Effects of endurance training on performance and metabolism during a repeated treadmill sprint in females

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EFFECTS OF ENDURANCE TRAINING ON PERFORMANCE AND METABOLISM DURING A REPEATED TREADMILL SPRINT IN FEMALES

by

ANTONIS TSAMPOUKOS

A Doctoral Thesis

Submitted in partial fulfilment of the requirements for the award of the Doctor of Philosophy of Loughborough University

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