trial</th>
<th>CV [%] 90 CL</th>
<th>Avg. % Change in Mean</th>
<th>Avg. SWC [%] 90 CL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2-1</td>
<td>2-1</td>
<td>0.19</td>
</tr>
<tr>
<td>2</td>
<td>3-2</td>
<td>1.1</td>
<td>0.91</td>
</tr>
<tr>
<td>3</td>
<td>4-3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **Conclusion:** Practitioners and researchers should note that the reliability of the self-paced match simulation performed on a curved, non-motorised treadmill is more reliable for speed/distance variables than power variables. However, the interpretation of individual responses should be made taking into account the specific CV% for each variable.
5. Chapter 5: Study Two - Reliability of Measures of Quadriceps Muscle Function Using Magnetic Stimulation

Publication statement:

This chapter is comprised of the following paper published in *Muscle and Nerve*

5.1. **Linking Paragraph**

The self-paced match simulation developed in Study 1 provides an avenue for laboratory-based assessments to be undertaken within- and post-match. A principal measure for the direct assessment of post-match fatigue is the interpolated twitch technique. This laboratory-based measure uses an electrical or magnetic current to superimpose a stimulation to the nerve innervating a maximally contracting muscle group. Calculations based on the resultant torque and electromyography data can provide insight into central and peripheral fatigue. Electrical stimulation is often used; although magnetic stimulation has been validated as a less painful alternative. Therefore, magnetic stimulation may be favoured for certain populations, or during periods of repeat stimulations. However, the reliability of magnetic stimulation has not been determined within and across multiple days. Chapter 5 aimed to establish the intra- and inter-day reliability of magnetic stimulation to the femoral nerve for assessing central and peripheral fatigue.
5.2. Abstract

Magnetic stimulation can be used to assess muscle function by calculating voluntary activation using an interpolated twitch during maximal voluntary contractions (MVC) and control twitches to potentiated muscle. We assessed the reliability of torque, electromyography (EMG), and voluntary activation variables. Fifteen men completed 5 testing sessions (2 familiarization; 3 reliability trials) to assess quadriceps femoris muscle function. Intra- and inter-day reliability of torque and EMG variables were estimated using typical error ± 90% confidence limits expressed as a percentage [coefficient of variation (CV)] and the intraclass correlation coefficient. The smallest worthwhile change was calculated as 0.2 x between-participant SD. Intra- and inter-day torque variables for MVC were reliable (CV<4%, ICC 0.98, and <5%, 0.99 respectively). EMG variables were less reliable than torque variables, with CVs ranging from 7–18%. Magnetic stimulation of the femoral nerve provides a reliable method for assessing muscle function.

**Key Words:** Skeletal muscle, magnetic stimulation, reliability, interpolated twitch, torque, EMG
5.3. Introduction

Understanding the contribution of central and peripheral factors \cite{81} of force production can be beneficial for a variety of reasons. For instance, identifying decrements in force output and the contribution of voluntary activation (level of voluntary drive during an effort) \cite{81} post-operatively may influence the prescription of specific rehabilitation regimens \cite{321}. Similarly, understanding the roles of central and peripheral fatigue following intense exercise may modify recovery strategies in athletes \cite{172}. Morphological (e.g., muscle fiber type and cross sectional area) and neuromuscular (e.g., motor unit recruitment and firing frequency) factors are responsible for maximal force production in skeletal muscle \cite{322}, both contributing to voluntary muscle activation.

To assess muscle activation, voluntary activation (%VA) and the central activation ratio (CAR) can be calculated \cite{117, 118, 323}. Performing such a calculation involves superimposing a stimulation to the motor nerve innervating a muscle group during a maximal voluntary contraction (MVC) and comparing the size of the superimposed twitch increment to a potentiated twitch soon after (~5 s) the MVC. A larger superimposed twitch in comparison to the potentiated twitch may indicate a lower %VA of the muscle \cite{117}. Alternatively, CAR \cite{109} compares the peak force output during an MVC to the peak force during the superimposed stimulation of the same MVC. However, this method assumes a maximal voluntary contraction which may not always occur and can result in erroneous assumptions of high voluntary activation levels \cite{180}.

Both calculations of %VA and CAR rely on the superimposed stimulation occurring during a plateau of the MVC, which is not always the case \cite{324}. Consequently, 2 corrections to the %VA calculation have been employed previously \cite{152, 182}. The first defines the superimposed twitch size as the difference between the force following the twitch to the mean force 100 ms prior \cite{152}. The second method divides the superimposed stimulation force by the actual MVC force \cite{182}. Therefore, if a superimposed stimulation was delivered while voluntary torque was declining or still rising, the result of the stimulation would be compared directly to the declining or rising torque, not the maximal torque measured during the entire contraction. Furthermore, the potentiated twitch alone can be compared pre- and post- intervention to assess isolated changes in peripheral locomotor muscle fatigue \cite{8}. The reliability of these various calculations has not been compared using the same dataset.
When torque output is measured to calculate voluntary activation, surface electromyography (EMG) is collected in synchrony. These EMG data can be used to assess neural drive to skeletal muscle, action potentials following stimulation (known as M-waves), and the ratio of rectified EMG signals to M-waves to assess muscle activation [113, 325-327]. An M-wave can occur when stimulation is applied to the nerve, and it increases as the stimulation intensity increases. At supramaximal stimulation intensities, maximal orthodromic and antidromic action potentials occur in the motorneuron axons, maximizing the M-wave (orthodromic action potential) and cancelling out other responses (H-reflex, antidromic action potential) [326]. Therefore, a plateau in the size of the M-wave with an increase in stimulation intensity can confirm that maximal stimulation has occurred. A plateau in M-wave amplitude is assessed using a ramp protocol (see methods). However, the reliability of this measure has not been assessed on consecutive days as an indicator of reproducibility.

While electrical stimulation is commonly used to stimulate the femoral nerve innervating the quadriceps femoris to produce muscle twitches for the assessment of muscle activation [8, 21, 115], this technique can be painful for participants and might not be suitable for certain populations [183]. Magnetic stimulation is an alternative method of stimulating the motor nerve [184-186] that is considered painless [183, 187], making it a potentially favourable alternative to electrical stimulation.

In order to accurately assess muscle activation, the reliability of the measures associated with magnetic stimulation must be established. Furthermore, reliability should be assessed as the tests are intended to be used e.g., within and across days. Little research is available that addresses reliability using magnetic stimulation to assess muscle activation, and no data are available regarding reliability across sequential days [23, 188]. The aim of this study was to investigate the reliability of torque and EMG parameters through different calculations acquired using magnetic stimulation of the femoral nerve.

5.4. Materials and Method

5.4.1. Study Design

Participants attended the laboratory on 5 occasions across 2 weeks. The first 2 sessions were for familiarization and occurred during week 1 (48 hours apart). In the following week, the testing protocol was repeated for 3 consecutive days at the same time of day. Data collection
was performed by the same researcher, who had approximately 300 hours of experience with the magnetic stimulation technique prior to study commencement.

5.4.2. Participants

Fifteen healthy and active men (23.5 ± 3.0 y, 181.8 ± 7.2 cm, and 80.7 ± 7.8 kg) were recruited. Participants were requested to abstain from caffeine and alcohol in the 24 hours prior to testing and throughout the testing period. Additionally, participants were asked to refrain from strenuous exercise on the day prior to initial testing and for the 3 consecutive testing days. All participants provided written informed consent, and the research was approved by the Australian Catholic University Human Research Ethics Committee in accordance with the Declaration of Helsinki.

5.4.3. Surface EMG

EMG was recorded from the vastus lateralis using surface electrodes (Duo-Trode, NAOL, New South Wales, Australia) connected directly to wireless probes that pre-amplified the signal (gain 400) and transmitted data in real-time to a wireless EMG system (Noraxon, Telemyo DTS, Arizona, USA). Data were collected at 1000 Hz through a custom software package (Labview 2013, National Instruments, Austin, TX). Prior to electrode placement, the measurement site was thoroughly prepared by shaving and abrading the skin, then cleaned with swabbing alcohol. A bipolar surface electrode was positioned on the vastus lateralis according to SENIAM guidelines \[293\], and the location was marked with indelible ink to ensure identical placement on subsequent sessions. The M-wave peak-to-peak amplitude (PPA) and duration (PPD) were measured during twitches, and the EMG signal during each maximal voluntary contraction (MVC) was quantified by using the root mean square (RMS) calculated over a 1-s period after the torque had reached a plateau (RMS\textsubscript{MVC}). During post-processing, the EMG signals were rectified and filtered (bandwidth frequency: 10–500 Hz). The RMS\textsubscript{MVC} was then normalized to the corresponding PPA by using the ratio RMS\textsubscript{MVC}/PPA.

5.4.4. Peripheral Magnetic Stimulation

Participants lay supine on an isokinetic dynamometer (Humac Norm, Computer Sports Medicine, Inc., Massachusetts, USA) with the right thigh resting flat on the bench, the angle of the right knee set to 90° of flexion, and the right ankle fixed to the torque arm of the
dynamometer just above the lateral malleolus. This knee angle lengthens the muscle, reducing the series elastic slackness that can influence control twitches [180]. The participant’s hips and right leg were secured to the dynamometer using non-compliant straps to minimize body movement. A magnetic coil (D70², The Magstim Company Ltd, UK) powered by a magnetic stimulator (Magstim BiStim, The Magstim Company Ltd, UK) was positioned over the femoral triangle of each participant. Torque was collected at 1000 Hz via the dynamometer and synchronized with the EMG signal and timing of magnetic stimulation through the aforementioned custom software package. In order to locate the optimal stimulation position, twitches were administered at various locations in the vicinity of the femoral triangle with the stimulator intensity set to 50% of maximum output. The optimal position was defined as the location where the greatest torque and M-Wave PPA occurred from these aforementioned twitches. This coil position was marked on the participant with indelible ink for subsequent stimulations and test days.

To ensure that maximal twitches were delivered with the magnetic stimulator (i.e., plateau in twitch torque and M-wave PPA) [294], a ramp test was performed at the beginning of every session (see Figure 5.1). The ramp protocol consisted of 2 non-potentiated stimulations every 30 s at increasing intensities (50, 60, 70, 80, 90, 95, 100% of stimulator output). At completion of the ramp test, participants performed a warm-up comprising 3 isometric contractions at approximately 50, 80, and 100% MVC, separated by 60 s. At least 3 minutes after the warm-up, participants performed 2 isometric, maximal voluntary contractions (MVC) lasting 5 s, separated by 3 minutes. Participants were manually administered a single twitch (stimulator intensity of 100%) when the researcher visually identified a torque plateau during the MVC (see Figure 5.2 A and B). This twitch was used to assess the completeness of muscle activation, calculated using 4 methods (outlined below). At 5-seconds post-MVC, participants were administered a potentiated twitch.
Figure 5.1: Vastus lateralis M-Wave amplitude (A, top) and quadriceps twitch torque (B, bottom) in response to magnetic stimulation. As part of a ramp protocol, participants were administered 2 non-potentiated stimulations to the femoral nerve every 30 s at increasing intensities (50, 60, 70, 80, 90, 95, 100% of stimulator output) to assess a plateau in M-Wave and torque outputs (i.e., whether a maximal stimulation has occurred). Data presented are means, error bars represent ± SD.

**Method 1:** Central activation ratio (CAR) was calculated using the equation:\(^{109}\)

\[ \text{CAR} = \frac{\text{MVC}}{\text{MVC} + \text{superimposed twitch}} \]

**Method 2:** Voluntary activation was calculated using the equation: \(^{295, 296}\)

\[ \% \text{VA} = (1 - \frac{\text{Superimposed Twitch}}{\text{Potentiated Twitch}}) \times 100 \]

**Method 3:** Voluntary activation was calculated by defining the superimposed twitch as the difference between the peak superimposed twitch force and the force averaged over 100 ms prior to that peak \(^{152}\).

**Method 4:** Voluntary activation was calculated when stimulation was applied prior to or following MVC.\(^{181, 182}\)

\[ \% \text{VA} = (1 - \left[ \frac{\text{superimposed twitch} \times (\text{force level at stimulation/MVC force})}{\text{potentiated twitch}} \right] \times 100 \]
Figure 5.2 (A) Representative output from the twitch interpolation method. The participants perform a maximal voluntary contraction, from which peak torque is obtained (a). Once a plateau in torque is observed, a superimposed twitch (depicted by the green line) is administered using a magnetic stimulator (b: maximal voluntary contraction + superimposed twitch; c: superimposed twitch). Approximately 4 seconds post-maximal voluntary contraction, a potentiated twitch is administered (d). These variables are input into a number of equations to calculate levels of voluntary activation. (B) Representative output from a resting twitch during the ramp stimulation protocol. The participants are given a series of incremental twitches at rest from 50 – 100% of the magnetic stimulator output to assess whether the twitch is maximal (100% stimulator output shown here). The magnetic stimulation creates an artifact in the EMG signal (e). However, this does not affect the interpretation of the M-wave variables (f: peak-to-peak duration; g: peak-to-peak amplitude)
5.4.5. Rate of Torque Development

Using the aforementioned custom software package, rate of torque development (\(\Delta\)torque/\(\Delta\)time) was calculated for the potentiated stimulations, as well as during the ramp protocol. Onset of torque was defined as 5% of the peak twitch torque, and the rate was calculated from onset to peak torque output.

5.4.6. Data Analysis

The researcher was blinded to the data until data collection and analysis were completed. All raw data collected were analyzed through a custom software package (Labview 2013, National Instruments, Austin, TX) to provide all variables presented in Table 5.1, Table 5.2 and Table 5.3. All variables were log-transformed to reduce bias because of non-uniformity of error, and analysis was performed using a custom spreadsheet.\(^{[291]}\) For clarity, all data are presented as raw values (mean ± SD) in the tables and text, unless otherwise stated. The peak of the best maximal voluntary contraction for each day was used to assess inter-day reliability. The intra-day (e.g., Trial 1 vs. Trial 2) and inter-day (e.g., Day 1 vs. Day 2) reliability of MVC, potentiated twitch, PPA, PPD, RMS\(_{\text{MVC}}\)/PPA, and twitch rate of torque development (RTD), and the various calculations of voluntary activation were estimated using the intra-class correlation coefficient [ICC (3,1)]\(^{[297]}\) and typical error ± 90% confidence limits (CL) expressed as a percentage [coefficient of variation (CV)].\(^{[297]}\) The smallest worthwhile change (SWC), defined as the smallest change of practical importance, was calculated as 0.2 \(\times\) the between-participant standard deviation (SD). Variables were considered capable of detecting the SWC if CV% \(\leq\) SWC.\(^{[298]}\) Inter-day reliability was calculated for the ramp protocol at each stimulator intensity (70-100%) for torque, PPA, PPD and RTD. Confirmation of a plateau during the ramp protocol (i.e., to confirm maximal stimulation) was assumed when the change from the last 2 stimulator intensities (95-100%) of the ramp protocol were smaller than CV% for the resting twitch.

5.5. Results

5.5.1. Torque

The reliability of MVC for both intra-day (CV% < 3.2) and inter-day (CV% < 4.3) was considered good, with ICC values > 0.98 (Table 5.1 and Table 5.2). Torque values from potentiated twitches were also repeatable within trials (CV% < 4.7, ICC > 0.92) and across the
3 days (CV% 6.8, ICC 0.82). The RTD was less reliable for intra-day (CV% 9.6-13.8, ICC 0.82-0.93) compared with inter-day (CV% 7.3, ICC 0.96). During the ramp protocol, twitch torque was more reliable than RTD (CV% 6.8-8.1, ICC 0.89-0.93 and CV% 12.6-17.0, ICC 0.75-0.84, respectively). Fourteen of the 15 participants reached a plateau in torque during the ramp protocol.

5.5.2. Voluntary Activation

Four different calculations of voluntary activation were assessed for reliability (see Table 5.1 and Table 5.2), as described in the methods. Intraclass correlation coefficients for both intra- and inter-day tests were low to moderate (ICC 0.43-0.82).

5.5.3. Surface Electromyography

All variables assessed via EMG were less reliable than torque-based measures when comparing CV% (see Table 5.1, Table 5.2 and Table 5.3), with the ramp protocol exhibiting the largest variations (CV% 12.9-22.7).
Table 5.1: The inter-day reliability for measures collected when magnetic stimulation was applied to the femoral nerve during a maximal voluntary contraction of the knee extensors and a control, potentiated twitch to the resting muscle.

<table>
<thead>
<tr>
<th>Raw Data</th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>Trial 3</th>
<th>Mean ± SD</th>
<th>Avg % Change in Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>MVC (N·m)</td>
<td>342.2 ± 79.1</td>
<td>344.5 ± 87.8</td>
<td>334.4 ± 85.2</td>
<td>340.4 ± 84.1</td>
<td>-1.3</td>
</tr>
<tr>
<td>Potentiated Twitch (N·m)</td>
<td>88.3 ± 12.0</td>
<td>85.0 ± 14.0</td>
<td>85.6 ± 12.6</td>
<td>86.3 ± 12.9</td>
<td>-1.5</td>
</tr>
<tr>
<td>PPA (mV)</td>
<td>2.2 ± 1.0</td>
<td>2.0 ± 0.8</td>
<td>2.1 ± 0.9</td>
<td>2.1 ± 0.9</td>
<td>-2.8</td>
</tr>
<tr>
<td>PPD (m·s)</td>
<td>7.5 ± 3.7</td>
<td>7.4 ± 2.7</td>
<td>7.0 ± 2.9</td>
<td>7.3 ± 3.1</td>
<td>-2.6</td>
</tr>
<tr>
<td>RMS\textsubscript{MVC}/PPA (m·s)</td>
<td>0.08 ± 0.05</td>
<td>0.07 ± 0.05</td>
<td>0.08 ± 0.05</td>
<td>0.08 ± 0.05</td>
<td>-1.8</td>
</tr>
<tr>
<td>RTD (N·m·s\textsuperscript{-1})</td>
<td>1408.0 ± 450.4</td>
<td>1393.7 ± 490.5</td>
<td>1387.6 ± 433.9</td>
<td>1396.4 ± 458.9</td>
<td>-0.6</td>
</tr>
<tr>
<td>Method 1 (%)</td>
<td>98.7 ± 4.7</td>
<td>104.3 ± 8.1</td>
<td>101.7 ± 8.7</td>
<td>101.7 ± 7.4</td>
<td>1.7</td>
</tr>
<tr>
<td>Method 2 (%)</td>
<td>97.5 ± 4.7</td>
<td>101.7 ± 6.3</td>
<td>100.2 ± 7.0</td>
<td>99.8 ± 6.1</td>
<td>1.3</td>
</tr>
<tr>
<td>Method 3 (%)</td>
<td>98.2 ± 1.8</td>
<td>98.0 ± 1.6</td>
<td>98.2 ± 1.4</td>
<td>98.2 ± 1.6</td>
<td>0.03</td>
</tr>
<tr>
<td>Method 4 (%)</td>
<td>93.2 ± 6.4</td>
<td>92.9 ± 5.0</td>
<td>93.4 ± 5.0</td>
<td>93.2 ± 5.5</td>
<td>0.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reliability Values</th>
<th>CV% 2-1</th>
<th>CV% 3-2</th>
<th>CV% (90% CL)</th>
<th>ICC (90% CL)</th>
<th>SWC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MVC (N·m)</td>
<td>4.5 (3.4 - 6.6)</td>
<td>5.4 (4.2 - 8.0)</td>
<td>4.3 (3.5 - 5.9)</td>
<td>(0.95 - 0.99)</td>
<td>5.65</td>
</tr>
<tr>
<td>Potentiated Twitch (N·m)</td>
<td>4.5 (3.1 - 5.9)</td>
<td>5.4 (6.0 - 11.7)</td>
<td>6.8 (5.5 - 9.4)</td>
<td>(0.65 - 0.92)</td>
<td>3.09</td>
</tr>
<tr>
<td>PPA (mV)</td>
<td>13.4 (10.1 - 20.1)</td>
<td>13.4 (10.3 - 20.4)</td>
<td>13.5 (10.9 - 18.9)</td>
<td>(0.87 - 0.97)</td>
<td>12.07</td>
</tr>
<tr>
<td>PPD (m·s)</td>
<td>12.4 (9.40 - 18.7)</td>
<td>12.4 (10.7 - 21.3)</td>
<td>13.3 (10.8 - 18.7)</td>
<td>(0.84 - 0.97)</td>
<td>10.20</td>
</tr>
<tr>
<td>RMS\textsubscript{MVC}/PPA (m·s)</td>
<td>17.8 (13.4 - 27.0)</td>
<td>17.8 (13.6 - 27.4)</td>
<td>17.9 (14.4 - 25.4)</td>
<td>(0.83 - 0.96)</td>
<td>14.20</td>
</tr>
<tr>
<td>RTD (N·m·s\textsuperscript{-1})</td>
<td>8.4 (6.4 - 12.5)</td>
<td>8.0 (6.1 - 11.8)</td>
<td>8.3 (7.2 - 14.2)</td>
<td>(5.9 - 10.1)</td>
<td>7.50</td>
</tr>
<tr>
<td>Method 1 (%)</td>
<td>6.6 (6.1 - 11.8)</td>
<td>6.6 (5.9 - 11.6)</td>
<td>6.7 (7.1 - 12.2)</td>
<td>(5.9 - 10.0)</td>
<td>1.44</td>
</tr>
<tr>
<td>Method 2 (%)</td>
<td>0.9 (5.0 - 9.8)</td>
<td>1.0 (0.9 - 1.7)</td>
<td>0.9 (0.9 - 1.7)</td>
<td>(0.8 - 1.4)</td>
<td>0.34</td>
</tr>
<tr>
<td>Method 3 (%)</td>
<td>4.3 (3.3 - 6.3)</td>
<td>4.5 (3.4 - 6.6)</td>
<td>4.5 (3.56 - 6.06)</td>
<td>(0.23 - 0.76)</td>
<td>1.24</td>
</tr>
</tbody>
</table>

Data presented are mean ± SD for all variables, CV (90% CL), average percent change in mean, average SWC, and average ICC (90% CL). CV: coefficient of variation; CL: confidence limit; SWC: smallest worthwhile change (0.2 x the between participant SD); ICC: intraclass correlation coefficient; PPA: peak-to-peak amplitude; PPD: peak-to-peak duration; RTD: rate of torque development, potentiated twitch.

*Note: refer to text for formulae used to calculate each method.
Table 5.2: The intra-day reliability for measures collected when magnetic stimulation was applied to the femoral nerve during a maximal voluntary contraction of the knee extensors and during a control, potentiated twitch to the resting muscle.

<table>
<thead>
<tr>
<th>Raw Data</th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>Mean ± SD</th>
<th>% Change in Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1 MVC (N·m)</td>
<td>336.0 ± 77.4</td>
<td>336.6 ± 80.4</td>
<td>336.3 ± 78.9</td>
<td>0.1</td>
</tr>
<tr>
<td>Potentiated Twitch (N·m)</td>
<td>87.3 ± 13.0</td>
<td>87.3 ± 11.1</td>
<td>87.3 ± 12.1</td>
<td>0.3</td>
</tr>
<tr>
<td>PPA (mV)</td>
<td>2.2 ± 1.0</td>
<td>2.2 ± 1.1</td>
<td>2.2 ± 1.0</td>
<td>-0.2</td>
</tr>
<tr>
<td>PPD (m·s)</td>
<td>7.7 ± 3.6</td>
<td>7.6 ± 3.9</td>
<td>7.6 ± 3.7</td>
<td>-3.0</td>
</tr>
<tr>
<td>RMS_{MVC}/PPA (m·s)</td>
<td>0.08 ± 0.06</td>
<td>0.08 ± 0.07</td>
<td>0.08 ± 0.06</td>
<td>-2.7</td>
</tr>
<tr>
<td>RTD (N·m·s^{-1})</td>
<td>1430.0 ± 445.6</td>
<td>1361.9 ± 366.6</td>
<td>1395.9 ± 408.0</td>
<td>-3.7</td>
</tr>
<tr>
<td>Method 1 (%)</td>
<td>99.1 ± 6.7</td>
<td>97.5 ± 6.2</td>
<td>98.3 ± 6.5</td>
<td>-1.6</td>
</tr>
<tr>
<td>Method 2 (%)</td>
<td>97.8 ± 6.3</td>
<td>96.1 ± 6.3</td>
<td>96.9 ± 6.3</td>
<td>-1.7</td>
</tr>
<tr>
<td>Method 3 (%)</td>
<td>98.1 ± 1.9</td>
<td>97.5 ± 2.4</td>
<td>97.8 ± 2.2</td>
<td>-0.6</td>
</tr>
<tr>
<td>Method 4 (%)</td>
<td>91.9 ± 7.8</td>
<td>91.1 ± 9.0</td>
<td>91.5 ± 8.4</td>
<td>-1.0</td>
</tr>
<tr>
<td>Day 2 MVC (N·m)</td>
<td>339.7 ± 84.0</td>
<td>338.6 ± 87.4</td>
<td>339.2 ± 85.7</td>
<td>-0.6</td>
</tr>
<tr>
<td>Potentiated Twitch (N·m)</td>
<td>85.7 ± 12.5</td>
<td>85.5 ± 12.8</td>
<td>85.6 ± 12.7</td>
<td>-0.2</td>
</tr>
<tr>
<td>PPA (mV)</td>
<td>2.0 ± 0.8</td>
<td>1.9 ± 0.8</td>
<td>2.0 ± 0.8</td>
<td>-5.1</td>
</tr>
<tr>
<td>PPD (m·s)</td>
<td>7.3 ± 2.8</td>
<td>7.8 ± 3.3</td>
<td>7.5 ± 3.1</td>
<td>5.8</td>
</tr>
<tr>
<td>RMS_{MVC}/PPA (m·s)</td>
<td>0.07 ± 0.04</td>
<td>0.08 ± 0.05</td>
<td>0.07 ± 0.04</td>
<td>8.7</td>
</tr>
<tr>
<td>RTD (N·m·s^{-1})</td>
<td>1413.9 ± 519.3</td>
<td>1436.4 ± 501.1</td>
<td>1425.2 ± 510.3</td>
<td>2.1</td>
</tr>
<tr>
<td>Method 1 (%)</td>
<td>105.3 ± 9.1</td>
<td>100.6 ± 7.5</td>
<td>103.0 ± 8.3</td>
<td>-4.4</td>
</tr>
<tr>
<td>Method 2 (%)</td>
<td>102.3 ± 6.6</td>
<td>99.0 ± 6.9</td>
<td>100.7 ± 6.7</td>
<td>-3.3</td>
</tr>
<tr>
<td>Method 3 (%)</td>
<td>98.0 ± 1.7</td>
<td>98.0 ± 2.0</td>
<td>98.0 ± 1.8</td>
<td>-0.1</td>
</tr>
<tr>
<td>Method 4 (%)</td>
<td>92.9 ± 5.0</td>
<td>92.9 ± 6.5</td>
<td>92.9 ± 5.8</td>
<td>-0.1</td>
</tr>
<tr>
<td>Day 3 MVC (N·m)</td>
<td>332.3 ± 86.5</td>
<td>328.6 ± 84.2</td>
<td>330.4 ± 85.3</td>
<td>-1.0</td>
</tr>
<tr>
<td>Potentiated Twitch (N·m)</td>
<td>85.6 ± 12.6</td>
<td>82.7 ± 13.3</td>
<td>84.2 ± 13.0</td>
<td>-3.6</td>
</tr>
<tr>
<td>PPA (mV)</td>
<td>2.0 ± 0.9</td>
<td>2.0 ± 0.9</td>
<td>2.0 ± 0.9</td>
<td>-4.9</td>
</tr>
<tr>
<td>PPD (m·s)</td>
<td>6.9 ± 2.7</td>
<td>7.7 ± 3.4</td>
<td>7.3 ± 3.1</td>
<td>9.4</td>
</tr>
<tr>
<td>RMS_{MVC}/PPA (m·s)</td>
<td>0.08 ± 0.05</td>
<td>0.08 ± 0.06</td>
<td>0.08 ± 0.05</td>
<td>1.1</td>
</tr>
<tr>
<td>RTD (N·m·s^{-1})</td>
<td>1386.8 ± 434.5</td>
<td>1410.5 ± 472.9</td>
<td>1398.6 ± 454.1</td>
<td>1.0</td>
</tr>
<tr>
<td>Method 1 (%)</td>
<td>102.5 ± 7.7</td>
<td>101.7 ± 8.4</td>
<td>102.1 ± 8.1</td>
<td>-0.8</td>
</tr>
<tr>
<td>Method 2 (%)</td>
<td>100.0 ± 6.2</td>
<td>99.5 ± 7.1</td>
<td>99.7 ± 6.7</td>
<td>-0.5</td>
</tr>
<tr>
<td>Method 3 (%)</td>
<td>97.6 ± 2.0</td>
<td>97.9 ± 1.7</td>
<td>97.8 ± 1.9</td>
<td>0.3</td>
</tr>
<tr>
<td>Method 4 (%)</td>
<td>91.3 ± 6.7</td>
<td>91.7 ± 7.1</td>
<td>91.5 ± 6.9</td>
<td>0.5</td>
</tr>
</tbody>
</table>
Table 5 continued:

<table>
<thead>
<tr>
<th>Reliability Values</th>
<th>CV% (90% CL)</th>
<th>ICC (90% CL)</th>
<th>SWC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MVC (N·m)</td>
<td>3.2 (2.4 - 4.7)</td>
<td>0.99 (0.97 - 0.99)</td>
<td>5.46</td>
</tr>
<tr>
<td>Potentiated Twitch (N·m)</td>
<td>4.1 (3.1 - 6.0)</td>
<td>0.92 (0.82 - 0.97)</td>
<td>2.85</td>
</tr>
<tr>
<td>PPA (mV)</td>
<td>7.4 (5.6 - 11.0)</td>
<td>0.98 (0.96 - 0.99)</td>
<td>13.98</td>
</tr>
<tr>
<td>PPD (m s)</td>
<td>9.0 (6.9 - 13.4)</td>
<td>0.97 (0.92 - 0.99)</td>
<td>10.98</td>
</tr>
<tr>
<td>RMS&lt;sub&gt;MVC/PPA&lt;/sub&gt; (m s)</td>
<td>13.8 (10.4 - 20.8)</td>
<td>0.96 (0.91 - 0.98)</td>
<td>16.52</td>
</tr>
<tr>
<td>RTD (N·m·s&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>13.8 (10.4 - 20.7)</td>
<td>0.82 (0.61 - 0.93)</td>
<td>6.70</td>
</tr>
<tr>
<td>Method 1 (%)</td>
<td>6.1 (4.6 - 9.0)</td>
<td>0.21 (-0.24 - 0.58)</td>
<td>1.36</td>
</tr>
<tr>
<td>Method 2 (%)</td>
<td>5.5 (4.2 - 8.2)</td>
<td>0.82 (0.63 - 1.19)</td>
<td>1.36</td>
</tr>
<tr>
<td>Method 3 (%)</td>
<td>1.3 (1.0 - 2.0)</td>
<td>0.69 (0.37 - 0.86)</td>
<td>0.45</td>
</tr>
<tr>
<td>Method 4 (%)</td>
<td>7.4 (5.6 - 10.9)</td>
<td>0.50 (0.09 - 0.76)</td>
<td>2.04</td>
</tr>
<tr>
<td>Day 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MVC (N·m)</td>
<td>2.7 (2.1 - 4.0)</td>
<td>0.99 (0.98 - 1.00)</td>
<td>5.82</td>
</tr>
<tr>
<td>Potentiated Twitch (N·m)</td>
<td>4.7 (3.6 - 6.9)</td>
<td>0.92 (0.80 - 0.97)</td>
<td>3.14</td>
</tr>
<tr>
<td>PPA (mV)</td>
<td>9.7 (7.3 - 14.4)</td>
<td>0.96 (0.91 - 0.98)</td>
<td>11.07</td>
</tr>
<tr>
<td>PPD (m s)</td>
<td>10.0 (7.6 - 15.0)</td>
<td>0.95 (0.88 - 0.98)</td>
<td>9.81</td>
</tr>
<tr>
<td>RMS&lt;sub&gt;MVC/PPA&lt;/sub&gt; (m s)</td>
<td>13.8 (10.4 - 20.8)</td>
<td>0.94 (0.86 - 0.98)</td>
<td>12.64</td>
</tr>
<tr>
<td>RTD (N·m·s&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>11.6 (8.8 - 17.4)</td>
<td>0.91 (0.79 - 0.96)</td>
<td>8.12</td>
</tr>
<tr>
<td>Method 1 (%)</td>
<td>8.0 (6.1 - 11.9)</td>
<td>0.07 (-0.37 - 0.48)</td>
<td>1.65</td>
</tr>
<tr>
<td>Method 2 (%)</td>
<td>6.3 (4.8 - 9.3)</td>
<td>0.19 (-0.26 - 0.57)</td>
<td>1.39</td>
</tr>
<tr>
<td>Method 3 (%)</td>
<td>1.1 (0.9 - 1.7)</td>
<td>0.68 (0.34 - 0.86)</td>
<td>0.39</td>
</tr>
<tr>
<td>Method 4 (%)</td>
<td>3.9 (3.0 - 5.7)</td>
<td>0.68 (0.35 - 0.86)</td>
<td>1.31</td>
</tr>
<tr>
<td>Day 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MVC (N·m)</td>
<td>2.9 (2.2 - 4.3)</td>
<td>0.99 (0.97 - 1.00)</td>
<td>5.81</td>
</tr>
<tr>
<td>Potentiated Twitch (N·m)</td>
<td>4.2 (3.2 - 6.1)</td>
<td>0.93 (0.84 - 0.97)</td>
<td>3.14</td>
</tr>
<tr>
<td>PPA (mV)</td>
<td>13.2 (10.0 - 19.9)</td>
<td>0.94 (0.86 - 0.98)</td>
<td>12.04</td>
</tr>
<tr>
<td>PPD (m s)</td>
<td>11.8 (8.9 - 17.6)</td>
<td>0.94 (0.85 - 0.97)</td>
<td>10.18</td>
</tr>
<tr>
<td>RMS&lt;sub&gt;MVC/PPA&lt;/sub&gt; (m s)</td>
<td>14.4 (10.9 - 21.7)</td>
<td>0.95 (0.87 - 0.98)</td>
<td>14.26</td>
</tr>
<tr>
<td>RTD (N·m·s&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>9.6 (7.3 - 14.4)</td>
<td>0.93 (0.83 - 0.97)</td>
<td>7.46</td>
</tr>
<tr>
<td>Method 1 (%)</td>
<td>5.9 (4.5 - 8.7)</td>
<td>0.48 (0.07 - 0.75)</td>
<td>1.61</td>
</tr>
<tr>
<td>Method 2 (%)</td>
<td>4.5 (3.4 - 6.6)</td>
<td>0.59 (0.22 - 0.81)</td>
<td>1.36</td>
</tr>
<tr>
<td>Method 3 (%)</td>
<td>1.3 (1.0 - 1.9)</td>
<td>0.57 (0.19 - 0.80)</td>
<td>0.39</td>
</tr>
<tr>
<td>Method 4 (%)</td>
<td>5.7 (4.3 - 8.4)</td>
<td>0.54 (0.15 - 0.79)</td>
<td>1.63</td>
</tr>
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</table>

Data presented are mean ± SD for all variables, CV (90% CL), average percent change in mean, average SWC, and average ICC (90% CL). CV: coefficient of variation; CL: confidence limit; SWC: smallest worthwhile change (0.2 x the between participant SD); ICC: intraclass correlation coefficient; PPA: peak-to-peak amplitude; PPD: peak-to-peak duration; RTD: rate of torque development, potentiated twitch. *Note: refer to text for formulae used to calculate each method.
Table 5.3: The inter-day reliability for the ramp protocol performed prior to testing on each day, including torque and EMG derived variables. The ramp protocol incorporated magnetic stimulation of the femoral nerve at increasing stimulator intensities.

<table>
<thead>
<tr>
<th>Raw Data</th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>Trial 3</th>
<th>Mean ± SD</th>
<th>Avg % Change in Mean</th>
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<tr>
<td>100% Stimulator output</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Torque (N·m)</td>
<td>53.4 ±11.0</td>
<td>53.3 ± 11.5</td>
<td>50.9 ± 10.7</td>
<td>52.5 ± 11.1</td>
<td>-2.5</td>
</tr>
<tr>
<td>PPA (mV)</td>
<td>2.2 ± 1.0</td>
<td>1.9 ± 0.8</td>
<td>2.0 ± 0.9</td>
<td>2.0 ± 0.9</td>
<td>-1.5</td>
</tr>
<tr>
<td>PPD (ms)</td>
<td>8.2 ±2.9</td>
<td>8.4 ±3.6</td>
<td>8.2 ±3.3</td>
<td>8.3 ±3.3</td>
<td>-0.8</td>
</tr>
<tr>
<td>RTD (N·m·s⁻¹)</td>
<td>803.2 ±166.0</td>
<td>836.7 ±267.6</td>
<td>748.7 ±251.1</td>
<td>795.3 ±231.6</td>
<td>-4.5</td>
</tr>
<tr>
<td>95%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Torque (N·m)</td>
<td>52.7 ± 11.0</td>
<td>52.2 ± 11.3</td>
<td>50.4 ± 10.7</td>
<td>51.8 ± 11.0</td>
<td>-2.3</td>
</tr>
<tr>
<td>PPA (mV)</td>
<td>2.2 ± 1.0</td>
<td>1.8 ± 0.8</td>
<td>2.0 ± 0.9</td>
<td>2.0 ± 0.9</td>
<td>-0.2</td>
</tr>
<tr>
<td>PPD (ms)</td>
<td>8.1 ±2.8</td>
<td>8.4 ±3.3</td>
<td>8.2 ±3.4</td>
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<tr>
<td>RTD (N·m·s⁻¹)</td>
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<td>757.9 ±215.1</td>
<td>777.8 ±215.1</td>
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<tr>
<td>Torque (N·m)</td>
<td>51.3 ± 11.1</td>
<td>51.2 ± 11.2</td>
<td>49.4 ± 10.7</td>
<td>50.6 ± 11.0</td>
<td>-1.9</td>
</tr>
<tr>
<td>PPA (mV)</td>
<td>2.2 ± 1.0</td>
<td>1.8 ± 0.8</td>
<td>2.0 ± 0.9</td>
<td>2.0 ± 0.9</td>
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<tr>
<td>PPD (ms)</td>
<td>8.2 ±2.9</td>
<td>8.3 ±3.4</td>
<td>8.4 ±3.4</td>
<td>8.3 ±3.2</td>
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<tr>
<td>RTD (N·m·s⁻¹)</td>
<td>755.2 ±168.1</td>
<td>771.6 ±256.7</td>
<td>739.1 ±215.8</td>
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<td>Torque (N·m)</td>
<td>49.5 ± 11.2</td>
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<td>47.6 ± 10.9</td>
<td>48.6 ± 11.2</td>
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<tr>
<td>PPA (mV)</td>
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<td>1.9 ± 0.9</td>
<td>2.0 ± 0.9</td>
<td>2.0 ± 0.9</td>
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</tr>
<tr>
<td>PPD (ms)</td>
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<td>8.4 ±3.5</td>
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<tr>
<td>RTD (N·m·s⁻¹)</td>
<td>726.8 ±157.8</td>
<td>728.6 ±247.7</td>
<td>695.0 ±213.6</td>
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<tr>
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<tr>
<td>PPA (mV)</td>
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<td>1.8 ± 0.9</td>
<td>1.9 ± 1.0</td>
<td>1.9 ± 1.0</td>
<td>2.7</td>
</tr>
<tr>
<td>PPD (ms)</td>
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<td>8.0 ±3.0</td>
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<tr>
<td>RTD (N·m·s⁻¹)</td>
<td>666.9 ±155.3</td>
<td>649.3 ±237.1</td>
<td>633.9 ±205.6</td>
<td>650.0 ±202.2</td>
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Table 6 continued:

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<th>CV% 2-1</th>
<th>CV% 3-2</th>
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<th>ICC</th>
<th>SWC (%)</th>
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<td>7.3 (5.6 - 10.9)</td>
<td>6.9 (5.2 - 10.2)</td>
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<td>PPA (mV)</td>
<td>21.9 (16.3 - 34.2)</td>
<td>20.7 (15.4 - 32.3)</td>
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<td>0.84 (0.68 - 0.93)</td>
<td>11.33</td>
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<tr>
<td>PPD (ms)</td>
<td>12.0 (9.1 - 18.4)</td>
<td>14.4 (10.8 - 22.2)</td>
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<td>16.2 (12.1 - 25.0)</td>
<td>17.9 (13.3 - 27.6)</td>
<td>17.0 (13.5 - 24.1)</td>
<td>0.75 (0.53 - 0.89)</td>
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<tr>
<td>Torque (N·m)</td>
<td>7.0 (5.3 - 10.4)</td>
<td>6.5 (5.0 - 9.7)</td>
<td>6.8 (5.5 - 9.4)</td>
<td>0.92 (0.83 - 0.96)</td>
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<td>PPA (mV)</td>
<td>24.4 (18.3 - 37.6)</td>
<td>20.9 (15.7 - 32.0)</td>
<td>22.7 (18.2 - 32.5)</td>
<td>0.82 (0.65 - 0.92)</td>
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<td>PPD (ms)</td>
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<td>13.3 (10.1 - 20.0)</td>
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<td>12.0 (9.1 - 18.0)</td>
<td>14.5 (11.7 - 20.4)</td>
<td>0.77 (0.56 - 0.89)</td>
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<tr>
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<td>8.3 (6.3 - 12.4)</td>
<td>7.2 (5.5 - 10.6)</td>
<td>7.8 (6.3 - 10.8)</td>
<td>0.89 (0.78 - 0.95)</td>
<td>4.73</td>
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<tr>
<td>PPA (mV)</td>
<td>21.7 (16.3 - 33.3)</td>
<td>19.6 (14.7 - 29.8)</td>
<td>20.7 (16.6 - 29.4)</td>
<td>0.86 (0.72 - 0.94)</td>
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<td>14.8 (11.2 - 22.4)</td>
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<tr>
<td>RTD (N·m·s⁻¹)</td>
<td>17.0 (12.8 - 25.7)</td>
<td>12.3 (9.4 - 18.5)</td>
<td>14.8 (11.9 - 20.9)</td>
<td>0.76 (0.55 - 0.89)</td>
<td>6.05</td>
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<tr>
<td>80%</td>
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<td></td>
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<tr>
<td>Torque (N·m)</td>
<td>7.3 (5.6 - 10.8)</td>
<td>7.6 (5.8 - 11.3)</td>
<td>7.4 (6.0 - 10.4)</td>
<td>0.91 (0.82 - 0.96)</td>
<td>5.04</td>
</tr>
<tr>
<td>PPA (mV)</td>
<td>18.6 (14.0 - 28.3)</td>
<td>14.2 (10.8 - 21.4)</td>
<td>16.6 (13.3 - 23.4)</td>
<td>0.92 (0.83 - 0.96)</td>
<td>12.86</td>
</tr>
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<td>PPD (ms)</td>
<td>14.1 (10.7 - 21.2)</td>
<td>11.8 (8.9 - 17.6)</td>
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<td>9.17</td>
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<td>12.6 (10.2 - 17.7)</td>
<td>0.82 (0.66 - 0.92)</td>
<td>6.09</td>
</tr>
<tr>
<td>70%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Torque (N·m)</td>
<td>6.8 (5.2 - 10.1)</td>
<td>9.2 (7.0 - 13.7)</td>
<td>8.1 (6.6 - 11.3)</td>
<td>0.93 (0.85 - 0.97)</td>
<td>6.07</td>
</tr>
<tr>
<td>PPA (mV)</td>
<td>20.4 (15.4 - 31.2)</td>
<td>18.4 (13.9 - 28.0)</td>
<td>19.5 (15.6 - 27.6)</td>
<td>0.93 (0.85 - 0.97)</td>
<td>17.20</td>
</tr>
<tr>
<td>PPD (ms)</td>
<td>13.3 (10.1 - 20.0)</td>
<td>12.5 (9.5 - 18.8)</td>
<td>12.9 (10.4 - 18.1)</td>
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<td>8.88</td>
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<tr>
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<td>15.3 (11.5 - 23.0)</td>
<td>10.7 (8.1 - 15.9)</td>
<td>13.1 (10.6 - 18.4)</td>
<td>0.84 (0.68 - 0.93)</td>
<td>6.58</td>
</tr>
</tbody>
</table>

Data presented are mean ± SD for all variables, CV (90% CL), average percent change in mean, average SWC, and average ICC (90% CL). CV: coefficient of variation; CL: confidence limit; SWC: smallest worthwhile change (0.2 x the between participant SD); ICC: intraclass correlation coefficient; PPA: peak-to-peak amplitude; PPD: peak-to-peak duration; RTD: rate of torque development, potentiated twitch.
Discussion

This study evaluated the reliability of magnetic stimulation and associated measures during maximal voluntary contractions with interpolated twitch and potentiated control twitches to resting muscle. These measures were used to assess central and peripheral factors of muscle contraction in the quadriceps femoris within a single day, and across 3 consecutive days. A majority of the measures assessed were reliable, and some were capable of detecting the smallest worthwhile change (i.e., CV% < SWC). This indicates that the sensitivity of these specific measures was high. However, EMG measures were typically less reliable than torque-based measures. As all data were collected by the same experienced researcher, this may have contributed to the reliability of the values.

Magnetic stimulation has been introduced relatively recently as an alternative to electrical stimulation for measuring muscle activation by comparing maximal voluntary contractions with interpolated twitches and potentiated control twitches to resting muscle [23, 188, 328]. However, unlike electrical stimulation [329, 330], the intra- and inter-day reliability of magnetic stimulation has not been assessed over consecutive days. The inter-day CV% for maximal voluntary contraction (MVC) and twitch torque (4.3 and 6.8%, respectively) compare well with recent research using a similar method for assessing quadriceps muscle function (CV% 5.1 and 5.7, respectively)[23]. However, in the study of Bachasson and colleagues [23], the interval between testing sessions varied from 5-19 days (mean 9 days) and was limited to 2 days of testing only. Furthermore, no reliability data for EMG measures were presented.

The inter-day variability for the EMG variables, PPA, PPD, and RMS_{MVC}/PPA (CV% 13.5, 13.3 and 17.9, respectively), are in similar to those obtained using electrical stimulation (CV% 14.5, 6.9 and 12.1, respectively)[331]. The ICCs obtained in our study for these 3 measures (all greater than 0.92) compare favourably with those from the aforementioned work (0.71, 0.97, and 0.78, respectively) [331], where the electrical stimulation protocol was performed over 2 days only, and with varying durations (3-5 days) between sessions. The similarities between data obtained using electrical stimulation and those reported here using magnetic stimulation are not unexpected, as previous research has shown favourable comparisons between the 2 stimulation techniques [328, 332].
It should be noted that EMG was collected from the vastus lateralis as a surrogate for quadriceps muscle activation, and this method has been validated previously [331].

Voluntary activation (%VA) is calculated to assess central and peripheral contributions of muscle activation [118]. However, the calculation of this ratio can be performed in various ways. The basic premise of calculating %VA is to compare the size of a superimposed stimulus (above an MVC) to a potentiated twitch of a resting muscle. However, if the stimulus is delivered before/after the MVC is reached, then estimation errors in %VA will occur (i.e., %VA exceeding 100%, see Table 5.1 and Table 5.2). As a result, correction equations [152, 182] have been employed for situations when the stimulation is delivered at the incorrect time, or with participants who are unable to maintain an isometric torque plateau. Both correction equations for %VA used in this study were in line with the literature for intra- and inter-day reliability, with CV% 1.1-1.3 and 1.0 observed in Marshall et al (2014) compared to 1.3 and 2.4, respectively [152]. Similarly, the intra- and inter-day reliability of Place et al (2007) [331] (CV% 4.4 and 3.9-7.4) compared well to our CV% values of 1.7 and 3.1, respectively. The ICC for %VA calculations in our research should be interpreted with caution due to the small variability between individual participant results. This small variability can underestimate reliability due to the ceiling effect of %VA, as seen in similar literature that does not report the ICC of %VA [23, 331].

Each day, prior to performing MVCs, the assessment of whether twitches were maximal was performed by completing a ramp protocol (increasing stimulator intensity) to assess a plateau in twitch torque. This plateau is required to confirm full spatial recruitment of motor axons [177]. Overall, reliability for the resting and potentiated twitch torque were good, with the latter (CV% intra-day 4.1-5.7, inter-day 6.8) similar to electrical stimulation twitch values of the hamstrings muscles (CV% intra-day 7.9, inter-day 4.9) [152]. One participant did not meet the threshold for plateau (change in twitch torque from 95-100% stimulator intensity less than twitch torque CV%). This is a potential limitation of using magnetic stimulation, as the stimulation intensity is limited by the unit’s output. We included this participant’s data in this research, as his observed outputs were similar to the other participants. This approach has been used previously in the scientific literature [190]. Other authors should investigate such data to ensure it is representative of other subjects (who have a twitch-torque plateau during a ramp protocol) and consider whether a participant would be discarded from analysis.
Rate of torque development measured from potentiated twitches was more reliable (CV% 7.3, ICC 0.96) than when it was measured during 100% stimulation of the ramp protocol (CV% 17.0, ICC 0.75). The RTD measure has been used to compare quadriceps femoris muscle function between young and older men [333], to assess the effect of potentiation [334], and to assess fatigue from a potentiated twitch in hamstrings following a simulated soccer match [152]. None of the aforementioned research assessed reliability of this measure within and across consecutive days. However, as we show good reliability for potentiated twitch RTD in the quadriceps femoris muscles, it may be another potential measure for muscle activation assessment following activity in various populations.

Peripheral magnetic stimulation is gaining popularity as a less painful alternative to electrical stimulation for assessing muscle activation in various populations. Our results demonstrate that magnetic stimulation provides reliable data for measuring muscle activation. The experience of the researcher performing this technique may be a factor in its reliability.
5.7. Addendums Following Review

- **Method:** An appropriate reference \([335]\) to support the use of a relative threshold for the onset of rate of torque development should be added to section 5.4.5 – Rate of Torque Development.

6. Chapter 6: Study Three - A Self-Paced Team Sport Match Simulation Results in Reductions in Voluntary Activation and Modifications to Biological, Perceptual and Performance Measures at Half-Time, and for up to 96 Hours Post-Match

Publication statement:

This chapter is comprised of the following paper published in the Journal of Strength and Conditioning Research

Tofari P, Kemp J, Cormack S. A Self-Paced Team Sport Match Simulation Results In Reductions In Voluntary Activation And Modifications To Biological, Perceptual And Performance Measures At Half-Time, And For Up To 96 Hours Post-Match. Journal of Strength and Conditioning Research. 2017: Publish Ahead of Print.
6.1. **Linking paragraph**

Studies 1 and 2 describe reliable methods for simulating team-sport running and assessing central and peripheral fatigue, respectively. The aim of Study 3 was to utilise these tests to quantify the half-time and post-match responses to a match simulation. These assessments, coupled with other biochemical, perceptual and performance tests, can provide a detailed, multi-faceted assessment of the within- and post-match responses to team-sport exercise. This data set allows for the assessment of associations between laboratory-based measures, performance tests and the activity profiles performed during the simulation protocol, of which many have not been elucidated to date. In addition, the influence of participant physical qualities on activity profiles and post-match responses can be explored.
6.2. Abstract

Assessing responses to soccer match-play is limited by match variability or unrealistic simulations. To address this, the biological, perceptual, and performance response were assessed using a self-paced, simulated soccer match protocol using a non-motorized treadmill. Twelve male team-sport athletes performed the 90-min simulation. Match activity; quadriceps twitch interpolation [voluntary activation (%VA) and potentiated twitch (POT)]; biochemical markers; strength and power performance; rating of perceived exertion (RPE) and self-report wellness were collected pre-, half-time, post-, and 2, 24, 48, 72 and 96-h post-match. Change compared to pre-match was calculated using effect size (ES) ±90% confidence limit, and relationships were assessed using regression analysis. Subjects covered 12445.8±768.7 m at 87.1±3.2% maximal HR (mean±SD). Reductions in %VA and POT was present at half-time (-0.38±0.46 and -0.79±0.30, respectively), and persisted post-match. Squat jump height decreased at half-time (-0.42±0.31) and was decreased until Post96. Perceptual fatigue, soreness (-0.92±0.88 and -1.49±0.76, respectively) and creatine kinase (CK, 1.11±0.43) peaked at Post24. Pre-test strength (N.kg⁻¹) correlated with changes in CK (r=−0.58 to -0.81), peak oxygen consumption (V̇O₂peak) correlated with reduced perceived wellness at Post24 (r=0.44 to 0.58) and RPE post (r=−0.71±0.28). High-speed running correlated with soreness (r²=0.42) and very high speed running with reduced POT (r²=0.61). Previously unreported half-time reductions in %VA and POT plateaued by post-match, suggesting a role in regulating second-half performance. Perceptual and neuromuscular responses appear related to running intensity. Greater lower-body strength and V̇O₂peak were associated with less CK (i.e., muscle damage) and perceptual responses post-match, respectively, suggesting a training focus should be placed on these capacities.

Keywords: central fatigue; peripheral fatigue; non-motorized treadmill; activity profile; physical characteristics
6.3. Introduction

The activity profile of team sports is characterized by high-intensity intermittent running interspersed with directional changes, rapid accelerations and decelerations \([3, 45]\). These activities are associated with acute and more prolonged exercise-induced fatigue following a match \([7]\), and might already be present after the first half as suggested by modified activity profiles in the second half of matches \([3, 8]\). With players commonly participating in multiple games per week and undertaking large volumes of training, the prolonged exercise-induced post-match fatigue becomes a major factor in athlete management. Therefore, understanding the biological and perceptual responses to match play is important for optimizing the management of match-day performance, training and recovery \([14]\).

There have been multiple reports outlining the biological and perceptual responses to team-sport exercise \([8, 9]\). Biochemical variables such as creatine kinase (CK), uric acid (UA) and cortisol (C) have been shown to increase in response to match play, with reductions in testosterone (T) also observed \([11, 33]\). Reductions in post-match physical performance [assessed via countermovement jumps, strength testing and sprinting performance \([6, 33]\)] and increased perceptual fatigue \([8, 19]\) have been detected following team-sport activity. While recovery times vary, reports of incomplete recovery are common even at 72 h post-game \([11]\). However, while a player’s running performance might be maintained with short (3-4 day) breaks between games, this frequency of match play can greatly increase their injury risk \([13]\).

The post-match responses to team-sport activity suggest muscle damage occurs in response to the activity profile performed \([11]\). However, while reductions in force output following team-sport matches are often attributed to peripheral factors (e.g., muscle damage, glycogen depletion), more recent observations suggest central fatigue also contributes to the reduced post-match output \([8, 9, 175]\). Central fatigue can be defined as a reduction in voluntary activation occurring above the neuromuscular junction, while peripheral fatigue relates to changes occurring at or distal to this point, \([81]\). Central fatigue can be measured with peripheral nerve stimulation (i.e., twitch interpolation) to assess voluntary activation (%VA) of specific muscle groups. Reductions in %VA have been identified to varying degrees in both the laboratory following a match simulation and after a friendly soccer match \([8, 175]\). However, assessments performed after soccer matches have
occurred with considerable delay (i.e., 40 min) which may underestimate the magnitude of %VA reductions [8]. Using twitch interpolation, alterations in peripheral fatigue can be also assessed by stimulating the peripheral nerve of a resting muscle [8]. Minor delays in this assessment may also underestimate the magnitude of peripheral fatigue, as significant recovery can occur within 2-min post-exercise [37]. Therefore, assessment of peripheral fatigue must occur within this short time frame to ensure the assessment is valid.

While competitive team-sport matches present the most ecologically-valid modality to assess these responses, experimental control is compromised due to their inherent variability (e.g., scoreline and tactics influencing high-intensity running) [9]. Furthermore, the proximity of sporting fields to laboratories can delay data collection beyond an acceptable timeframe to obtain valid outcomes (as mentioned above), and limits the measures that can be collected. To overcome this limitation, half-time responses to match simulations have been assessed in a laboratory, but these responses are limited to muscle force and peripheral fatigue and are compromised by externally-paced match simulations utilizing motorized treadmills that limit both peak speed and acceleration [153]. Furthermore, traditional methods of statistical analysis (i.e., significance testing) may substantially under estimate the practical importance of observed decrements and result in incorrect conclusions regarding the presence of central and peripheral fatigue [153].

Hence, an appropriate laboratory-based match simulation [such as Tofari, McLean [300]] would allow for the collection of a variety of biological and perceptual responses following an ecologically valid performance within a timeframe that maximizes the opportunity for detecting change. The timely collection of these data would also allow the assessment of interactions between complex laboratory-based measures (i.e., twitch interpolation), simple performance tasks (e.g., countermovement jump) and activity performed during the match simulation (e.g., high speed running). Many of these relationships have not been elucidated to date. Therefore, the aim of this research was to assess neuromuscular, biochemical, performance and perceptual responses to a self-paced match simulation [300] by collecting this comprehensive data set immediately at half- and full-time, 2 hours post-match and at daily intervals for 4 days.
6.4. Methods

6.4.1. Experimental Approach to the Problem

Subjects were required to attend the laboratory on seven occasions over a two-week period. Week 1 consisted of two familiarization sessions separated by at least 48 hours. Week 2 consisted of a match-simulation protocol, followed by 4 consecutive days of follow-up testing. Subjects were requested to abstain from alcohol in the 24-hours prior to testing and caffeine 12-hours prior to the testing session and asked to consume a similar diet throughout the study.

6.4.2. Familiarization 1 and 2

Subjects performed a standardized warm-up including 3-min of self-paced running on the non-motorized treadmill (NMT) as well as dynamic lower-body stretches. During the first session, subjects undertook familiarization in the following order: magnetic stimulations and isometric knee extensions, countermovement jump (CMJ), squat jump (SJ), isometric mid-thigh pull (IMTP) and a 15-min portion of the non-motorized treadmill protocol. The second familiarization session began with the peak oxygen consumption ($\dot{V}O_{2\text{peak}}$) test (as described below). Ten minutes after the $\dot{V}O_{2\text{peak}}$ test, subjects completed the same familiarization as session 1. Familiarization during the isometric knee extension was continued until consistent maximal contractions (i.e., plateaued torque curve) were performed. Two sessions have been shown to be suitable for this technique [299]. Equally, two sessions of running on the NMT were performed to ensure confidence on the apparatus. However, one session has been demonstrated to provide sufficient familiarization [300].

6.4.3. Main Testing Session

Subjects completed a self-report wellness questionnaire (as described below) prior to performing the standardized warm-up. Pre-testing included: magnetic stimulation ramp protocol, quadriceps twitch interpolation assessment, CMJ, SJ, IMTP and blood sampling. Following pre-testing, subjects towel-dried and body mass (PW-200KGL, A&D Weighing, Kensington, Australia) was obtained while wearing shorts only. Heart rate (HR) was monitored throughout the match simulation. Subjects then performed the first half (45 min) of the NMT match simulation. Within
2-min post the first half, subjects completed the quadriceps twitch interpolation assessment, followed by CMJ, SJ and IMTP (total time ~5 min). Once the half-time testing was complete, subjects had a 15-min break prior to the second half. With 5-min remaining in the half-time break, subjects completed a submaximal self-paced 3-min treadmill run as a re warm-up. Approximately 1-min prior to beginning the second half, subjects gave their RPE for the first half. At the completion of the second half, the same testing as half-time was repeated. In addition, a second blood sample was obtained. Participant RPE for the full match-simulation was obtained 15-min after completion of the second half. At 2-h post the match-simulation, subjects completed the standardized warm-up followed by the quadriceps twitch interpolation assessment, CMJ, SJ, IMTP and a blood sample.

Subjects presented to the laboratory at the same time of day to perform follow-up testing for four consecutive days following the match-simulation protocol. This included quadriceps twitch interpolation assessment, CMJ, SJ, IMTP, blood sample and self-report questionnaire.

6.4.4. Subjects

Twelve amateur male team-sport athletes (24.5 ± 3.9 y, 76.8 ± 5.1 kg, \( \dot{V}O_{2\text{peak}} \) 52.3 ± 4.0 ml.kg\(^{-1}.\text{min}^{-1} \)) were recruited to participate in this study. These athletes had various sporting backgrounds (i.e., Australian Football, Soccer), and therefore, were experienced in completing intermittent team-sport activity. All subjects provided written informed consent and the research was approved by the University Research Ethics Committee.

6.4.5. Procedures

6.4.5.1. Peak oxygen consumption (\( \dot{V}O_{2\text{peak}} \)): Subjects completed an incremental run to exhaustion on a treadmill (Pulsar 3p, HP Cosmos, Nussdorf-Traunstein, Germany) while monitored via open-circuit spirometry (TrueOne 2400, Parvo Medics, Utah, USA) for assessment of \( \dot{V}O_{2\text{peak}} \) and for maximal HR using a HR monitor (Polar Team System, Polar Electro, Kempele, Finland). A run to exhaustion test was used as described previously \(^{[301]} \), which involved initial speed being set to 10 km\( \cdot \)h\(^{-1} \) with a grade of 1%
to more closely mimic overground running \cite{302}. Thereafter, speed was increased by 1 km•h\(^{-1}\) every min until volitional exhaustion.

6.4.5.2. **Neuromuscular function**

The method of neuromuscular function, comprising of a quadriceps twitch interpolation assessment, used in the current research has been described in detail previously and the variables associated with this method have acceptable reliability \cite{299}. Subjects were secured in a supine position on an isokinetic dynamometer (Humac Norm, Computer Sports Medicine, Inc., Massachusetts, USA) with their right knee set to 90° of flexion and right ankle fixed to the torque arm of the dynamometer just above the lateral malleolus. A magnetic coil (D70\(^2\), The Magstim Company Ltd, UK) powered by a magnetic stimulator (Magstim Bistim, The Magstim Company Ltd, UK) was positioned over the femoral triangle of each participant. The supine position allowed for proper placement of the magnetic coil in the femoral triangle. Torque, electromyography (EMG) signal and timing of magnetic stimulation was synchronized and collected through a custom software package (Labview 2013, National Instruments, Austin, TX).

To ensure the optimal magnetic stimulation location, twitches were administered on the femoral triangle at 50% of stimulator output to locate the highest torque and M-Wave peak-to-peak amplitude (PPA). This position was marked with indelible ink for subsequent stimulations. To confirm maximal twitches were delivered with the magnetic stimulator (i.e., plateau in twitch torque and M-wave PPA), a ramp test (consisting of two non-potentiated stimulations every 30 s at increasing intensities) was performed prior to the first testing session. Confirmation of a plateau during the ramp protocol (i.e., to confirm maximal stimulation) was assumed when the change from the last 2 stimulator intensities (95-100%) of the ramp protocol were smaller than the CV\% (4%) for the resting twitch \cite{299}.

EMG was recorded from the vastus lateralis using surface electrodes (Duo-Trode, NAOL, New South Wales, Australia) positioned according to SENIAM guidelines \cite{303}, connected directly to wireless probes that pre-amplified the signal (gain 400) and transmitted data in real-time to a wireless EMG system (Noraxon, Telemyo DTS, Arizona, USA). The measurement site was thoroughly prepared prior to collection by shaving and abrading the skin, and then cleaned with
swabbing alcohol and the location was marked with indelible ink to ensure identical placement on subsequent sessions. The M-wave PPA was measured during twitches and the EMG signal during each maximal voluntary contraction (MVC) was quantified by using the root mean square (RMS) calculated over a 1-s period after the torque had reached a plateau (RMS\textsubscript{MVC}). During post-processing, the EMG signals were rectified and filtered (bandwidth frequency =10–500 Hz). The RMS\textsubscript{MVC} was then normalized to the corresponding PPA by using the ratio RMS\textsubscript{MVC}/PPA.

The assessment required subjects to perform an isometric MVC lasting 5 s. Subjects were manually administered a single twitch (stimulator intensity of 100%) at a visually identified torque plateau during the MVC. The twitch was used to assess voluntary activation (%VA). At five seconds post-MVC, subjects were administered a potentiated twitch (POT). The following equation was used to calculate %VA: %VA = (1 – Superimposed Twitch/Potentiated Twitch) x 100, where the superimposed twitch was defined as the difference between the peak superimposed twitch force and the force averaged over 100 ms prior to that peak \cite{152}.

### 6.4.5.3. Performance testing

Subjects performed a CMJ on a force plate sampling at 600 Hz (400 Series Force Plate, Fitness Technologies, South Australia). Subjects were instructed to maintain their hands on hips throughout the jump, and jump as high as possible \cite{212}. Variables recorded included: jump height (cm), peak power relative to body mass (W\textperiodcentered kg\textsuperscript{-1}), peak force per kg (N\textperiodcentered kg\textsuperscript{-1}), flight time (s), and flight time:contraction time as per Cormack et al.\cite{6}. Following the CMJ, a SJ was performed on the force plate to determine the subjects concentric-only jump performance. The subjects maintained their hands on their hips and were instructed to squat down to a self-selected depth and hold the position for three seconds prior to a maximal jump \cite{212}. Variables recorded included: jump height (cm), peak power relative to body mass (W\textperiodcentered kg\textsuperscript{-1}), peak force per kg (N\textperiodcentered kg\textsuperscript{-1}) and flight time (s).

Maximal isometric strength (N\textperiodcentered kg\textsuperscript{-1}) was recorded using an isometric mid-thigh pull (IMTP) \cite{304}. Subjects stood on the force plate and held an immovable barbell fixed at mid-thigh height. The height of the bar was adjusted for each participant to allow a hip angle of ~155-165˚ and a knee angle of 125-135˚ and kept constant throughout the testing period. Subjects wore wrist straps to
assist their grip, and were instructed to pull up as hard and as fast as possible for approximately 5 s. All force-plate data was collected and analyzed using proprietary software (Ballistic Measurement System; Fitness Technology, Adelaide, Australia).

6.4.5.4. Biochemical analysis

An 8-mL sample of venous blood was drawn from an antecubital vein into serum-separating tubes on seven occasions (pre-, post-, 2h-post match simulation, and on days 1-4 following testing). The sample was left to clot at room temperature for 30 min before being centrifuged at 1500 G for 10 min at 4 °C. Serum samples were aliquoted into Eppendorf vials and frozen at -80 °C. The samples were returned to room temperature prior to analysis of testosterone, cortisol and uric acid (oxidative stress) using enzyme-linked immunosorbent assays (Abcam and Abnova corporations). Creatine kinase (CK) as a marker of muscle damage was determined using a Reflotron analyzer (Reflotron Plus, Bohringer-Mannheim, Indianapolis, IN, USA). The coefficient of variation (CV%) was calculated using 39 duplicate samples for creatine kinase, testosterone, cortisol and uric acid as 3.9, 4.8, 6.5 and 15.9%, respectively. The sensitivity of the testosterone assay was 0.05 ng/mL. The intra- and inter-assay reliability was <10% and <8.4% CV, respectively. The sensitivity of the cortisol assay was 1.5 ng/mL. The intra- and inter-assay reliability was <9.4% and <15% CV, respectively.

6.4.5.5. Perceptual response

Subjects provided a rating of perceived exertion (RPE) using Borg's category ratio 10-scale 15-min after the first half and following the match-simulation protocol [305]. Prior to the match-simulation and at the beginning of subsequent monitoring sessions, subjects were asked to complete a self-report questionnaire that assessed their fatigue, sleep quality, general muscle soreness, stress levels and mood on a five-point scale (scores of 1; poor to 5; very good), with overall well-being determined by summing the five scores [19].
6.4.5.6. **Match-simulation protocol**

Subjects performed a 90-min (two 45-min halves with 15-min rest between halves) match-simulation protocol on a non-motorized treadmill (Woodway Curve 3.0, Woodway, USA). This protocol was an extended version of a previously described, reliable protocol [300]. The protocol used visual and audible commands to direct participant locomotor speed (i.e., ‘Stand Still’, ‘Walk’, ‘Jog’, ‘Run’, ‘Sprint’), however, actual locomotor speeds were self-selected. This self-selected pacing allowed subjects to maintain their own pacing strategy throughout the match. Furthermore, the non-motorized treadmill allowed for maximal accelerations, decelerations and maximum speed running, not possible on a motorized treadmill. Modifications from the initial protocol [300] were to ensure the proportions of the match activity (e.g., run, sprint etc.) were equivalent to a soccer match. Before commencing the protocol, the visual cues and audible commands were explained to subjects and they were instructed that during ‘run’ periods they should perform a “hard run, as if attempting to reach the next contest within a game” and to “sprint maximally” during the defined sprint periods. This initial guidance was provided to assist subjects in differentiating between the discrete speed categories. Subjects were offered water on three occasions throughout each half. To allow for direct comparison with published literature, the following variables of the activity profile were assessed: total distance (TD), low-speed activity [under 14.4 km.h\(^{-1}\) (LSA)], high-speed running [over 14.4 km.h\(^{-1}\) (HSR)] and very-high speed running [over 20 km.h\(^{-1}\) (VHSR)] [306].

6.4.5.7. **Statistical Analyses**

All data were log-transformed to reduce bias because of non-uniformity of error and ES and % change ± 90% CL were calculated using a custom spreadsheet to assess the magnitude of change in all variables from initial pre-test values compared to other time points [307]. Effects of 0.2, 0.6 and > 1.2 were considered small, moderate and large, respectively. Effects of less than 0.2 were considered trivial and where the 90% CI overlapped the positive and the negative thresholds simultaneously the effect was deemed unclear [308]. Regression analysis was performed between match-simulation outcomes and performance variables. The magnitude of r ± 90% CL was classified as 0.1 to 0.3 small, 0.3 to 0.5 moderate, 0.5 to 0.7 large, 0.7 to 0.9 very large, and 0.9 to 0.99 nearly perfect [308], using an Excel spreadsheet [309].
6.5. **Results**

All data are displayed as ES or % change ± 90% CL unless otherwise stated.

6.5.1. *Match Simulation*

Subjects covered 12445.8 ± 768.7 m (mean ± SD) during the match-simulation protocol at 87.1 ± 3.2% (mean ± SD) maximal heart rate. Table 6.1 outlines changes between first and second half. There was a moderate reduction in total distance covered during the second half (-0.48 ± 0.28), including a reduction in very high-speed distance (-0.57 ± 0.47). There was a -2.3% ± 0.1% reduction in body mass Post compared to Pre (-0.32 ± 0.02).
Table 6.1: First and second half activity profile, heart rate, body mass (mean ± SD) and change from first to second half (ES ± 90% CI).

<table>
<thead>
<tr>
<th>Match Specific Variables</th>
<th>First Half Mean (± SD)</th>
<th>Second Half Mean (± SD)</th>
<th>Effect size (± 90% CL)</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Distance (m)</td>
<td>6321.17 (391.62)</td>
<td>6124.64 (409.50)</td>
<td>-0.48 (0.28) decrease</td>
<td>12</td>
</tr>
<tr>
<td>High-Speed Distance [(m) &gt;14.4 km.h⁻¹]</td>
<td>2430.83 (772.37)</td>
<td>2254.04 (650.22)</td>
<td>-0.19 (0.18) trivial</td>
<td>12</td>
</tr>
<tr>
<td>Very High-Speed Distance [(m) &gt;20 km.h⁻¹]</td>
<td>704.89 (308.04)</td>
<td>549.13 (343.07)</td>
<td>-0.57 (0.47) decrease</td>
<td>12</td>
</tr>
<tr>
<td>Low Speed Activity [(m) &lt;14.4 km.h⁻¹]</td>
<td>3890.34 (572.62)</td>
<td>3870.60 (497.04)</td>
<td>-0.02 (0.1) trivial</td>
<td>12</td>
</tr>
<tr>
<td>RPE(0-10)</td>
<td>6.13 (1.33)</td>
<td>7.50 (1.19)</td>
<td>0.84 (0.45) increase</td>
<td>12</td>
</tr>
<tr>
<td>HR Peak (bpm)</td>
<td>183 (6)</td>
<td>179 (7)</td>
<td>-0.59 (0.46) decrease</td>
<td>11</td>
</tr>
<tr>
<td>HR mean (bpm)</td>
<td>162 (6)</td>
<td>161 (6)</td>
<td>-0.16 (0.37) unclear</td>
<td>11</td>
</tr>
<tr>
<td>HR %max (%)</td>
<td>87.43 (3.44)</td>
<td>86.85 (3.39)</td>
<td>-0.15 (0.35) unclear</td>
<td>11</td>
</tr>
</tbody>
</table>

Descriptors in italics represent an increase, decrease, trivial or unclear change between first and second halves. Effects of 0.2, 0.6 and > 1.2 are considered small, moderate and large, respectively. Heart rate (HR), rating of perceived exertion (RPE), beats per minute (BPM), metres (m).
6.5.2. Neuromuscular Assessment

Table 6.2 and Figure 6.1 display changes observed during the quadriceps twitch interpolation assessment. All subjects showed a plateau in both torque and PPA during the ramp protocol. There was a reduction in MVC performance from half time through to Post24 compared to Pre (range, -0.32 to -0.58). There was a small reduction in %VA at half time, Post, Post2, Post48 and Post96 compared to Pre (% decrease -1.0 ± 1.2, -1.1 ± 1.7, -1.5 ± 1.3, -2.0 ± 2.0 and -1.1 ± 1.3, respectively). RMS\(_{MVC}/PPA\) calculated via EMG was reduced at half time, Post and Post2 compared to Pre (range: -0.29 ± 0.28 - -0.43 ± 0.28, see Table 6.2).

![Figure 6.1](image)

Figure 6.1: A) Maximal voluntary contraction, B) Voluntary activation, C) RMS\(_{PPA}/Mwave\) and D) Potentiated twitch Pre, half time (HT) and up to 4-days post a self-paced, match simulation protocol. Data are mean ± SD.
Table 6.2: Effect Size (± 90% CL) changes at half time to Post96 compared to Pre.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Half time</th>
<th>Post</th>
<th>Post1</th>
<th>Post24</th>
<th>Post48</th>
<th>Post72</th>
<th>Post96</th>
</tr>
</thead>
<tbody>
<tr>
<td>MVC (Nꞏm)</td>
<td>-0.49 (0.12)</td>
<td>decrease</td>
<td>-0.58 (0.25)</td>
<td>decrease</td>
<td>-0.32 (0.20)</td>
<td>decrease</td>
<td>-0.11 (0.26)</td>
</tr>
<tr>
<td>%VA (%)</td>
<td>-0.38 (0.46)</td>
<td>decrease</td>
<td>-0.55 (0.47)</td>
<td>decrease</td>
<td>-0.08 (0.33)</td>
<td>decrease</td>
<td>-0.75 (0.76)</td>
</tr>
<tr>
<td>Potentiated Twitch (Nꞏm)</td>
<td>-0.79 (0.30)</td>
<td>decrease</td>
<td>0.30 (0.36)</td>
<td>increase</td>
<td>-0.26 (0.42)</td>
<td>decrease</td>
<td>0.09 (0.42)</td>
</tr>
<tr>
<td>RMSMVC/PPA (mꞏs)</td>
<td>-0.29 (0.28)</td>
<td>decrease</td>
<td>-0.43 (0.28)</td>
<td>decrease</td>
<td>-0.09 (0.46)</td>
<td>decrease</td>
<td>0.12 (0.42)</td>
</tr>
<tr>
<td>CMJ Height (cm)</td>
<td>0.18 (0.25)</td>
<td>decrease</td>
<td>-0.14 (0.17)</td>
<td>decrease</td>
<td>-0.37 (0.16)</td>
<td>decrease</td>
<td>-0.14 (0.02)</td>
</tr>
<tr>
<td>SJ Height (cm)</td>
<td>0.42 (0.31)</td>
<td>increase</td>
<td>-0.32 (0.33)</td>
<td>decrease</td>
<td>-0.43 (0.20)</td>
<td>decrease</td>
<td>-0.20 (0.22)</td>
</tr>
<tr>
<td>IMTP Force (N.kg)</td>
<td>0.40 (0.20)</td>
<td>decrease</td>
<td>-0.35 (0.24)</td>
<td>decrease</td>
<td>-0.24 (0.30)</td>
<td>decrease</td>
<td>-0.13 (0.22)</td>
</tr>
<tr>
<td>Overall Wellness*</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Muscle Soreness*</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Fatigue*</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Testosterone</td>
<td>0.20 (0.17)</td>
<td>increase</td>
<td>-0.59 (0.35)</td>
<td>decrease</td>
<td>-0.18 (0.52)</td>
<td>decrease</td>
<td>-0.18 (0.38)</td>
</tr>
<tr>
<td>Cortisol</td>
<td>0.31 (0.32)</td>
<td>increase</td>
<td>-0.80 (0.41)</td>
<td>decrease</td>
<td>-0.28 (0.50)</td>
<td>decrease</td>
<td>-0.43 (0.53)</td>
</tr>
<tr>
<td>Uric Acid</td>
<td>0.66 (0.42)</td>
<td>increase</td>
<td>0.23 (0.50)</td>
<td>decrease</td>
<td>-0.22 (0.66)</td>
<td>decrease</td>
<td>-0.35 (0.65)</td>
</tr>
<tr>
<td>Creatine Kinase</td>
<td>0.85 (0.18)</td>
<td>increase</td>
<td>0.92 (0.26)</td>
<td>increase</td>
<td>1.11 (0.43)</td>
<td>increase</td>
<td>0.56 (0.41)</td>
</tr>
</tbody>
</table>

Descriptors in italics represent an increase, decrease, trivial or unclear change from Half time to Post96, when compared to Pre.
Effects of 0.2, 0.6 and > 1.2 are considered small, moderate and large, respectively. n/a indicates no data were collected for that time period.

*note: lower values indicate a negative response
6.5.3. Performance Testing

IMTP relative force was lower compared to Pre at half time through to Post24 (range, -0.24 to -0.41). CMJ height was reduced at Post24 compared to Pre (-0.37 ± 0.16). Squat jump height was lowest compared to Pre at Post24 (-0.43 ± 0.20), but was also decreased from half time through to Post96 (Table 6.2 and Figure 6.2).

Figure 6.2: Performance response Pre, half time (HT), and up to 4-days post in A) Countermovement jump (CMJ) height, B) Squat jump (SJ) height, and C) Isometric mid-thigh pull (IMTP) relative force. Data are mean ± SD.
6.5.4. Perceptual

Participant RPE at the end of the match was 7.5 (± 1.2) out of 10 (mean ± SD). There was a moderate reduction in overall wellness at Post24 (-0.50 ± 0.63), with unclear and trivial changes at all other time points. Muscle soreness and fatigue had large and moderate reductions up to Post48 (see Table 6.2).

6.5.5. Biochemical

Table 6.2 and Figure 6.3 display the biochemical response. There was an increase compared to Pre in CK at Post through to Post48, peaking at Post24 (1.11 ± 0.43). Uric acid increased moderately Post compared to Pre (0.66 ± 0.42), while changes from Post2 through to Post96 were unclear. Cortisol was elevated at Post (0.31 ± 0.32) but was suppressed at all other time points compared to Pre.

Figure 6.3: Biochemical response Pre, and up to 4-days post for A) Creatine Kinase, B) Uric Acid, C) Testosterone, D) Cortisol and E) T:C Ratio. Data are mean ± SD.
6.5.6. Correlations

Figure 6.4 displays correlations between CK and neuromuscular, performance and perceptual response. There were moderate correlations between change in CK and soreness at Post24 and Post48 compared to pre (r = 0.4 ± 0.44 and 0.34 ± 0.45, respectively). Changes in POT showed large to very large correlations with VHSR (range, r=-0.5 to -0.78) at half time – Post48, while IMTP relative force showed small to large correlations with POT at the same time points (r=-0.25 to -0.52). There were small to moderate negative correlations between POT and SJ Height changes at half time – Post2 (r=-0.23 to -0.50), becoming positive after this time point until Day3 (r=0.17 to 0.47). Change in SJ height also had a large correlation with change in %VA at Post (r=-0.61), with small correlations at all other time points (r=-0.01 to -0.23). Reductions in %VA at Post2 and Post48 correlated with TD (r=-0.42 and -0.5, respectively). Furthermore, reductions in MVC at Post had a very large correlation with reductions in %VA (r=0.74), but only small with POT (r=0.25). The reduction in CMJ height at Post24 had a small correlation with POT (r=-0.21). There were moderate to large correlations observed between $\hat{V}O_2$peak and perceptual responses at Post24 ($r = 0.44$ to 0.58), and $\hat{V}O_2$peak with RPE at the Post ($r = -0.71 \pm 0.28$). The amount of LSA performed was negatively associated with changes in overall wellness ($r=-0.40$ to -0.70), fatigue ($r=-0.16$ to -0.62) and soreness ($r=-0.20$ to -0.61).
Discussion

This work provides a comprehensive account of the responses to a self-paced match simulation that closely mimics the activity profile of soccer. The volume and intensity of running performed in the match-simulation protocol, as well as peak and mean HR, were similar or slightly greater than observed in midfielders during competitive soccer matches [8, 306]. Similarly, reductions in TD, HSR and VHSR during the second half compared to the first half of the protocol were similar to those reported in match play [3, 8]. As a result, it is likely that the findings from this work have implications for competitive on-field performance. The self-paced protocol allowed detection of previously unreported reductions in %VA and POT at half-time. In addition, the degree of reduction in %VA and POT induced by the first-half activity was maintained after the second half. Reduced performance during strength and power tasks (i.e., IMTP and SJ) were observed at half time, and persisted until Post24 and Post72, respectively. An important observation was the relationship between a reduced overall perceived wellness for those who performed more running under 14.4 km.h\(^{-1}\) (i.e., LSA). Furthermore, subjects with...
higher maximal lower-body strength and $\dot{V}O_{2\text{peak}}$ experienced less muscle damage (assessed via POT and CK) and lower levels of perceived exertion post-match, respectively.

This study is the first to assess and observe reductions in %VA using quadriceps twitch interpolation at half time. Critically however, the magnitude of this reduction did not change post-match. This suggests that central fatigue might have impacted second-half activity profiles (as observed by reductions in HSR and VHSR), resulting in the degree of %VA being maintained from half-time to post-match. Similar to %VA, performance variables (i.e., MVC, SJ, IMTP) were reduced equally or by a greater amount at half-time than full time. Likewise, POT was reduced more at half time than at full time. One study has previously assessed peripheral fatigue at half-time of a match-simulation (via electrical stimulation) which suggested no change in the potentiated twitch response \[153\]. However, the authors made inferences based on statistical significance (i.e., $p<0.05$) and, while this approach is common, it may underestimate practically meaningful effects \[153, 336\]. When their data \[153\] are calculated as an ES, it demonstrates a moderate (-0.73) and trivial (-0.16) reduction in potentiated twitch torque at half time and post-match, respectively. This magnitude of change is similar to our half-time data (-0.79 ± 0.30), although their post-match reduction was less. The smaller post-match reduction may be explained by the lower overall intensity of the externally paced protocol compared to the self-paced version used here, as demonstrated by the lower mean HR (83.9 ± 6.4% vs. 87.1 ± 3.2% HR max, respectively). The unique observation of reduced %VA and POT at half time shown here warrants investigation into their role in regulating second-half performance, including specific half-time interventions focused on recovering voluntary activation and potentiated twitch that may allow maintenance of second half exercise intensity \[337\].

The duration of reduced %VA in the current study is the longest reported, with a small decrease at Post, no change at Post24, a moderate secondary drop at Post48 and a small decline at Post96 (see Table 6.2). Although previous research \[8\] has assessed %VA, POT and RMS$_{\text{MVC}}$/PPA following a soccer match, their findings might underestimate the practical importance of the match-play changes due to the statistical approach used. For example, the authors showed reduced %VA at Post and Post24, but not beyond \[8\]. The prolonged reduction of %VA in our study might be due to the higher total distance covered (~12.5 km), compared to Rugby players covering ~6 km during an 80-min match who showed no changes in %VA post-match \[194\]. Interestingly TD ultimately correlated to reductions in %VA in the current study (see later discussion). The prolonged reductions in %VA (up to 96-h post-match) may have implications...
for athletes who compete in multiple games in a short time frame (i.e., less than 3 days), as research suggests match performance (i.e., high-intensity distance, sprint distance etc.) can be maintained with as little as 72-96 hours between matches, but the injury rate of the second match is much higher [13]. Future research assessing the impact of reduced %VA on subsequent training and/or match performance, as well as recovery modalities to restore %VA between matches, is warranted.

With respect to performance variables (i.e., SJ, IMTP, MVC), the half-time declines are similar to previous findings [153]. However, the degree of these reductions at half time was not accentuated after the second half, possibly due to reductions in %VA, POT and RMSMVC/PPA impacting second half exercise intensity and therefore limiting further decrements [7]. IMTP relative force and MVC had not recovered at Post24, which may be the result of muscle damage [227]. This contention is supported by the relationship between IMTP relative force and CK (see Figure 6.4). Squat jump and CMJ performance showed a markedly different pattern following the match simulation. While the change in CMJ height was overwhelmingly trivial (except for a small decrease at Post24), SJ height was decreased by a small magnitude from half time until Post72. This disparity may be due to the different contraction modes employed in the SJ and CMJ [153]. Utilization of the stretch shortening cycle (SSC) may allow the maintenance of performance in the CMJ despite reduced force production capacity, as evidenced by reductions in IMTP and MVC occurring up to Post24 [338]. The correlation between SJ and POT (r=-0.5 at Post) and CK (r=-0.6 at Post) up until Post24 suggest muscle damage follows a similar time-course to the suppression of SJ performance. However, the highest shared variance between SJ and CK (approximately 36% at Post2) implies factors other than muscle damage influence explosive performance following simulated team sport match play. The magnitude of reductions seen in SJ height are similar to those in %VA (r² = 37.5% at Post) potentially indicating a central role in mediating performance on this task. Importantly, both microtrauma and central factors seem to have less impact on CMJ performance, and this may be due to similar mechanisms mentioned above. Moreover, maximal lower-body strength is suggested to limit muscle damage caused by repetitive SSC activity, and may have moderated the response in SJ and CMJ to the match simulation [45]. While previous research assessing CMJ height following a match simulation showed reductions up to 72 h post, the maximal lower-body strength of subjects was much higher in our study (36.2 ± 4.6 N.kg⁻¹ vs ~ 21.1 N.kg⁻¹), hence potentially moderating the deleterious effect of the repetitive SSC activities in the match [67].
Reductions in MVC observed post-match were mainly related to central ($r^2 = 55\%$) rather than peripheral factors ($r^2 = 6.3\%$), which is in agreement with previous findings [$r^2 = 74\%$ and $7.3\%$, respectively, $^{[8]}$]. Furthermore, the volume of VHSR was related to peripheral fatigue, explaining $47\%$ and $61\%$ of the decrease in POT at half time and Post24, respectively. This is most likely due to the high-intensity SSC movements involved in VHSR which have resulted in muscle damage $^{[215]}$. Greater reduction in %VA after the match were associated with higher TD, which has previously been shown following a soccer match $^{[8]}$. Given stronger athletes in our study also incurred less muscle damage, taken together this highlights the importance of an athlete’s underlying physical capacity on the biological and perceptual response to a match play.

Changes in CK have been used as an indirect marker of muscle damage following a soccer match, and generally display an immediate and often persistent (multiple days post) increase $^{[9, 11]}$. In this study, subjects showed a similar response, with the increase peaking (ES $1.11 \pm 0.43$) at Post24, and returning to pre-match levels at Post72. While CK has been previously been shown to relate to the volume of HSR $^{[22]}$, this was not the case here ($r = 0.09$ at Post24). As mentioned previously, the greater lower-body strength of the subjects in this study may have attenuated the CK response to HSR $^{[45]}$. In fact, baseline values of IMTP relative force were able to predict between 33-65\% of the variance in CK following the match simulation, supporting the suggestion that greater lower-body strength has a protective effect on muscle damage $^{[45]}$.

Testosterone, cortisol, and uric acid all showed increases immediately following the match simulation. Beyond Post, the magnitude of change in testosterone and uric acid was mostly unclear due to large individual variation $^{[336]}$. However, contrary to our findings, reductions in testosterone are common following team-sport exercise $^{[6]}$. Work assessing sub-elite soccer players suggests that increases in testosterone are possible, potentially due to the stimulus obtained from sprinting efforts performed (similar to a strength training response) $^{[22]}$. The similar caliber of athletes and number of sprints performed (84 times during the 90 min) in the current work might explain the observed increase in testosterone post-match. In respect to cortisol, there was an initial post-match increase, similar to that seen in a competitive match $^{[11]}$, followed by a decrease at all other post-match time points when compared to pre-test values. However, given cortisol was not measured prior to pre-match, it is unclear whether these initial values were a true representation of basal levels $^{[339]}$. The amateur status of the athletes might have induced greater pre-match, stress-related elevation of cortisol than would occur in more elite performers $^{[339]}$. Therefore, elevated pre-match cortisol may have accentuated the
measured post-match reductions observed from 2 hours onwards. Elevations in uric acid can indicate oxidative stress as part of the inflammatory response post-exercise \[^{10}\], and the results of the current study are consistent with those from simulations and competitive matches \[^{10, 11, 67}\]. Compared to actual soccer matches, the elevation of uric acid in our study and other treadmill-based simulations was more transient and may be due to the absence of soccer-specific actions (e.g., jumping, kicking, tackling) \[^{67}\]. It should be noted that there was a lack of meaningful relationships observed between biochemical markers and the activity profile of the match simulation, and this is likely the result of large individual variation in the biochemical response (see Figure 6.3).

A unique finding of this study was the association between low speed activity and a reduced perception of overall wellness and increased soreness at Post\(_{24}\) \(r^2 = 48\%\) and 37\%, respectively). It is plausible that athletes who self-selected a higher volume of LSA did so because of an inability to maintain a faster pace, but paid a relatively higher price (i.e., increased soreness) for this intensity compared to fitter athletes \[^{45}\]. Similarly, athletes with a lower \(\dot{V}_O^{\text{peak}}\) reported higher RPE values and greater soreness, fatigue and reduced wellness at Post\(_{24}\). There is support for this in an investigation with Futsal players, reporting lower RPE values in players with higher aerobic fitness \[^{278}\].

In conclusion, this study provides a comprehensive account of the post-match responses to a self-paced simulation, highlighting responses and unique relationships not previously reported due to limitations of earlier protocols. Due to the nature of the self-paced protocol, it is likely that these findings will have implications for competitive on-field performance. A unique finding is the half-time reductions in %VA (central fatigue) and POT (peripheral fatigue). Subsequently, the activity profile of the second half was modified without further reductions in these measures. Critically, VHSR running distance was associated with higher levels of peripheral fatigue (POT) while total distance was associated with reductions in %VA. Furthermore, %VA was still reduced at Post\(_{96}\), which could have implications for subsequent training sessions and matches scheduled within a short period of time. Reduced central drive was also associated with lower post-match IMTP force. This work also suggests that SJ height and perceptual soreness may be valuable to assess post-match, as they correlate with variations in exercise intensity. The high variability in other biochemical variables limits their usefulness for monitoring athlete status, but might be of interest if assessed over a longer period (i.e., multiple weeks or a season).
6.7. Addendums Following Review

- **Results:** No testosterone:cortisol ratio data was included in the original table that was published. However, the data was presented in figure 6.3, panel E. Below are these data as they would have appeared in table 6.2.

<table>
<thead>
<tr>
<th></th>
<th>Half time</th>
<th>Post</th>
<th>Post2</th>
<th>Post24</th>
<th>Post48</th>
<th>Post72</th>
<th>Post96</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone:Cortisol Ratio</td>
<td>-0.09 (0.31)</td>
<td>0.22 (0.53)</td>
<td>0.18 (0.62)</td>
<td>0.22 (0.66)</td>
<td>0.27 (0.54)</td>
<td>0.46 (0.58)</td>
<td></td>
</tr>
</tbody>
</table>

- **Results:** Some correlations that appeared in section 6.5.6 did not contain their confidence intervals. Where these correlations appeared individually, and not as part of the description of a range of correlations, the confidence intervals have been added below and are underlined.

Change in SJ height also had a large correlation with change in %VA at Post ($r=-0.61 \pm 0.35$), with small correlations at all other time points ($r=-0.01$ to -0.23). Reductions in %VA at Post2 and Post48 correlated with TD ($r=-0.42 \pm 0.43$ and -0.5 ± 0.40, respectively). Furthermore, reductions in MVC at Post had a very large correlation with reductions in %VA ($r=0.74 \pm 0.26$), but only small with POT ($r=0.25 \pm 0.48$). The reduction in CMJ height at Post24 had a small correlation with POT ($r=-0.21 \pm 0.48$).

- **Discussion:** As mentioned in section 6.4.4, the athletes who completed this study were amateur team-sport athletes. As a result, it is possible that their response to the match simulation protocol completed herein may vary from professional athletes.

- **Discussion:** The manuscript currently states: “The unique observation of reduced %VA and POT at half time shown here warrants investigation into their role in regulating second-half performance…” For clarity, it should be noted that the authors refer to central (%VA) and peripheral (POT) fatigue as the factors warranting further investigation, as %VA and POT are the indices used to assess those variables.
7. Chapter 7: Study 4 - Biological and Perceptual Responses to Simulated Fixture Congestion in Soccer

Publication statement:

This chapter is comprised of the following paper under review in *Frontiers: Physiology*.

7.1. Linking Paragraph

The outcomes of Study 3 suggest that central and peripheral fatigue are present at half-time in a match simulation, which has not been previously described. Furthermore, greater lower-body strength and aerobic capacity are associated with less muscle damage and smaller reductions in perceived wellness, respectively. Additionally, the activity profiles and post-match responses observed in Study 3 are similar to those reported following competitive match play. This highlights the ability of the self-paced match simulation to replicate a soccer match. In professional soccer, players are often required to compete in multiple matches during a short period of time (e.g., 72-96 hours). However, a multi-faceted analysis of the within- and post-match responses to fixture congestion have not been explored. Study 4 aimed to determine the biological and perceptual responses to a period of simulated fixture congestion using the match simulation and procedures developed in Studies 1, 2 and 3.
7.2. Abstract

Competitive soccer matches result in acute biological and perceptual disturbances, lasting up to 96-h. Yet, multiple games are often played with as little as 72-h rest. Contextual factors cause match variability, limiting comparisons between competitive matches. And, the assessment of within- and post-match responses are compromised in a competitive environment. To overcome these issues, this work assessed the within- and post-match responses to two self-paced match simulations in a 72-hour period. Eleven amateur male team-sport athletes (24.4±3.8 y, 82.4±8.4 kg, $\dot{V}O_{2\text{peak}}$ 53.8±3.5 ml.kg$^{-1}$.min$^{-1}$) attended eight consecutive testing sessions, after familiarisation. Assessments occurred at pre-, half-time, post- and 2 h post-match simulation, daily between matches and for four-days after match 2. Change compared to pre-match 1 was calculated using effect size ±90% confidence limit, and relationships were assessed using regression analysis. Central and peripheral fatigue were evident at half time in both matches, defined as reduced voluntary activation (match 1: -1.52±1.41 and match 2: -0.50±0.58) and potentiated twitch torque (match 1: -0.50±0.37 and match 2: -0.31±0.37), respectively. These half-time changes may be responsible for decreased running volume and intensity in the second halves. The difference in activity profiles between matches was unclear. Both matches resulted in acute neuromuscular, biochemical, perceptual and performance decrements, similar in magnitude and duration to competitive matches. Additionally, the magnitude and duration of the response to the second match was lower than the first. Higher lower-body strength was associated with reduced perceived general muscle soreness and fatigue at all time points (range: $r = 0.27$ to 0.69), as well as lower peripheral fatigue. Velocity at $V_{O2\text{peak}}$ showed a negative association with circulating creatine kinase levels ($r$ range = -0.28 to -0.70). These results suggest 72-h recovery is sufficient to maintain activity profiles between matches. Further, lower-body strength and aerobic capacity displayed protective qualities against post-match decrements, which may have implications for training prescription.
7.3. Introduction

The high-intensity and intermittent nature of soccer activity profiles are associated with exercise-induced fatigue [5, 9]. Reductions in total and high-intensity running distances in the second half of matches compared to the first suggests players experience acute fatigue within matches [8]. Numerous investigations have profiled the biochemical, perceptual and peripheral (e.g., muscle damage) responses to soccer matches and match simulations [22, 63, 234]. These studies describe a variety of outcomes, including: reductions in lower body strength and power; alterations to biochemical markers of muscle damage and hormonal status; and increased perceived soreness and fatigue [22, 63, 234]. There changes can be present from immediately post-match, and are often still significantly altered for 24 to at least 72 hours post-match [10, 11].

The aforementioned responses are indicative of muscle impairment occurring during soccer match play [11]. Traditionally, these responses have been attributed to peripheral alterations; that is, changes occurring at or distal to the neuromuscular junction, such as modifications in excitation-contraction coupling, ionic fluctuations, neuromuscular propagation failure, etc. [81, 340]. However, spinal and supraspinal factors (i.e., central fatigue) also contribute to reduced force production post-exercise [172]. To assess peripheral and central contributions of neuromuscular fatigue, procedures such as transcranial magnetic stimulation and the interpolated twitch technique can be used [118, 172]. The interpolated twitch technique involves superimposing an electrical or magnetic stimulation to a peripheral nerve innervating a muscle group of interest during a maximal contraction and comparing the superimposed twitch amplitude to a potentiated resting twitch performed 2 to 5 s after, establishing the level of voluntary activation (%VA) [8]. This method, though a surrogate measure of central fatigue due to the location of the stimulation in relation to the motor cortex, can still provide valuable information regarding central fatigue [341]. Additionally, as force production during the potentiated twitch occurs without volition, data recorded can be interpreted as peripheral muscle function [37].

The time-course of central and peripheral fatigue assessed via peripheral nerve stimulation following soccer matches and match simulations has recently been described [8, 78, 342, 343]. Alterations in force production, %VA and potentiated twitch torque were present following the soccer matches, generally recovering by 48 h [8, 343]. Similar responses were observed following match simulations; however, some variables (such as resting twitch and %VA) were reduced for up to 96 h post-match [78, 342]. While soccer matches provide the most ecologically-valid
approach to assess post-match fatigue, the delay in performing assessments and variation in activity profiles from match to match may problematic for data collection and interpretation \[198\]. Consequently, match simulations based on soccer activity profiles are conducted to allow prompt laboratory-based post-match data collection, together with the potential for half-time measures to be assessed. However, these simulations are often externally paced; that is, speeds are dictated to the athletes \[64\]. Pacing in competitive soccer matches can be influenced by the playing standard, opposition, score line and match importance \[9, 70\]. Additionally, when matched for intensity and duration, externally-paced exercise can increase physiological strain over self-paced exercise \[154\]. Therefore, a self-paced protocol [e.g., Tofari, Kemp \[342\]] may be beneficial for replicating competitive match demands and assessing the post-match response in a laboratory environment. Furthermore, due to the consistency afforded by match simulations, they may be useful for assessing repeat measures, such as the impact of recovery interventions or periods of fixture congestion.

In high-level soccer competitions, athletes are often required to play multiple games separated by only 48 to 72 hours \[34\]. Based on the time course of recovery following soccer matches, it is plausible that these players may still be experiencing residual fatigue due to the first match. However, research assessing fixture congestion in soccer players suggests that the volume and intensity of running is maintained between matches, but the risk of injury is greater during the second game \[13, 344, 345\]. There is limited work outlining the concomitant perceptual, biochemical and neuromuscular responses to fixture congestion \[48\]. Finally, no data exist describing the central and peripheral contributions of post-match fatigue in periods of fixture congestion.

This research aims to describe the cause and time course of biochemical, perceptual, performance and neuromuscular responses during and following a period of simulated fixture congestion. Interactions between the post-match responses, as well as interactions between the activity profile performed and these responses will be examined. Furthermore, the impact of pre-test measures of physical qualities on the activity profile performed will be assessed, as well as the subsequent response to the matches. To achieve this, a self-paced soccer match simulation on a curved, non-motorised treadmill will be used, allowing untethered and unrestricted running to better simulate the activity profile of competitive match play.
7.4. Method

7.4.1. Experimental Approach to the Problem

Subjects were required to attend the laboratory on twelve occasions over a three-week period. The two initial sessions consisted of familiarisation separated by at least 48 hours. The experimental component consisted of eight consecutive days and began at least 4 days after familiarisation. This consisted of two match-simulation protocols separated by 72 h. Follow-up testing occurred between matches, and for four consecutive days after the second match. Subjects were requested to abstain from alcohol in the 24 hours and caffeine in the 12-hours prior to the testing session and asked to consume a similar diet throughout the study. No other exercise was permitted during the experimental period.

7.4.2. Familiarization 1 and 2

Subjects performed a standardized warm-up including 3-min of self-paced running on the non-motorized treadmill (NMT; Woodway Curve 3.0, Woodway, USA) as well as dynamic lower-body stretches. During the first session, subjects undertook familiarization in the following order: magnetic stimulations and isometric knee extensions, countermovement jump (CMJ), squat jump (SJ), isometric mid-thigh pull (IMTP) and a 15-min portion of the non-motorized treadmill protocol. The second familiarization session began with the peak oxygen consumption (VO2peak) test. At least ten minutes after the VO2peak test, subjects completed the same familiarization as session 1. Familiarization during the isometric knee extension was continued until consistent maximal contractions (i.e., plateaued torque curve) were performed. Two sessions have been shown to be suitable for this technique [299]. Similarly, two sessions of running on the NMT were performed to ensure confidence on the apparatus, although it has been demonstrated that only one is required [300].

7.4.3. Simulated Match Sessions

Subjects completed a self-report wellness questionnaire (as described below) prior to performing the standardized warm-up. Pre-testing included: blood sampling, magnetic stimulation ramp protocol (match 1 only), quadriceps interpolated twitch assessment, CMJ, SJ and IMTP. Following pre-testing, subjects towel-dried and body mass (PW-200KGL, A&D Weighing, Kensington, Australia) was obtained while wearing shorts only. Heart rate (HR) was monitored throughout the match simulation. Subjects then performed the first half (45 min) of
the NMT match simulation. Within 2-min post first half, subjects completed the quadriceps twitch interpolation assessment, followed by CMJ, SJ and IMTP (total time ~5 min). Once the half-time testing was complete, subjects had a 15-min break prior to the second half. With 5 min remaining in the half-time break, subjects completed a submaximal self-paced 3-min treadmill run as a re warm-up. Approximately 1-min prior to beginning the second half, subjects gave their RPE for the first half. At the completion of the second half, the same testing as half-time was repeated, with the addition of a blood sample. Rating of perceived exertion specific to the second half and to the full match-simulation was obtained 15-min after completion of the second half. Participants were provided with a standardized carbohydrate (1.2 g of CHO per kg of body mass) and protein (30 g) beverage to control their initial post-match meal. At 2-h post match-simulation a blood sample was taken, then subjects completed the standardized warm-up followed by the quadriceps interpolated twitch assessment, CMJ, SJ and IMTP.

Subjects presented to the laboratory at the same time of day as the start of the match simulation to perform follow-up testing for the two days between matches, and for four consecutive days following the second match-simulation protocol. This testing included a self-report questionnaire (see 3.5.5 below), blood sample, quadriceps interpolated twitch assessment, CMJ, SJ and IMTP.

7.4.4. Subjects

Eleven amateur male team-sport athletes (24.4 ± 3.8 y, 82.4 ± 8.4 kg, \( \dot{\text{VO}}_{2\text{peak}} \) 53.8 ± 3.5 ml.kg\(^{-1}\).min\(^{-1}\)) were recruited to participate in this study. These athletes had various team sport backgrounds (i.e., Australian Football, Soccer) and, therefore, were experienced in completing intermittent team-sport activity. This study was carried out in accordance with the recommendations of the Australian Government, National Health and Medical Research Council with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Australian Catholic University Human Research Ethics Committee (2013 191V).

7.4.5. Procedures

7.4.5.1. Peak oxygen consumption (\( \dot{\text{VO}}_{2\text{peak}} \)):

Subjects completed an incremental run to exhaustion on a treadmill (Pulsar 3p, HP Cosmos, Nussdorf-Traunstein, Germany) while monitored via open-circuit spirometry (TrueOne 2400, Parvo Medics, Utah, USA) for assessment of \( \dot{\text{VO}}_{2\text{peak}} \) and for maximal HR using a HR monitor.
(Polar Team System, Polar Electro, Kempele, Finland). A run to exhaustion test was used as described previously [301], which involved initial speed being set to 10 km•h\(^{-1}\) with a grade of 1\% to more closely mimic overground running [302]. Thereafter, speed was increased by 1 km•h\(^{-1}\) every min until volitional exhaustion.

7.4.5.2. Neuromuscular function

The method of neuromuscular function, comprising of a quadriceps interpolated twitch assessment, used in the current research has been described in detail previously and the variables associated with this method have acceptable reliability [299]. Subjects were secured in a supine position on an isokinetic dynamometer (Biodex System 4 Quick Set, Biodex Medical Systems Inc, New York, USA) with their right knee set to 90˚ of flexion and right ankle fixed to the torque arm of the dynamometer just above the lateral malleolus. A magnetic coil (D702, The Magstim Company Ltd, UK) powered by a magnetic stimulator (Magstim BiStim, The Magstim Company Ltd, UK) was positioned over the femoral triangle of each participant. The supine position allowed for proper placement of the magnetic coil in the femoral triangle. Torque, electromyography (EMG; Noraxon, Telemyo DTS, Arizona, USA) signal and timing of magnetic stimulation was synchronized and collected through a custom software package (Labview 2013, National Instruments, Austin, TX).

To ensure the optimal magnetic stimulation location, twitches were administered on the femoral triangle at 50\% of stimulator output to locate the highest torque and M-Wave peak-to-peak amplitude (PPA). This position was marked with indelible ink for subsequent stimulations. To confirm maximal twitches were delivered with the magnetic stimulator (i.e., plateau in twitch torque and M-wave PPA), a ramp test (consisting of two non-potentiated stimulations every 30 s at increasing intensities) was performed prior to the first testing session. Confirmation of a plateau during the ramp protocol (i.e., to confirm maximal stimulation) was assumed when the change from the last 2 stimulator intensities (95-100\%) of the ramp protocol were smaller than the CV\% (4\%) for the resting twitch [299].

EMG was recorded from the vastus lateralis using surface electrodes (Duo-Trode, NAOL, New South Wales, Australia) positioned according to SENIAM guidelines [303], connected directly to wireless probes that pre-amplified the signal (gain 400) and transmitted data in real-time to a wireless EMG system (Noraxon, Telemyo DTS, Arizona, USA). The measurement site was thoroughly prepared prior to collection by shaving and abrading the skin, and then cleaned with
swabbing alcohol, and the location was marked with indelible ink to ensure identical placement on subsequent sessions. The M-wave PPA was measured during twitches, and the EMG signal during each maximal voluntary contraction (MVC) was quantified by using the root mean square (RMS) calculated over a 1-s period after the torque had reached a plateau (RMS\text{MVC}). During post-processing, the EMG signals were rectified and filtered (bandwidth frequency =10–500 Hz). The RMS\text{MVC} was then normalized to the corresponding PPA by using the ratio RMS\text{MVC}/PPA.

The assessment required subjects to perform an isometric MVC lasting 5 s. Subjects were manually administered a single twitch (stimulator intensity of 100%) at a visually identified torque plateau during the MVC. The twitch was used to assess voluntary activation (%VA). At five seconds post-MVC, subjects were administered a potentiated twitch (POT). The following equation was used to calculate %VA: %VA = (1 – Superimposed Twitch/Potentiated Twitch) x 100, where the superimposed twitch was defined as the difference between the peak superimposed twitch force and the force averaged over 100 ms prior to that peak \cite{152}. If the twitch was administered prior to or following the plateau in MVC, a correction was applied to the equation as per Strojnik and Komi \cite{182}.

7.4.5.3. Performance testing

Subjects performed a CMJ on a force plate sampling at 600 Hz (400 Series Force Plate, Fitness Technologies, South Australia). Subjects were instructed to maintain their hands on hips throughout the jump, and jump as high as possible \cite{212}. Variables recorded included: jump height (cm), peak power relative to body mass (W·kg\text{−1}), peak force per kg (N·kg\text{−1}), flight time (s), and flight time:contraction time \cite{6}. Following the CMJ, a SJ was performed on the force plate to determine concentric-only jump performance. The subjects maintained their hands on their hips and were instructed to squat down to a self-selected depth and hold the position for three seconds prior to a maximal jump \cite{212}. Variables recorded included: jump height (cm), peak power relative to body mass (W·kg\text{−1}), peak force per kg (N·kg\text{−1}) and flight time (s).

Maximal isometric strength (N·kg\text{−1}) was recorded using an IMTP \cite{304}. Subjects stood on the force plate and held an immovable barbell fixed at mid-thigh height. The height of the bar was adjusted for each participant to allow a hip angle of 155-165° and a knee angle of 125-135° and kept constant throughout the testing period. Subjects wore wrist straps to assist their grip, and were instructed to pull up as hard and as fast as possible for approximately 5 s. All force-plate
data were collected and analyzed using proprietary software (Ballistic Measurement System; Fitness Technology, Adelaide, Australia).

7.4.5.4. **Biochemical analysis**

An 8-mL sample of venous blood was drawn from an antecubital vein into serum-separating tubes on twelve occasions (pre-, post-, 2h-post match simulations, every other day during the experimental period). The sample was left to clot at room temperature for 30 min before being centrifuged at 1500 G for 10 min at 4 °C. Serum samples were aliquoted into Eppendorf vials and frozen at -80 °C. The samples were returned to room temperature prior to analysis of uric acid and creatine kinase via enzymatic assay, and testosterone and cortisol via immunoassay (Siemens Healthcare Diagnostics). The coefficient of variation (CV%) was < 1.3%, < 4.2%, < 20% and < 6% for uric acid, creatine kinase, testosterone and cortisol, respectively.

7.4.5.5. **Perceptual response**

Participants provided a rating of perceived exertion (RPE) using Borg's category ratio 10-scale at 15-min after the first half and 15 min following the match-simulation protocol [305]. Post-match, participants were asked to independently rate their RPE for the second half and for the whole match.

Prior to the match-simulation and at the beginning of subsequent monitoring sessions, subjects were asked to complete a self-report questionnaire that assessed their fatigue, sleep quality, general muscle soreness, stress levels and mood on a five-point scale (scores of 1; poor to 5; very good), with overall well-being determined by summing the five scores [19].

7.4.5.6. **Match-simulation protocol**

Subjects performed a 90-min (two 45-min halves with 15-min rest between halves) match-simulation protocol on a curved non-motorized treadmill (Woodway Curve 3.0, Woodway, USA). This protocol was an extended version of a previously described, reliable protocol [300]. The protocol used visual and audible commands to direct participant locomotor speed (i.e., ‘Stand Still’, ‘Walk’, ‘Jog’, ‘Run’, ‘Sprint’), however, actual locomotor speeds were self-selected. This self-selected pacing allowed subjects to maintain their own pacing strategy throughout the match. Furthermore, the non-motorized treadmill allowed for maximal
accelerations, decelerations and maximum speed running, not possible on a motorized treadmill. Modifications from the initial protocol [300] were to ensure the proportions of the match activity (e.g., run, sprint, etc.) were equivalent to a soccer match. Before commencing the protocol, the visual cues and audible commands were explained to subjects and they were instructed that during ‘run’ periods they should perform a “hard run, as if attempting to reach the next contest within a game” and to “sprint maximally” during the defined sprint periods. This initial guidance was provided to assist subjects in differentiating between the discrete speed categories. Subjects were offered water on three occasions throughout each half. To allow for direct comparison with published literature, the following variables of the activity profile were assessed: total distance (TD), low-speed activity [under 14.4 km.h\(^{-1}\) (LSA)], high-speed running [14.4 km.h\(^{-1}\) to 20 km.h\(^{-1}\) (HSR)], very-high speed running [over 20 km.h\(^{-1}\) (VHSR)] and hard accelerations (>2.78 m.s\(^{2}\)) [59, 306].

7.4.5.7. Statistical Analyses

All data were log-transformed to reduce bias because of non-uniformity of error and effect size (ES) ± 90% confidence limits (CL) were calculated using a custom spreadsheet to assess the magnitude of change in all variables from initial pre-test values compared to other time points [307]. Effects of 0.2, 0.6 and > 1.2 were considered small, moderate and large, respectively. Effects of less than 0.2 were considered trivial, and where the 90% CL overlapped the positive and the negative thresholds simultaneously the effect was deemed unclear [308]. Regression analysis was performed between match-simulation outcomes and physical qualities. The magnitude of r ± 90% CL was classified as 0.1 to 0.3 small, 0.3 to 0.5 moderate, 0.5 to 0.7 large, 0.7 to 0.9 very large, and 0.9 to 0.99 nearly perfect [308], using an Excel spreadsheet [309].
7.5. Results

7.5.1. Match running variables

Table 7.1 outlines the activity profile variables (mean ±SD) during both match simulations and Table 7.2 displays the ES ±90% CL change in physiological and perceptual variables relating to the two match simulations. Total distance, very high-speed running, high-speed running, average acceleration and deceleration < -2.78 m.s\(^{-2}\) and > 2.78 m.s\(^{-2}\) was greater in the first half than the second half in both matches (small to moderate effect, see Figure 7.5). Total distance was greater in match 2 than match 1 (ES 0.71 ± 0.64). However, changes in low-speed activity, high-speed running and very high-speed running in match 2 compared to match 1 were unclear (see Figure 7.1).

Average heart rate as a percent of maximum was lower in match 2 than match 1, (ES -1.06 ± 0.54), while between half differences were unclear and trivial for match 1 and match 2, respectively. Rating of perceived exertion was higher in the second than the first half in both matches, but the difference between match 1 and match 2 was unclear (see Figure 7.1).
Table 7.1: Match variables recorded during the two match simulations separated by 72 h.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Match 1 First Half</th>
<th>Match 1 Second Half</th>
<th>Match 1 Total</th>
<th>Match 2 First Half</th>
<th>Match 2 Second Half</th>
<th>Match 2 Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Distance (m)</td>
<td>6482.81 ± 245.40</td>
<td>6267.10 ± 494.34</td>
<td>12749.91 ± 717.68</td>
<td>6700.64 ± 456.11</td>
<td>6441.80 ± 482.51</td>
<td>13142.44 ± 923.64</td>
</tr>
<tr>
<td>High-Speed Running [(m) 14.4 to 20 km.h⁻¹]</td>
<td>1625.26 ± 513.20</td>
<td>1424.49 ± 598.97</td>
<td>3049.75 ± 1052.31</td>
<td>1691.06 ± 735.36</td>
<td>1515.04 ± 700.18</td>
<td>3206.10 ± 1413.46</td>
</tr>
<tr>
<td>Very High-Speed Running [(m) &gt; 20 km.h⁻¹]</td>
<td>1038.55 ± 210.42</td>
<td>937.71 ± 387.48</td>
<td>1976.26 ± 585.16</td>
<td>1103.53 ± 283.52</td>
<td>982.62 ± 356.32</td>
<td>2086.15 ± 616.90</td>
</tr>
<tr>
<td>Low-Speed Activity [(m) &lt; 14.4 km.h⁻¹]</td>
<td>3819.01 ± 418.37</td>
<td>3904.90 ± 377.62</td>
<td>7723.90 ± 775.85</td>
<td>3906.05 ± 545.17</td>
<td>3944.13 ± 498.91</td>
<td>7850.19 ± 1012.51</td>
</tr>
<tr>
<td>Mean Heart Rate (%Max)</td>
<td>86.16 ± 2.35</td>
<td>85.49 ± 3.36</td>
<td>85.83 ± 2.57</td>
<td>83.32 ± 3.14</td>
<td>83.12 ± 3.57</td>
<td>83.22 ± 3.31</td>
</tr>
<tr>
<td>RPE [(AU) 0 – 10]</td>
<td>6.55 ± 1.06</td>
<td>7.00 ± 1.41</td>
<td>7.09 ± 1.22</td>
<td>6.32 ± 1.55</td>
<td>7.05 ± 1.93</td>
<td>6.95 ± 1.71</td>
</tr>
<tr>
<td>Mean Acceleration and Deceleration (m.s²)</td>
<td>1.10 ± 0.09</td>
<td>1.07 ± 0.10</td>
<td>1.09 ± 0.09</td>
<td>1.14 ± 0.10</td>
<td>1.11 ± 0.09</td>
<td>1.13 ± 0.09</td>
</tr>
<tr>
<td>Acceleration/Deceleration &gt;2.78 m.s² (s)</td>
<td>122.63 ± 28.55</td>
<td>114.69 ± 28.98</td>
<td>237.32 ± 56.42</td>
<td>129.43 ± 28.04</td>
<td>120.62 ± 28.65</td>
<td>250.05 ± 56.24</td>
</tr>
</tbody>
</table>

Data presented are mean ± SD.
Table 7.2: Effect size ± CL at all time points compared to match 1 pre-test values.

<table>
<thead>
<tr>
<th></th>
<th>M1half</th>
<th>M1post</th>
<th>M12h</th>
<th>M124h</th>
<th>M148h</th>
<th>M2pre</th>
<th>M2half</th>
<th>M2post</th>
<th>M22h</th>
<th>M224h</th>
<th>M248h</th>
<th>M272h</th>
<th>M296h</th>
</tr>
</thead>
<tbody>
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<td><strong>PERFORMANCE</strong></td>
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<td></td>
</tr>
<tr>
<td>IMTP N.kg⁻¹</td>
<td>-0.38</td>
<td>-0.41</td>
<td>-0.52</td>
<td>-0.30</td>
<td>-0.32</td>
<td>-0.13</td>
<td>-0.48</td>
<td>-0.56</td>
<td>-0.68</td>
<td>-0.37</td>
<td>-0.35</td>
<td>-0.02</td>
<td>0.07</td>
</tr>
<tr>
<td>SJ Height (cm)</td>
<td>-0.34</td>
<td>-0.22</td>
<td>-0.16</td>
<td>-0.20</td>
<td>-0.18</td>
<td>-0.12</td>
<td>-0.27</td>
<td>-0.18</td>
<td>-0.14</td>
<td>-0.14</td>
<td>-0.15</td>
<td>-0.01</td>
<td>0.07</td>
</tr>
<tr>
<td>CMJ (N.kg⁻¹)</td>
<td>-0.27</td>
<td>0.09±</td>
<td>-0.27</td>
<td>-0.37</td>
<td>-0.24</td>
<td>-0.22</td>
<td>-0.47</td>
<td>-0.31</td>
<td>-0.27</td>
<td>-0.44</td>
<td>-0.23</td>
<td>-0.15</td>
<td>-0.21</td>
</tr>
<tr>
<td>CMJ Height (cm)</td>
<td>-0.08</td>
<td>-0.02</td>
<td>-0.04</td>
<td>-0.04</td>
<td>0.05</td>
<td>0.09</td>
<td>-0.12</td>
<td>-0.11</td>
<td>0.11</td>
<td>0.01</td>
<td>0.02</td>
<td>0.12</td>
<td>0.16</td>
</tr>
<tr>
<td>CMJ FT:CT</td>
<td>0.01</td>
<td>0.12</td>
<td>0.03</td>
<td>-0.12</td>
<td>0.03</td>
<td>-0.01</td>
<td>-0.19</td>
<td>-0.14</td>
<td>0.05</td>
<td>-0.14</td>
<td>-0.10</td>
<td>-0.03</td>
<td>0.07</td>
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<tr>
<td><strong>BIOCHEMICAL</strong></td>
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<td></td>
</tr>
<tr>
<td>Creatine Kinase (U/L)</td>
<td>-0.86</td>
<td>0.99</td>
<td>1.36</td>
<td>0.70</td>
<td>0.27</td>
<td>-</td>
<td>1.05</td>
<td>1.19</td>
<td>1.49</td>
<td>0.88</td>
<td>0.46</td>
<td>0.45</td>
<td></td>
</tr>
<tr>
<td>Testosterone (nmol/L)</td>
<td>-0.46</td>
<td>-0.73</td>
<td>0.14</td>
<td>0.14</td>
<td>0.12</td>
<td>-</td>
<td>0.47</td>
<td>-0.60</td>
<td>-0.12</td>
<td>0.10</td>
<td>0.15</td>
<td>0.29</td>
<td></td>
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<tr>
<td>Testosterone:Cortisol (au)</td>
<td>-0.79</td>
<td>-0.43</td>
<td>0.59</td>
<td>0.49</td>
<td>0.27</td>
<td>-</td>
<td>-0.27</td>
<td>0.01</td>
<td>-0.02</td>
<td>0.42</td>
<td>0.42</td>
<td>0.57</td>
<td></td>
</tr>
<tr>
<td>Cortisol (nmol/L)</td>
<td>1.19</td>
<td>-0.23</td>
<td>-0.46</td>
<td>-0.35</td>
<td>-0.16</td>
<td>-</td>
<td>0.68</td>
<td>-0.54</td>
<td>-0.09</td>
<td>-0.33</td>
<td>-0.29</td>
<td>-0.30</td>
<td></td>
</tr>
<tr>
<td>Uric Acid (mmol/L)</td>
<td>0.89</td>
<td>0.85</td>
<td>0.25</td>
<td>-0.07</td>
<td>-0.18</td>
<td>-</td>
<td>0.53</td>
<td>0.49</td>
<td>0.15</td>
<td>-0.10</td>
<td>-0.18</td>
<td>0.06</td>
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</tr>
</tbody>
</table>
Table 10: continued:

<table>
<thead>
<tr>
<th></th>
<th>M₁half</th>
<th>M₁post</th>
<th>M₁₂h</th>
<th>M₁₄₈h</th>
<th>M₂pre</th>
<th>M₂half</th>
<th>M₂post</th>
<th>M₂₂h</th>
<th>M₂₄₈h</th>
<th>M₂₇₂h</th>
<th>M₂₉₆h</th>
</tr>
</thead>
<tbody>
<tr>
<td>WELLNESS</td>
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<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Fatigue</td>
<td>- - -</td>
<td>- -</td>
<td>-0.97</td>
<td>-0.26</td>
<td>-0.01</td>
<td>-0.74</td>
<td>-0.16</td>
<td>-0.05</td>
<td>0.15</td>
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<tr>
<td></td>
<td>± 0.69</td>
<td>± 0.65</td>
<td>± 0.65</td>
<td>± 0.65</td>
<td></td>
<td>± 0.64</td>
<td>± 0.63</td>
<td>± 0.72</td>
<td>± 0.65</td>
<td></td>
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</tr>
<tr>
<td>Sleep</td>
<td>- - -</td>
<td>- -</td>
<td>-1.14</td>
<td>-1.10</td>
<td>-1.59</td>
<td>-3.05</td>
<td>-1.53</td>
<td>-1.4</td>
<td>-0.69</td>
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<td></td>
<td>± 1.02</td>
<td>± 1.01</td>
<td>± 0.66</td>
<td></td>
<td></td>
<td>± 2.15</td>
<td>± 1.34</td>
<td>± 1.02</td>
<td>± 0.68</td>
<td></td>
<td></td>
</tr>
<tr>
<td>General Muscle Soreness</td>
<td>- - -</td>
<td>- -</td>
<td>-2.59</td>
<td>-1.55</td>
<td>-0.69</td>
<td>-1.86</td>
<td>-1.08</td>
<td>-0.44</td>
<td>-0.12</td>
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<tr>
<td></td>
<td>± 1.16</td>
<td>± 1.20</td>
<td>± 0.87</td>
<td></td>
<td></td>
<td>± 1.06</td>
<td>± 1.2</td>
<td>± 0.92</td>
<td>± 0.83</td>
<td></td>
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</tr>
<tr>
<td>Overall</td>
<td>- - -</td>
<td>- -</td>
<td>-1.29</td>
<td>-0.69</td>
<td>-0.48</td>
<td>-1.43</td>
<td>-0.83</td>
<td>-0.48</td>
<td>-0.03</td>
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<tr>
<td></td>
<td>± 0.76</td>
<td>± 0.81</td>
<td>± 0.47</td>
<td></td>
<td></td>
<td>± 0.75</td>
<td>± 0.91</td>
<td>± 0.85</td>
<td>± 0.56</td>
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<tr>
<td>NEUROMUSCULAR</td>
<td></td>
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</tr>
<tr>
<td>MVC (N.m⁻¹)</td>
<td>-0.15</td>
<td>-0.11</td>
<td>-0.02</td>
<td>0.06</td>
<td>0.03</td>
<td>-0.14</td>
<td>-0.18</td>
<td>-0.08</td>
<td>0.07</td>
<td>0.08</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>± 0.17</td>
<td>± 0.13</td>
<td>± 0.13</td>
<td>± 0.14</td>
<td>± 0.15</td>
<td>± 0.15</td>
<td>± 0.14</td>
<td>± 0.19</td>
<td>± 0.2</td>
<td>± 0.17</td>
<td>± 0.19</td>
</tr>
<tr>
<td>Voluntary activation (%)</td>
<td>-1.52</td>
<td>-1.29</td>
<td>-0.41</td>
<td>-0.64</td>
<td>-0.57</td>
<td>-0.15</td>
<td>-0.50</td>
<td>-0.94</td>
<td>-0.40</td>
<td>0.18</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>± 1.41</td>
<td>± 0.73</td>
<td>± 0.45</td>
<td>± 0.62</td>
<td>± 1.14</td>
<td>± 0.54</td>
<td>± 0.74</td>
<td>± 0.60</td>
<td>± 0.62</td>
<td>± 0.66</td>
<td>± 0.45</td>
</tr>
<tr>
<td>Potentiated twitch (N.m⁻¹)</td>
<td>-0.50</td>
<td>-0.19</td>
<td>0.06</td>
<td>-0.07</td>
<td>-0.19</td>
<td>0.13</td>
<td>-0.31</td>
<td>0.39</td>
<td>0.31</td>
<td>0.10</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>± 0.37</td>
<td>± 0.34</td>
<td>± 0.22</td>
<td>± 0.21</td>
<td>± 0.50</td>
<td>± 0.24</td>
<td>± 0.37</td>
<td>± 0.54</td>
<td>± 0.17</td>
<td>± 0.21</td>
<td>± 0.24</td>
</tr>
</tbody>
</table>

Isometric mid-thigh pull (IMTP), squat jump (SJ), countermovement jump (CMJ), maximal voluntary contraction (MVC). “ – “ indicates no data were collected for that time period.
Figure 7.1: Effect size changes ± 90%CL for within match variables across halves within matches, across halves between matches and overall between matches. ACC = acceleration, LSA = low-speed activity, HSR = high-speed running, VHSR = very high-speed running, TD = total distance, HR = heart rate, RPE = rate of perceived exertion.
7.5.1.1. Neuromuscular fatigue

Figure 7.2 displays the individual change in neuromuscular fatigue variables. Changes in maximal voluntary contraction torque were trivial at all time points compared to M1 pre (see Table 7.2). Potentiated twitch torque was reduced at M1 half compared to M1 pre (ES -0.34 ± 0.29); however, the change at M1 post was trivial. There was a small reduction at M2 half and M2 post compared to M1 pre (ES -0.31 ± 0.37 and -0.39 ± 0.54, respectively). There was a reduction in %VA at M1 half, M1 post, M124 h, M2 half and M2 post compared to M1 pre (range: 2.8 – 8.3% reduction). All other changes in %VA were unclear.

Figure 7.2: Changes in variables associated with the quadriceps femoris assessed via the interpolated twitch technique after two matches played within 72 hours. MVC = maximal voluntary contraction, %VA = voluntary activation.
7.5.1.2. Performance tests

Squat jump height was reduced at M1\(_{\text{half}}\), M1\(_{\text{post}}\), M1\(_{24\text{h}}\) and M2\(_{\text{half}}\) compared to M1\(_{\text{pre}}\) (ES range -0.20 to -0.34). Countermovement jump relative peak force was reduced at various points throughout the experimental period compared to pre-testing (see Table 7.2). However, countermovement jump height remained trivial.

Figure 7.3: Response in performance tests assessed using a force plate following two matches played in 72 hours. IMTP = isometric mid-thigh pull, SJ = squat jump, CMJ = countermovement jump.
7.5.1.3. **Biochemical markers**

Figure 7.4 presents the individual responses in biochemical variables. Table 7.2 displays the ES ± 90% CL change in biochemical variables compared to M1pre. Creatine kinase increased after the first match and remained elevated throughout the study, peaking at M124h and M224h (ES 1.36 ± 0.5 and 1.49 ± 0.57, respectively). Uric acid was elevated for 24 h after match 1, and 2-h after match 2 and was unclear or trivial at other time points (see Table 7.2). Testosterone was elevated immediately following both matches compared to pre-testing, but was clearly reduced at 2-h post in both cases (see Table 7.2). Cortisol was increased at M1post and M2post compared to M1pre (ES 1.19 ± 0.59 and 0.68 ± 0.59, respectively).

7.5.1.1. **Wellness questionnaire**

Participants reported a reduction in their overall perceived wellness compared to M1pre for all time points, except M296h (see Table 7.2). Moderate to large negative reductions in sleep were also reported at all time points compared to M1pre (ES range: -0.69 to -3.05). An increase in fatigue and general muscle soreness was reported up to 24-h and 48-h post both matches compared to M1pre, respectively.
Figure 7.4: Biochemical response to two match simulations separated by 72 hours.
7.5.1.2.  Correlations

Pre-game wellness and activity profiles

Table 7.3 displays the relationship between pre-match wellness scores and activity profiles completed during both matches. There was a moderate positive correlation between overall wellness at M1_pre and total distance, high-speed running, and both acceleration variables in match 1 (r range: 0.34 – 0.47; see Table 7.3). Overall wellness at M2_pre was positively correlated with HSR (r = 0.36) and acceleration variables (r = 0.49) in match 2, and there was a large negative correlation (r = -0.52) between low-speed activity in match 2 and overall wellness at M2_pre (see Table 7.3).

Physical qualities and activity profile

Table 7.4 displays the correlations between pre-match physical qualities and the activity profiles of both simulations. A large and very large relationship between VO2peak and high-speed running in match 1 (r = 0.52 ± 0.41) and match 2 (0.72 ± 0.29) was observed, respectively. There was a very large relationship between vVO2 with total distance and very-high speed running (r range: 0.72 to 0.80). Absolute IMTP force at M1_pre was negatively correlated with low-speed activity in both matches (r = -0.52 and -0.56 for match 1 and 2, respectively), and positively correlated with acceleration > 2.78 m.s^-2 (r = 0.45 and 0.53 for match 1 and 2, respectively).

Correlations between physical qualities and percent changes in activity profile variables between first and second halves are shown in Table 7.5. The change in very-high speed running was positively correlated with vVO2 in both match 1 and match 2 (r = 0.66 and 0.60, respectively). Isometric mid-thigh pull force relative to body mass was positively correlated with low-speed activity in both match 1 (r = 0.42 ± 0.45) and match 2 (0.73 ± 0.29).
Table 7.3: Relationship between pre-match wellness scores and activity profile variables

<table>
<thead>
<tr>
<th>MATCH 1</th>
<th>Total Distance (m)</th>
<th>Low-speed activity (m)</th>
<th>High-speed running (m)</th>
<th>Mean acceleration (m.s²)</th>
<th>Acceleration &gt;2.78 m.s²</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1_pre Wellness</td>
<td>0.39</td>
<td>0.06</td>
<td>0.34</td>
<td>-0.22</td>
<td>0.45</td>
</tr>
</tbody>
</table>

Table 7.4: Relationship between physical qualities and activity profile variables

<table>
<thead>
<tr>
<th>Physical quality</th>
<th>V̇O₂peak</th>
<th>vVO₂</th>
<th>IMTP (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activity variable</td>
<td>VHSR</td>
<td>TD</td>
<td>VHSR</td>
</tr>
<tr>
<td>Match 1</td>
<td>0.52</td>
<td>0.72</td>
<td>0.80</td>
</tr>
<tr>
<td>Match 2</td>
<td>0.72</td>
<td>0.78</td>
<td>0.77</td>
</tr>
</tbody>
</table>

Very high-speed running (VHSR), Total distance (TD), Acceleration (accel), Low-speed activity (LSA).
**Physical qualities and activity profile with post-match responses**

Figure 7.5 displays correlations between baseline levels of strength and fitness (i.e., IMTP N.kg\(^{-1}\) and \(v\text{VO}_2\)) with various post-match responses. Lower-body strength was positively correlated at all time points with perceived general muscle soreness and fatigue (range: \(r = 0.27\) to 0.69). Velocity at \(\text{VO}_2\text{peak}\) showed a negative association with creatine kinase levels (range = -0.28 to -0.70).

There was a small to large inverse relationship between total distance covered in each match with change in %VA at M1\(_{\text{post}}\) (\(r = -0.28\)) and M2\(_{\text{post}}\) (\(r = -0.59\)). A small to moderate relationship existed between very high-speed running and potentiated twitch torque at M1\(_{\text{post}}\) (-0.18) and M2\(_{\text{post}}\) (-0.34).

**Different methods of post-match assessment**

There was a large relationship between creatine kinase and general muscle soreness 24-h after both matches (\(r = 0.61\) and 0.68). Changes in squat jump height were related to changes in %VA at M1\(_{2\text{h}}\) and M2\(_{2\text{h}}\) (\(r = 0.29\) and 0.61, respectively).

Table 7.5: Relationship between physical qualities and between-half changes in running variables

<table>
<thead>
<tr>
<th></th>
<th>(\text{VO}_2\text{peak})</th>
<th>(v\text{VO}_2)</th>
<th>IMTP (N.kg(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Match 1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\Delta) Total distance</td>
<td>0.12</td>
<td>0.71</td>
<td>-0.16</td>
</tr>
<tr>
<td>(\Delta) LSA</td>
<td>0.03</td>
<td>-0.26</td>
<td>0.48</td>
</tr>
<tr>
<td>(\Delta) VHSR</td>
<td>0.53</td>
<td>0.66</td>
<td>0.07</td>
</tr>
<tr>
<td><strong>Match 2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\Delta) Total distance</td>
<td>0.43</td>
<td>0.30</td>
<td>0.57</td>
</tr>
<tr>
<td>(\Delta) LSA</td>
<td>0.06</td>
<td>-0.36</td>
<td>0.73</td>
</tr>
<tr>
<td>(\Delta) VHSR</td>
<td>0.34</td>
<td>0.60</td>
<td>0.04</td>
</tr>
</tbody>
</table>
Figure 7.5: Correlations ($r$) between pre-match physical qualities and changes in perceptual, neuromuscular and biochemical markers. IMTP = isometric mid-thigh pull, GMS = general muscle soreness, Pot = potentiated twitch torque.
7.6. Discussion

This study assessed the magnitude and duration of biochemical, perceptual, performance and neuromuscular responses to a period of simulated fixture congestion in soccer. To achieve this, a reliable self-paced match simulation was performed twice, separated by 72 h, in a laboratory setting allowing the collection of data at half-time, as well as immediately post-match, without considerable time delays (i.e., < 2 mins). Meaningful changes were observed in the majority of variables assessed, including central and peripheral fatigue at half-time in both matches, with most recovering by 72 h following both match 1 and match 2. Additionally, the study design allowed for relationships to be assessed between physical qualities, activity profile and post-match recovery data which highlighted associations that may have implications for athlete preparation.

7.6.1. Match Running Variables

Reductions in total distance and high-intensity running from the first to second half of a match have been observed in elite soccer, especially in players who perform more running in the first half [162, 306, 346]. There was a similar pattern during both matches in this study. Previous work has suggested that alterations between match halves may be due to a combination of pacing and fatigue [155]. In the present work, we observed a reduction in all locomotor speed categories between halvess, except low-speed activity (see Figure 7.5), with the magnitude of the responses assessed at half-time being similar to post-match (e.g., %VA, see Table 7.2). It is possible that the participants modified their second-half activity to avoid any further deleterious physiological effects, which is supported by models of fatigue models that suggest exercise intensity is regulated by conscious decisions (such as the Psychobiological Model of fatigue) or subconscious processes (Central Governor Model of fatigue) [79, 138].

Research pertaining to fixture congestion has generally focused on activity profiling [346]. Similar to previous findings, participants in the current study were able to maintain the volume and intensity of running from match to match [34, 347]. Although the participants experienced considerable perturbations to homeostasis following the first match simulation, the majority of these had returned to baseline levels (i.e., trivial difference) before the beginning of the second match (see Table 7.2). It is plausible that the similarity in activity profiles between matches is a function of adequate recovery occurring in the 72 h between matches. Alternatively, the participants’ ability to maintain their running volume may be attributed to adopting a pacing strategy within games that allowed them to manage their fatigue levels [348].
Based on our data, it is difficult to determine whether fatigue or pacing is responsible for reduced second half running in each match and the maintenance of activity profiles between matches. However, it is likely that a combination of these factors contribute to changes in activity profiles within and across matches [155].

7.6.2. Neuromuscular Function

In line with previous findings, central fatigue (as measured by %VA) was present at half-time in the first match [342]. After 24 h, the change in %VA became unclear and remained so until pre-testing prior to match 2. Previous work assessing central fatigue post-match in competitive soccer match performance has displayed recovery by 48 h post-match [8, 343], but longer in other match simulations [78, 342]. One explanation for the observed response following competitive matches may be that the interpretation of the results were performed using traditional statistical analysis (i.e., p values), underestimating the practical importance of the outcomes. Additionally, previous work that has presented effect size changes between variables did so without displaying the confidence limits, which are important for interpretation [343]. While another match simulation observed prolonged declines in %VA [78], they incorporated forced, maximal decelerations in their protocol that potentially increased the demand above actual soccer match play. Two previous studies have identified a relationship between overall running volume and the magnitude of %VA decrements post-match [8, 342]. We also observed an association between the time course of %VA changes and total distance; however, the distance performed by our participants was ~8% higher than in a competitive match [8], which may further explain the difference in duration of the %VA decrement. Additionally, the unclear outcomes seen in the present work may be a function of the individual variation in response to the activity profiles performed (see Figure 7.2). For example, three of our participants displayed reduced %VA at M2pre (range: -3.8 to -12.8%), which is near or exceeds the CV% of this measure from our lab [CV% = 4.4%, see Tofari, Opar [299]]. As a result, the assessment of responses following competitive matches should be interpreted on an individual basis.

Reduced potentiated twitch torque was evident at half-time, but the change at M1post was unclear. Previous research has shown a larger reduction at half-time than post-match [342]. This may be due to the reduced activity profile of the second half in response to the fatigue incurred in the first half, or the adoption of a pacing strategy to reduce any further fatigue being induced from that observed at half-time [79]. There was a trivial difference in potentiated twitch torque by 24 h following match 1, which is in line with the response to an actual soccer match [8]. However, other studies have measured reduced twitch torque for up to 48 h, attributing the
reductions to muscle damage occurring during soccer match play [78, 343]. This disparity may be explained by the lower-body strength of the participants in the present work. Although no data exist to directly compare the studies, the participants in our work had similar lower-body strength to collegiate weightlifters [349]. Such lower-body strength may have protected the quadriceps from muscle damage associated with eccentric muscle actions performed during running [45]. This is further supported by only trivial changes observed in maximal voluntary contractions in this specific muscle group (see Table 7.2).

Voluntary activation was reduced from half-time in match 2 until 2-h post-match compared to M1 pre. The magnitude of the response to match 2 was lower than match 1 at half time (ES ± 90%CL - 0.50 ± 0.35) and the difference between matches was unclear at post-match (ES ± 90%CL 0.20 ± 0.71). This suggests, at least at half time, there was a smaller reduction of %VA to match 2 despite the similar activity profile performed during both matches. Limited work exists to explain this blunting of the post-match responses to a second match in a short time frame. One study that assessed the soreness and strength of youth soccer players following two matches in 72 h described reduced soreness and strength deficits following the second match [350]. However, they did not quantify the activity profiles performed. Another explanation could be that a repeated bout effect occurred following the first match, blunting some of the decrements to match 2 [351]. Previous work with trained team-sport athletes has observed a blunted perceptual pain response in two intermittent sprint tests separated by 14 days, but similar responses in other variables [352]. Although the current study does not confirm the presence of a repeated bout effect, further work may be warranted to establish whether this phenomenon exists in trained athletes following intermittent exercise, as they are still susceptible to muscle damage and soreness even though they are accustomed to the stimulus.

The second match simulation also resulted in reduced potentiated twitch torque, similar to that observed following match 1, suggesting some peripheral alterations occurred following both match simulations. However, towards the end of the data collection period, a small increase in potentiated twitch torque was observed (M272h and M296h compared to M1 pre). Increases in potentiated twitch torque have been observed following an 8-week strength training program [353]. It is possible that the athletes gained a small training effect throughout the study period resulting in some supercompensation towards the end of data collection [354].
7.6.3. Performance Tests

There was a reduction in relative IMTP force for 48 h after both matches. While force output is not a direct measure of muscle damage, it has been purported as a valid and reliable indicator of muscle damage \[^{227}\]. The post-match response seen here is similar to previous work using an identical protocol \[^{342}\]. However, others have reported no change in IMTP force following an externally-paced protocol on a tethered, non-motorised treadmill \[^{67}\]. The version of the non-motorised treadmill used in the aforementioned study [Woodway Force \[^{67}\]] requires the participant to wear a tether to assist them in overcoming the large inertial load of the treadmill belt \[^{311}\]. As a result, the requirement to forcefully decelerate may be reduced on that apparatus due to the high inertial load, in turn reducing muscle damage related to forceful eccentric muscle actions \[^{9}\]. Furthermore, the protocol used in the current study incorporated 714 speed changes, including 84 sprints. In comparison, the aforementioned study included 493 speed changes with 44 sprints \[^{67}\]. It is possible that the combination of reduced deceleration force, as well as fewer accelerations and decelerations, may have jointly contributed to the difference in lower-body force changes post-match between the two studies.

Small reductions in squat jump height were observed at various time points (see Table 7.1). Previous work has suggested that squat jump performance may be centrally mediated, as the time-course of responses were similar to changes in %VA \[^{342}\]. The present work also shows an association between the time-course of changes in the two measures. Together, these responses suggest that jumping performed without the stretch-shortening cycle provides a sensitive measure of post-match fatigue. On the other hand, previous work has suggested that activities incorporating the stretch-shortening cycle (e.g., countermovement jumps) may be useful for detecting neuromuscular fatigue \[^{215}\]. However, changes in countermovement jump height were trivial throughout our study period. Therefore, although our data set indicated that athletes experienced central and peripheral fatigue within- and post-match, countermovement jump height was not sensitive enough to detect this. As previous researchers have found, other variables assessed from countermovement jumps, such as the flight time:contraction time ratio, may be more sensitive than jump height alone \[^{214}\]. This is evidenced by unclear results in this metric in the current study, highlighting individual variation. Another explanation for the trivial response in countermovement jump height that we observed may be the lower-body strength of our participants, mitigating some of the damage incurred by repetitive stretch-shortening cycle exercise such as running \[^{46, 355}\]. In fact, peak lower-body force in the present study was 39.5
N.kg\(^{-1}\), almost 25% higher than that of participants who displayed reduced countermovement-jump performance after a match simulation [\(\sim 31\) N.kg\(^{-1}\); Nedelec, Wisloff [67]].

7.6.4. Biochemical Responses

Both match simulations resulted in large and prolonged biochemical alterations, consistent with previous findings following soccer matches. For example, acute increases in creatine kinase and uric acid were observed in response to the activity profiles. Although often assessed as a marker of muscle damage, interpretation of creatine kinase concentrations for this purpose is problematic, due largely to the variability in release rates from the muscle and clearance rates in the bloodstream [227]. In this study, creatine kinase was elevated after match 1 and remained so until M296h. However, the activity profiles of match 2 were similar to match 1 in spite of the prolonged, elevated creatine kinase concentrations. This may suggest that, although creatine kinase is responsive to the activity profiles performed in soccer matches, other simpler and more affordable tools, such as performance measures, may provide adequate information relating to muscle damage.

The acute increase in uric acid concentrations provide evidence of oxidative stress, which is common following soccer matches [10, 67, 240]. While acute oxidative stress is considered an important trigger for the body’s adaptive processes, chronic increases may potentially block this adaptation [241, 242]. We observed unclear results for uric acid concentrations at the end of data collection (i.e., M296h), suggesting some participants may still have had elevated levels at this time. Therefore, monitoring oxidative stress over a longer period may be useful for assessing the athletes’ training status and informing modifications to training load.

Acute disturbances to hormonal status were also present following both matches, similar to those measured in professional soccer players [214]. The testosterone:cortisol ratio has been suggested as a potential marker for monitoring overreaching, and providing an indication of the anabolic/catabolic state of the athlete [273]. We observed larger increases in cortisol compared to testosterone suggesting that the participants were in a catabolic state following the initial match [273]. However, these markers are subject to large individual variation, resulting in multiple unclear results in this study, and others [214, 342]. Furthermore, evidence supporting the use of the testosterone:cortisol to determine training status is conflicting [356]. Nonetheless, monitoring biochemical markers such as uric acid, testosterone and cortisol longer-term (e.g., over a competitive season), may be useful to instigate interventions aimed at recovering
oxidative stress or hormonal status [16, 357]. Importantly, the individual variation observed here highlights the need to assess the responses on an individual basis.

7.6.5. Perceptual Responses

A simple and cost-effective method to monitor an athlete’s response to training and competition load is by having them complete a wellness questionnaire [287]. Short, custom questionnaires, such as the five-question survey used here, are purported to be more practical than longer alternatives (e.g., Profile of Mood State questionnaire) [19, 282]. Using this five-question survey, reduced perceived wellness was observed throughout the study period, which is an important finding given the negative association with subsequent performance [123]. In fact, pre-match perceived wellness in the present study had a small to moderate positive association with high-speed running and hard accelerations (see Table 7.3). A recent investigation in Australian Football players supports this notion, highlighting the utility of simple wellness questionnaires to ascertain player readiness to upcoming sessions [282]. Therefore, these questionnaires present a useful monitoring tool for coaches. However, the subjective nature of this tool may be manipulated by players in order to indicate readiness for match selection, and, therefore, should be interpreted in combination with other monitoring tools (e.g., performance tests) [170].

The participants’ rating of perceived exertion was higher in the second half of both matches than the first half (see Figure 7.5). This occurred alongside a lower running volume performed in the both second halves (see Table 7.1). While this reduction in running volume is likely related to fatigue induced by the first half of the match, it is also likely that the fatigue modified the participants’ perception of effort for a given workload in the second half [32]. Previous work assessing the impact of mental fatigue, induced via the AX-continuous performance test, on intermittent running performance has described higher perceived exertion for a lower volume of running when compared to a non-fatigued state [32]. While we did not assess mental fatigue in the present work, it is likely that the perceptual fatigue contributed to the biological fatigue experienced during the first half and played a role in the reductions in running volume and intensity during the second half of the matches [32].

7.6.6. Influence of Physical Qualities

Physical qualities, such as aerobic capacity and lower-body strength, are known to positively influence team-sport related activities, including distances covered at high intensities, play
involvement, technical skills, acceleration and sprinting \[^{39, 358-360}\]. Our data support this, demonstrating associations between physical qualities and these activity profile measures (see Table 4). Given the ability to perform these activities is considered important for professional soccer players \[^{39, 59}\]. These findings suggest that practitioners should specifically focus some training on the development of these physical qualities.

Although there has been considerable exploration of the influence that physical qualities have on activity profiles during team-sports, there is a paucity of data concerning the effect of these qualities on the post-match response \[^{45, 342}\]. In this study, both vVO\(_2\) and lower-body force were related to post-match responses, suggesting that these physical qualities may mitigate some of the deleterious effects of a soccer match simulation (see Figure 7.2). Similar outcomes have been observed in other work using an identical protocol, demonstrating reduced muscle damage and lower perceived exertion in stronger and more aerobically fit participants, respectively \[^{342}\]. Specific to the present work, greater relative lower-body force was associated with less perceived soreness and smaller reductions in potentiated twitch torque. Similarly, there were lower circulating creatine kinase concentrations in participants who possessed a higher vVO\(_2\). Both outcomes suggest that lower-body strength and aerobic capacity can protect athletes from peripheral muscle damage associated with stretch-shortening cycle exercise, and these findings are in line with others assessing rugby league players \[^{45}\]. Therefore, these associations suggest developing lower-body strength and vVO\(_2\) may not only positively influence the activity profile, but also limit post-match perturbations to homeostasis. However, further work is required to determine whether these associations will still present with continued development of these physical capacities, or if there is a ceiling effect to the protective effect.

### 7.6.7. Implications

This research presents a comprehensive examination of the activity profiles and subsequent time-course of responses following a period of simulated fixture congestion in soccer. Perturbations were observed in most variables measured. Interestingly, although performing a similar activity profile across the two matches, the response to the second match was generally reduced in magnitude and duration compared to the first match simulation. This suggests the presence of an acute repeated bout effect following the first match. Physical qualities such as vVO\(_2\) and lower-body force were associated with very high-speed running volume, hard accelerations, peripheral muscle damage, and perceptual responses. This finding suggests that the development of vVO\(_2\) and lower-body strength are not only useful to improve match
running, but also to reduce the deleterious effects of soccer-match play. Furthermore, our data support the utility of simple wellness questionnaires to interpret player readiness for match performance. In combination with sensitive performance measures, such as squat jump height or isometric mid-thigh pull force, these tools may provide a cost-effective and less invasive avenue for athlete monitoring than nerve stimulation or blood sampling. Importantly, there were inter-individual variations in post-match responses. This confirms that monitoring athletes in an applied setting should be performed on an individual athlete level as opposed to relying on group outcomes.

Although reliable, this match simulation does not provide the contextual influences (such as scoring or opposition) present in competitive soccer match play. While this is a potential limitation, the self-paced nature of the simulation on a curved treadmill, that allows more natural accelerations and decelerations than flat non-motorised treadmills or motorised treadmills, provides greater ecological validity than other simulations in the literature. Furthermore, given the activity profile and the within- and post-match responses observed more closely match those of competitive match play than other simulations, it is likely that the findings presented in this work have direct implications for competitive match play and training [8, 306, 361].
8. Chapter 8: Discussion and Conclusions

This research program was designed to provide greater insight into the mechanisms, responses and potential mitigating factors of team-sport fatigue. A more detailed understanding of these variables can benefit athletes and practitioners alike by optimising player management and informing the training process. Numerous investigations have assessed the response to team-sport exercise via the measurement of neuromuscular, biochemical, performance and perceptual markers. Competitive matches present the most ecologically-valid modality to assess these responses. However, matches can be influenced by contextual factors, such as score and opposition, which reduces experimental control. Furthermore, the proximity of playing fields to laboratories can introduce delays in laboratory-based assessments that are time sensitive. To overcome these issues, match simulations are commonly used to assess the within- and post-match response. While these simulations are free from contextual factors, current protocols are criticised for being externally paced (i.e., dictating speeds to participants), and for unrepresentative acceleration and deceleration profiles due to the equipment used (e.g., tethered non-motorised or motorised treadmills). Study 1 of this work developed a reliable, self-paced match simulation on a curved, non-motorised treadmill. The curved treadmill design allows for more realistic accelerations and decelerations, as well as maximal speed to be reached. As a result, this protocol represents a more ecologically-valid alternative to previous versions.

A contemporary method of fatigue assessment following team-sport exercise is the measurement of central and peripheral fatigue using the interpolated twitch technique with magnetic or electrical stimulation. Magnetic stimulation represents a less painful and, hence, for repeated measures, a more viable alternative to more commonly used electrical stimulation. Therefore, to ensure experimental rigour of the technique across multiple time points, Study 2 established the intra- and inter-day reliability of magnetic stimulation for performing this assessment.

Study 3 used the protocols established in Studies 1 and 2 to assess the response at half-time, post-match, 2-h post, and daily for four days following a soccer match simulation. The volume and intensity of running performed in the match-simulation protocol, as well as peak and mean heart rate, were similar to competitive soccer matches. Similarly, reductions in second-half activity compared to the first half were similar to those reported in match play, supporting the ecological validity of our protocol design. Therefore, the findings from Study 3 likely have implications for competitive on-field performance. A principal finding of this study was that central and peripheral fatigue were evident at half-time, which has not been reported
previously. Furthermore, central and peripheral fatigue were evident post-match, but the magnitude of the change was no greater than the half-time measurement. This might suggest that the reductions in the second-half activity profile observed may be attributed to the fatigue experienced during the first half of the match.

The laboratory-based match simulation allowed for the assessment of various biochemical, performance and perceptual measures in series with central and peripheral fatigue assessments. Our data demonstrate an association between a simple performance test (i.e., squat jump height) with biochemical markers of muscle damage and with peripheral fatigue assessed via interpolated twitch. This information is valuable to practitioners, as squat jump height might provide a cost-effective and less invasive avenue for athlete monitoring than nerve stimulation or blood sampling. The participants’ physical qualities, such as lower-body strength and power and aerobic capacity, were also determined prior to the match simulation. This approach allowed for associations to be elucidated between various post-match responses, and determine whether physical qualities influenced these responses. Our data suggest that strength and aerobic fitness can reduce the magnitude of circulating CK and the severity of undesirable perceptual responses, respectively. This information may have direct implications for the prescription of athletic training, as the development of these capacities may improve the athletes’ ability to cope with the deleterious effects of soccer-match play.

Professional soccer players are often required to participate in multiple games within a week, generally with only 48 – 72 h between matches. Previous research has focused on the activity profiles performed during fixture congestion and established that running volume is maintained between matches, but the risk of injury can increase in the second match [346]. However, we, along with others (see review: [361]), have established that various post-match responses can persist for at least 72 h. As a result, it is possible that players undertake the second match in a short time-frame while still experiencing the deleterious effects of the first match. Currently, published literature reporting the concomitant perceptual, biochemical and neuromuscular responses to fixture congestion is limited. The reliable laboratory-based match simulation protocol, as well as the within- and post-match assessments established in Studies 1, 2 and 3, provided the experimental framework for determining the neuromuscular, biochemical, performance and perceptual responses to simulated fixture congestion.

The activity profiles completed in both matches during the period of simulated fixture congestion were similar, confirming previous reports assessing fixture congestion in competitive soccer. Like Study 3, central and peripheral fatigue was evident at half-time in both
matches, and the magnitude of these responses did not worsen by the end of the matches. Coupled with the reduced running volume and intensity in the second half of both matches, this provides further evidence that fatigue sustained during the first half of matches may influence the second-half activity profile.

A finding of note in Study 4 was that the post-match responses to match 1 had returned to baseline (i.e., trivial difference) at 72-h post, representing a hastier recovery than that observed in Study 3. The participants in Study 4 had greater lower-body strength than those in Study 3, further supporting the proposition that strength provides a protective mechanism that reduces the magnitude of muscle damage associated with eccentric muscle actions performed during running and other post-match perturbations. Furthermore, the responses measured following the second match simulation in Study 4 were generally of lesser magnitude than the first match, despite the activity profiles in both matches being similar. This might represent the occurrence of a repeated bout effect after the first match, blunting some of the perturbations following match 2. Further work is warranted to establish whether this phenomenon exists in trained athletes following intermittent exercise, as they are still susceptible to muscle damage and soreness even though they are accustomed to the stimulus.

The associations between physical qualities and post-match responses were assessed in Study 4, extending on the work of Study 3. For example, higher overall perceived wellness prior to the match was positively associated with the volume of high-speed running performed. Additionally, greater lower-body strength and aerobic fitness were related to (i) a higher volume of running performed during the matches, (ii) a smaller decrement in running volume between first and second halves, and (iii) a lower magnitude of change of some deleterious post-match responses. These findings are in agreement with Study 3, and highlight the importance of developing lower-body strength and aerobic fitness to not only maximise running volume and intensity during matches, but to also assist in mitigating some of the deleterious effects of match running.

This body of work presents some key outcomes that inform the assessment, mechanisms and possible mitigating factors of fatigue following team sport exercise. These include:

- The creation of a self-paced match simulation protocol that is reliable, ecologically-valid, and laboratory-based, allowing detailed match-related assessments to be undertaken.
- Assessment and confirmation of the reliability of magnetic stimulation to assess measures of central and peripheral fatigue within and across multiple days.
- Observing central and peripheral fatigue as early as half-time in a soccer match simulation, not previously reported.
- Similar activity profiles completed in team-sport match simulations performed 72 hours apart.
- Associations between neuromuscular, biochemical, performance and perceptual responses to single and multiple match simulations.
- Associations between lower-body strength and aerobic fitness and a reduction in the deleterious effects of soccer match simulations.

**Limitations**

Both Studies 3 and 4 assessed the response to a self-paced match simulation. Although the activity profile and physiological responses closely match those of competitive soccer, the simulation protocol does not include match-specific actions (e.g., kicking, tackling) that may further impact the post-match response. Additionally, it is possible that decision making, along with other contextual factors not present in the current simulation, may modify the response to the activity performed during the match simulation. Furthermore, it is unlikely that team-sport athletes would participate in a competitive match followed by complete rest for multiple days. Therefore, training or recovery interventions that are utilised in professional settings likely influence the magnitude and duration of the post-match response.

**Practical applications**

There are practical applications that can be derived from the findings of this thesis. Firstly, the influence of physical qualities on performance and subsequent post-match responses have implications for athletic training prescription. Larger volumes of running were observed in participants with a higher VO2peak and vVO2, and these participants maintained their very high-speed running volume between match halves better than less aerobically fit participants. This suggests that practitioners may focus specific training on increasing aerobic capacity, given very high-speed running is a critical component of team-sport performance. Furthermore, greater lower-body strength was positively associated with the volume of hard accelerations performed. Additionally, these physical qualities were associated with various post-match responses. For example, undesirable perceptual responses were reduced, as were direct and indirect markers of muscle damage, in athletes with greater aerobic capacity and lower-body strength. Therefore, increasing lower-body strength and aerobic capacity should be a priority for team-sport athletes.
The techniques used in this work to quantify the post-match responses represent a spectrum of mechanistic to performance-based assessments. Although fatigue from both central and peripheral origins was observed, it is unlikely that the interpolated twitch technique would be feasible in a practical setting. However, a relationship between squat jump height and the measure for central fatigue, voluntary activation, was established. Furthermore, squat jump height was more sensitive to the soccer match-simulation than other post-match responses assessed (e.g., countermovement jump height). Therefore, the assessment of squat jump height might provide an appropriate surrogate measure for identifying central fatigue related to team-sport exercise.

Subjective wellness questionnaires used in this work provided insight into the perceived wellbeing of the participants following the match simulations, highlighted by their association with other post-match responses. These data support the use of wellness questionnaires for providing a cost-effective tool for athlete monitoring. Biochemical markers were measured in both Studies 3 and 4. While perturbations in these variables were apparent for prolonged periods following all matches, large individual variation was present. It may be more beneficial to track the chronic variation in some of these variables, such as testosterone and cortisol, as opposed to their acute responses. This could provide more detailed information about athlete training status.

The individual variation observed in the post-match responses throughout Studies 3 and 4 resulted in numerous unclear statistical outcomes. To some extent, this is a function of the sample size in these studies (i.e., 12 and 11 participants, respectively). However, although the participants were not from the same playing squad, the sample sizes of Studies 3 and 4 closely represent a soccer playing squad, suggesting that these unclear results have practical implications if the testing battery was applied in an everyday setting. Namely, the group response to any measure within- and post-match is unlikely to be representative of all individual responses. Therefore, in a competitive team-sport setting, it is imperative to assess individuals, as opposed to groups. Practitioners should establish baseline levels for each player and monitor their responses compared to a function of the coefficient of variation and the smallest worthwhile change of the test.

**Future Research Directions**

The observation of half-time fatigue, and potential influence on reductions in second half activity profiles, suggest that future work should focus on half-time recovery interventions to reduce the decrement in second-half running volume and intensity. In addition, post-match
interventions directed at hastening the recovery of central fatigue may be warranted due to the prolonged reduction observed in this measure. Associations were observed between the participants’ physical qualities and their performance of and response to the match simulations. A training intervention focused on specifically improving aerobic capacity and lower-body strength may provide further insight into whether there is an upper limit to the benefits of developing these qualities.
Chapter 9: References


Appendix I – Research Portfolio

Publications


*Authors made an equal contribution to the manuscript

Contribution statement: PT and BM were jointly responsible for the design, implementation and overall management of this project. All authors were involved in the concept and design of the study, data analysis and preparation of the manuscript. PT and BM were responsible for the data collection. All authors agree that PT and BM made an equal contribution to the manuscript as lead authors (which was noted on the publication submission).

Approximate percentage contributions – P. Tofari 45%, B. D. McLean 45%, J. Kemp 5%; S. Cormack 5%.

I acknowledge that my contribution to the above publication is 45%:

[Signature]

Paul Tofari Date 06/03/2018

As principal supervisor, I certify that the above contributions are true and correct:

[Signature]

Stuart Cormack Date 06/03/2018

Co-author signatures:

[Signature]

Blake McLean Date 06/03/2018

[Signature]

Justin Kemp Date 06/03/2018

**Contribution statement:** PT was primarily responsible for the design, implementation and overall management of this project. All authors were involved in the concept and design of the study and preparation of the manuscript. PT was responsible for the data collection. PT and DO were responsible for data analysis.

Approximate percentage contributions – P. Tofari 75%, D. Opar 5%; J. Kemp 5%, F. Billaut 5%, S. Cormack 10%.

I acknowledge that my contribution to the above publication is 75%:

[Signature]

Paul Tofari
Date 06/03/2018

As principal supervisor, I certify that the above contributions are true and correct:

[Signature]

Stuart Cormack
Date 06/03/2018

**Co-author signatures:**

[Signature]

David Opar
Date 06/03/2018

[Signature]

Justin Kemp
Date 06/03/2018

[Signature]

François Billaut
Date 04/03/2018
3. **Tofari P, Kemp J, Cormack S.** A Self-Paced Team Sport Match Simulation Results In Reductions In Voluntary Activation And Modifications To Biological, Perceptual And Performance Measures At Half-Time, And For Up To 96 Hours Post-Match. *Journal of Strength and Conditioning Research*. 2017: **Publish Ahead of Print**.

*Contribution statement:* PT was primarily responsible for the design, implementation and overall management of this project. All authors were involved in the concept and design of the study and preparation of the manuscript. PT was responsible for the data collection and data analysis.

Approximate percentage contributions – P. Tofari 80%, J. Kemp 10%, S. Cormack 10%.

I acknowledge that my contribution to the above publication is 80%:

\[signature\]

Paul Tofari  
Date 06/03/2018

As principal supervisor, I certify that the above contributions are true and correct:

\[signature\]

Stuart Cormack  
Date 06/03/2018

**Co-authors signature:**

\[signature\]

Justin Kemp  
Date 06/03/2018

**Contribution statement:** PT was primarily responsible for the design, implementation and overall management of this project. All authors were involved in the concept and design of the study and preparation of the manuscript. PT was responsible for the data collection and data analysis.

Approximate percentage contributions – P. Tofari 80%, J. Kemp 10%, S. Cormack 10%.

I acknowledge that my contribution to the above publication is 80%:

Paul Tofari

Date 06/03/2018

As principal supervisor, I certify that the above contributions are true and correct:

Stuart Cormack

Date 06/03/2018

**Co-authors signature:**

Justin Kemp

Date 06/03/2018
Conference Presentations


   *Contribution statement:* This presentation was based on the work from publication one (see above for author contributions). The presentation was primarily compiled by PT and subsequently reviewed by JK and SC. The oral presentation was delivered by PT.


   *Contribution statement:* This presentation was based on the work from publication three (see above for author contributions). The presentation was primarily compiled by PT and subsequently reviewed by JK and SC. The oral presentation was delivered by SC.
Appendix II – Published paper which forms the basis of Chapter 4

Reference:

Please view the published version online at: http://jssm.org/abstresearchajssm-14-62.xml.xml
Appendix III – Published paper which forms the basis of Chapter 5

Reference:

Please view the published version online at:
Appendix IV – Published paper which forms the basis of Chapter 6

Reference:
Please view the published version online at: https://journals.lww.com/nsca-jscr/Abstract/publishahead/A_Self_Paced_Team_Sport_Match_Simulation_Results.96092.aspx
Appendix V – Ethics approvals, letters to participants and consent forms
Study 1: Letter to participants and consent forms
ACU Human Ethics Committee Approval Number: 2012 257V
INFORMATION LETTER TO PARTICIPANTS

TITLE OF PROJECT: The reliability of running performance during simulated team-sport running on a non-motorised treadmill

INVESTIGATOR 1: Dr Stuart Cormack
INVESTIGATOR 2: Professor Justin Kemp
STUDENT RESEARCHER: Mr Blake McLean
PROGRAMME IN WHICH ENROLLED: PhD – Exercise Science

Dear Participant,

You are invited to participate in the research project ‘The reliability of running performance during simulated team-sport running on a non-motorised treadmill’ being conducted by Dr Stuart Cormack, Associate Professor Justin Kemp, and Mr Blake McLean. The project aims to determine the reliability of a simulated team sport protocol on the newly developed Curve 3 Non-motorised treadmill (NMT). This will be achieved with a running protocol on the NMT designed to simulate a team sport match. For this, the study will involve five visits to the laboratory, with the first visit involving physiological testing and familiarisation with running on the NMT. Visits 2-5 will involve a 30min simulated team sport protocol on the NMT. During the first visit, oxygen consumption will be measured via analyses of expired air (i.e. you will have a mask attached to you, measuring what you breathe out) and eight to ten finger-prick blood samples will be collected during the exercise protocols for immediate analysis of blood lactate.

Should you choose to participate in the study, you will be required to complete treadmill exercise protocols on five separate visits to the laboratory and provide fingerprick blood samples during these exercise tests.

There are a number of small risks associated with these practices, which include: risk of injury during maximal exercise testing (however, this is minimal as you are used to high exercise intensities), and a very small risk of infection with blood sampling (this is unlikely due to strict aseptic techniques being used); however every precaution will be taken to ensure safe sampling.

Your participation in this study will help with understanding the reliability of testing team sport athletes on a NMT. These findings will help future projects determine what effect different training protocols, supplement interventions etc. have on team sport running performance, and ultimately lead to optimal training strategies for team sport athletes.

Participation in the study is voluntary and if you do choose to participate but later change your mind for any reason, you may withdraw without any consequences.
Your personal information and any data collected during this study will be kept completely confidential. The only people who will have access to this information are the researchers (Stuart Cormack, Blake McLean, and Justin Kemp). After all data have been collected and analysed, average scores for the group as a whole will also be used in sports science presentations and publications; however, no individually identifiable data will be apparent in such presentations/publications.

Any questions regarding this project should be directed to investigators and the student researcher:

Dr Stuart Cormack Ph: 03 9953 3133
Professor Justin Kemp Ph: 03 9953 3031
Mr Blake McLean Ph: 0468 646 765

School of Exercise
Science Australian Catholic University
115 Victoria Pde
Fitzroy, Victoria, 3065

Upon the completion of the project all of your results will be available to you and also given to your in a printed summary.

This study has been approved by the Human Research Ethics Committee at Australian Catholic University. In the event that you have any complaint or concern, or if you have any query that the Supervisor or Student Researcher have not been able to satisfy, you may write to the Chair of the Human Research Ethics Committee care of the nearest branch of the Research Services Office:

Chair, HREC
C/- Research Services Australian Catholic University
Melbourne Campus
Locked Bag 4115
FITZROY VIC 3065
Tel: 03 9953 3158
Fax: 03 9953 3315

Any complaint or concern will be treated in confidence and fully investigated. The participant will be informed of the outcome.

If you agree to participate in this project, you should sign both copies of the Consent Form, retain one copy for your records and return the other copy to the Investigator or Student Researcher.

………………………………………
……………………………………
……………………………………………………………………
……………………………………………………………………

Investigator 1 Student Researcher
CONSENT FORM

TITLE OF PROJECT: The reliability of running performance during simulated team-sport running on a non-motorised treadmill

INVESTIGATOR 1: Dr Stuart Cormack

INVESTIGATOR 2: Prof Justin Kemp

STUDENT RESEARCHER: Mr Blake McLean

I ................................................... have read and understood the information provided in the Letter to Participants. Any questions I have asked have been answered to my satisfaction. I agree to participate in this study involving blood collections and treadmill performance test over five laboratory visits, realising that I can withdraw my consent at any time without any adverse consequences. I agree that research data collected for the study may be published or may be provided to other researchers in a form that does not identify me in any way.

NAME OF PARTICIPANT: .................................................................

SIGNATURE: .................................................. DATE: .................................

SIGNATURE OF INVESTIGATOR 1: ............................................................... DATE: .................................

SIGNATURE OF INVESTIGATOR 2: ............................................................... DATE: .................................

SIGNATURE OF STUDENT RESEARCHER: .................................................. DATE: .................................
Study 2: Letter to participants and consent forms
ACU Human Ethics Committee Approval Number: 2014 88V
Dear Participant,

You are invited to participate in the research project titled ‘The reliability of peripheral, femoral nerve stimulation using magnetic stimulation to assess contractile function of the quadriceps muscles.’ being conducted by Dr Stuart Cormack, Prof Justin Kemp and Mr Paul Tofari (PhD student).

**What is the project about?**
The project aims to determine the reliability of a simple laboratory-based test of muscle fatigue (magnetic stimulation).

**What will I be asked to do?**
You will be required to attend the laboratory for two sessions to familiarise yourself with the testing protocol, including some maximal voluntary contractions of your right quadriceps muscles, and to become comfortable with the magnetic stimulation. The week following familiarisation, you will be required to attend the laboratory for three consecutive days. It’s important that you do not intake caffeine (coffee, energy drinks, coca-cola etc.) and alcohol in the 24 hours prior to testing, and throughout the testing period. Also, you must refrain from exercise on the day prior to initial testing session, and for the three days of testing. Each day you will follow an identical protocol of magnetic stimulation and maximal voluntary contractions.

The protocol will involve:
- A series of magnetic stimulations of the quadriceps (front thigh) muscle group at increasing intensity.
- Maximal voluntary contractions of the quadriceps (front thigh) muscle group with superimposed magnetic stimulation.
- Electrodes will be placed on your skin, on the outside of your thigh, to assess muscle activation during contractions and nerve stimulation.

**What’s magnetic stimulation? Does it hurt?**
Magnetic stimulation of the quadriceps involves having a coil placed near the top of your thigh. When activated, this coil sends a very brief (less than a second) electrical current to your thigh to activate the muscle. This procedure can cause minor discomfort. However, magnetic stimulation is used extensively in research and is well tolerated by participants, particularly when compared with the alternative (e.g., electrical stimulation). Magnetic stimulation is a safe procedure, and harmless to the individual.

**Are there any risks associated with participating in this project?**
There is a very small chance of the following risks:
Risk of injury during maximal exercise testing (however, this is minimal as you are a healthy and active individual).

Mild discomfort during magnetic stimulation (however, magnetic stimulation has been chosen over electrical stimulation to reduce this risk).

**How much time will the project take?**
All testing sessions will be identical, and take between 30-60 minutes. This will add up to a total of approximately 3 hours (up to a maximum of 5 hours).

**What are the benefits of the research project?**
This reliability study is required in order to perform a future study aimed at assessing the performance and fatigue response of males to a simulated team-sport match-simulation protocol. Therefore, it is important that the reliability of magnetic stimulation is understood.

**Can I withdraw from the study?**
Participation in this study is completely voluntary. You are not under any obligation to participate. If you agree to participate, you can withdraw from the study at any time without adverse consequences. If you are an Exercise Science student at ACU, participation or withdrawal from this study will not affect your academic progress.

**Will anyone else know the results of the project?**
Your personal information and any data collected during this study will be kept confidential. The only people who will have access to this information are the researchers (Stuart Cormack, Justin Kemp and Paul Tofari). After all data have been collected and analysed, average scores for the group as a whole will also be used in sports science presentations and publications; however, no individually identifiable data will be apparent in such presentations/publications.

**Will I be able to find out the results of the project?**
Once you have completed your participation in the project, the researchers will provide you with a summary of your individual results from the testing session.

**Who do I contact if I have questions about the project?**
If you have any questions or queries about the project please do not hesitate to contact Stuart Cormack.
E-mail address: Stuart.Cormack@acu.edu.au
Contact phone number: 0418 323 915
What if I have a complaint or any concerns?
The study has been approved by the Human Research Ethics Committee at Australian Catholic University (approval number 2014 88V). If you have any complaints or concerns about the conduct of the project, you may write to the Chair of the Human Research Ethics Committee care of the Office of the Deputy Vice Chancellor (Research).

Research Ethics Manager (ResEthics.Manager@acu.edu.au)
Office of the Deputy Vice-Chancellor (Research)
Australian Catholic University
North Sydney Campus
PO Box 968
North Sydney NSW 2059

Any complaint or concern will be treated in confidence and fully investigated. You will be informed of the outcome.

I want to participate! How do I sign up?
Two consent forms have been sent to you along with this information letter. Please fill out the details and sign both consent forms and send them to Paul Tofari.

Email: ptofari@gmail.com
Postal Address: Locked Bag 4115
             Fitzroy MDC
             Fitzroy
             VIC 3065

Yours sincerely,

Dr Stuart Cormack, Prof Justin Kemp, and Mr Paul Tofari
CONSENT FORM

TITLE OF PROJECT: THE RELIABILITY OF MAGNETIC NERVE STIMULATIONS
PRINCIPAL INVESTIGATOR: Dr Stuart Cormack
CO-INVESTIGATOR: Professor Justin Kemp
STUDENT RESEARCHER (if applicable): Mr Paul Tofari

I ................................................... (the participant) have read and understood the information provided in the Letter to Participants. Any questions I have asked have been answered to my satisfaction. I agree to participate in this research project over a 14 day period, understanding my participation could require up to 5 hours of contact time. I am aware and agree to participate in physical performance tests, realising that I can withdraw my consent at any time, without adverse consequences. I agree that research data collected for the study may be published or may be provided to other researchers in a form that does not identify me in any way. If applicable, as an Exercise Science student at Australian Catholic University, my participation or withdrawal from this research project will not negatively affect my academic progress.

NAME OF PARTICIPANT: ..........................................................................................................................

SIGNATURE ................................................................. DATE ..............................................

SIGNATURE OF PRINCIPAL INVESTIGATOR (or SUPERVISOR):.................................................................

DATE:............................................................

SIGNATURE OF STUDENT RESEARCHER:.................................................................................................

DATE:............................................................
Study 3 and 4: Letter to participants and consent forms
ACU Human Ethics Committee Approval Number: 2013 191V
PARTICIPANT INFORMATION LETTER (Study 3)

PROJECT TITLE: The fatigue response following a team-sport match simulation protocol.
INVESTIGATOR 1: Dr Stuart Cormack
INVESTIGATOR 2: Prof Justin Kemp
STUDENT RESEARCHER: Mr Paul Tofari
STUDENT’S DEGREE: Enrolled in PhD, Exercise Science

Dear Participant,

You are invited to participate in the research project titled ‘The fatigue response following a team-sport match simulation protocol’ being conducted by Dr Stuart Cormack, Prof Justin Kemp and Mr Paul Tofari (PhD student).

What is the project about?
The project aims to determine the time-course of the male fatigue response following a single match simulation performed on a non-motorised treadmill (NMT)

What will I be asked to do?
You will be required to attend the laboratory for three sessions to assess your levels of fitness and become familiar with the testing procedures. This testing will include a maximal treadmill-based running test of aerobic capacity, and performing maximal strength and power testing. One week following familiarisation, you will be required to attend the laboratory for six consecutive days. On the first of these days, you will perform a 90-min team-sport match simulation on the NMT. On each of the six consecutive days we will assess your markers of fatigue in response to the exercise activity.

The way we assess the fatigue response is using various markers. These markers are:
- Tests of strength and power of your lower body (including jumping and sprinting)
- Blood and saliva samples to measure the biochemical response to exercise
- Magnetic stimulation of the quadriceps (front thigh) muscle group
- You will also be asked to rate your level of exertion on a scale of 0-10.

What’s magnetic stimulation? Does it hurt?
Magnetic stimulation of the quadriceps involves having a coil placed near the top of your thigh. When activated, this coil sends a very brief (less than a second) electrical current to your thigh to activate the muscle. This procedure can cause minor discomfort. However, magnetic stimulation is used extensively in research and is well tolerated by participants, particularly when compared with alternative, (e.g., electrical stimulation).

Are there any risks associated with participating in this project?
There is a very small chance of the following risks:
- Risk of injury during maximal exercise testing (however, this is minimal as you are used to high exercise intensities)
- Mild discomfort normally associated with blood drawn from the anticubital vein (vein in the forearm)
Mild discomfort during magnetic stimulation (however, magnetic stimulation has been chosen over electrical stimulation to reduce this risk).

**How much time will the project take?**
Baseline testing sessions will take approximately two hours to complete. Sessions incorporating the match-simulation protocol will take approximately four hours. Measures of fatigue on the days following the match simulation will require approximately 45 min. This equates to ~14 hours in total for the study.

**What are the benefits of the research project?**
As a participant in this study, you will have the opportunity to be involved in complementary, professional and scientific testing providing insight into your level of fitness. Furthermore, you will learn what happens to you (fatigue response) after performing a 90 min team-sport match-simulation. In the long-term, the hope is that the research will inform sports scientists and strength and conditioning coaches, as well as team-sport athletes in understanding the fatigue response following a match. This in turn will provide guidance as to the best management of exercise-induced fatigue in athletes, allowing for the best recovery processes and informing strength and conditioning training.

**Can I withdraw from the study?**
Participation in this study is completely voluntary. You are not under any obligation to participate. If you agree to participate, you can withdraw from the study at any time without adverse consequences. If you are an Exercise Science student at ACU, participation or withdrawal from this study will not affect your academic progress.

**Will anyone else know the results of the project?**
Your personal information and any data collected during this study will be kept confidential. The only people who will have access to this information are the researchers (Stuart Cormack, Justin Kemp and Paul Tofari). After all data have been collected and analysed, average scores for the group as a whole will also be used in sports science presentations and publications; however, no individually identifiable data will be apparent in such presentations/publications.

**Will I be able to find out the results of the project?**
Once you have completed your participation in the project, the researchers will provide you with a summary of your individual results from the testing session.

**Who do I contact if I have questions about the project?**
If you have any questions or queries about the project please do not hesitate to contact Stuart Cormack.
E-mail address: Stuart.Cormack@acu.edu.au
Contact phone number: 0418 323 915
What if I have a complaint or any concerns?
The study has been approved by the Human Research Ethics Committee at Australian Catholic University (approval number 2013 191v). If you have any complaints or concerns about the conduct of the project, you may write to the Chair of the Human Research Ethics Committee care of the Office of the Deputy Vice Chancellor (Research).

Chair, HREC
c/o Office of the Deputy Vice Chancellor (Research)
Australian Catholic University
Melbourne Campus
Locked Bag 4115
FITZROY, VIC, 3065
Ph: 03 9953 3150
Fax: 03 9953 3315
Email: res.ethics@acu.edu.au

Any complaint or concern will be treated in confidence and fully investigated. You will be informed of the outcome.

I want to participate! How do I sign up?
Two consent forms have been sent to you along with this information letter. Please fill out the details and sign both consent forms and send them to Paul Tofari.

Email: ptofari@gmail.com
Postal Address: Locked Bag 4115
Fitzroy MDC
Fitzroy
VIC 3065

Yours sincerely,

Dr Stuart Cormack, Prof Justin Kemp, and Mr Paul Tofari
PARTICIPANT INFORMATION LETTER (Study 4)

PROJECT TITLE: The fatigue response following a team-sport match simulation protocol.
INVESTIGATOR 1: Dr Stuart Cormack
INVESTIGATOR 2: Prof Justin Kemp
STUDENT RESEARCHER: Mr Paul Tofari
STUDENT’S DEGREE: Enrolled in PhD, Exercise Science

Dear Participant,

You are invited to participate in the research project titled ‘The fatigue response following a team-sport match simulation protocol’ being conducted by Dr Stuart Cormack, Prof Justin Kemp and Mr Paul Tofari.

What is the project about?
The project aims to determine the time-course of the male fatigue response following two match simulations on a non-motorised treadmill (NMT) in a 72 hour period.

What will I be asked to do?
You will be required to attend the laboratory for three sessions to assess your levels of fitness and become familiar with the testing procedures. This testing will include a maximal treadmill-based running test of aerobic capacity, and performing maximal strength and power testing. One week following familiarisation, you will be required to attend the laboratory for nine consecutive days. On the first of these days, you will perform a 90-min team-sport match simulation on the NMT. You will perform a second 90-min match-simulation 72 hours after the first. You will be given a standardised carbohydrate and protein beverage following the match simulations. On each of the nine consecutive days we will assess your markers of fatigue in response to the exercise activity.

The way we assess the fatigue response is using various markers. These markers are:
- Tests of strength and power of your lower body (including jumping and sprinting)
- Blood and saliva samples to measure the biochemical response to exercise
- Magnetic stimulation of the quadriceps (front thigh) muscle group
- You will also be asked to rate your level of exertion on a scale of 0-10.

What’s magnetic stimulation? Does it hurt?
Magnetic stimulation of the quadriceps involves having a coil placed near the top of your thigh. When activated, this coil sends a very brief (less than a second) electrical current to your thigh to activate the muscle. This procedure can cause minor discomfort. However, magnetic stimulation is used extensively in research and is well tolerated by participants, particularly when compared with alternatives (e.g., electrical stimulation).

Are there any risks associated with participating in this project?
There is a very small chance of the following risks:
Risk of injury during maximal exercise testing (however, this is minimal as you are used to high exercise intensities)
Mild discomfort normally associated with blood drawn from the anticubital vein (vein in the forearm)
Mild discomfort during magnetic stimulation (however, magnetic stimulation has been chosen over electrical stimulation to reduce this risk).

**How much time will the project take?**
Baseline testing sessions will take approximately two hours to complete. Sessions incorporating the match-simulation protocol will take approximately four hours. Measures of fatigue on the days following the match simulation will require approximately 45 min. This equates to ~18 hours in total for the study.

**What are the benefits of the research project?**
As a participant in this study, you will have the opportunity to be involved in complementary, professional and scientific testing providing insight into your level of fitness. Furthermore, you will learn what happens to you (fatigue response) after performing two 90 min team-sport match-simulations in a 72 hour period. In the long-term, the hope is that the research will inform sports scientists and strength and conditioning coaches, as well as team-sport athletes in understanding the fatigue response following a match. This in turn will provide guidance as to the best management of exercise-induced fatigue in athletes, allowing for the best recovery processes and informing strength and conditioning training.

**Can I withdraw from the study?**
Participation in this study is completely voluntary. You are not under any obligation to participate. If you agree to participate, you can withdraw from the study at any time without adverse consequences. If you are an Exercise Science student at ACU, participation or withdrawal from this study will not affect any your academic progress.

**Will anyone else know the results of the project?**
Your personal information and any data collected during this study will be kept confidential. The only people who will have access to this information are the researchers (Stuart Cormack, Justin Kemp and Paul Tofari). After all data have been collected and analysed, average scores for the group as a whole will also be used in sports science presentations and publications; however, no individually identifiable data will be apparent in such presentations/publications.

**Will I be able to find out the results of the project?**
Once you have completed your participation in the project, the researchers will provide you with a summary of your individual results from the testing session.

**Who do I contact if I have questions about the project?**
If you have any questions or queries about the project please do not hesitate to contact Stuart Cormack.
E-mail address: Stuart.Cormack@acu.edu.au
Contact phone number: 0418 323 915
What if I have a complaint or any concerns?
The study has been approved by the Human Research Ethics Committee at Australian Catholic University (approval number 2013 191v). If you have any complaints or concerns about the conduct of the project, you may write to the Chair of the Human Research Ethics Committee care of the Office of the Deputy Vice Chancellor (Research).

Chair, HREC
c/o Office of the Deputy Vice Chancellor (Research)
Australian Catholic University
Melbourne Campus
Locked Bag 4115
FITZROY, VIC, 3065
Ph: 03 9953 3150
Fax: 03 9953 3315
Email: res.ethics@acu.edu.au

Any complaint or concern will be treated in confidence and fully investigated. You will be informed of the outcome.

I want to participate! How do I sign up?
Two consent forms have been sent to you along with this information letter. Please fill out the details and sign both consent forms and send them to Paul Tofari.

Email: ptofari@gmail.com
Postal Address: Locked Bag 4115
   Fitzroy MDC
   Fitzroy
   VIC 3065

Yours sincerely,

Dr Stuart Cormack, Prof Justin Kemp, and Mr Paul Tofari
CONSENT FORM (Study 3 and 4)

TITLE OF PROJECT: THE FATIGUE RESPONSE FOLLOWING A TEAM-SPORT MATCH SIMULATION

PRINCIPAL INVESTIGATOR: Dr Stuart Cormack
CO-SUPERVISOR: Professor Justin Kemp
STUDENT RESEARCHER (if applicable): Mr Paul Tofari

I ................................................... (the participant) have read and understood the information provided in the Letter to Participants. Any questions I have asked have been answered to my satisfaction. I agree to participate in this research project over a 14 day period, understanding my participation could require up to 18 hours of contact time. I am aware and agree to participate in two days of fitness testing, match simulation protocol(s), including physical and performance tests, as well as providing blood and saliva samples, realising that I can withdraw my consent at any time, without adverse consequences. I agree that research data collected for the study may be published or may be provided to other researchers in a form that does not identify me in any way. If applicable, as an Exercise Science student at Australian Catholic University, my participation or withdrawal from this research project will not negatively affect my academic progress.

NAME OF PARTICIPANT: ...................................................................................................................................................

SIGNATURE ........................................................................................................ DATE ...........................................

SIGNATURE OF PRINCIPAL INVESTIGATOR (or SUPERVISOR): ...................................................................................

DATE: ........................................................................

SIGNATURE OF STUDENT RESEARCHER: .................................................................................................................

DATE: ........................................................................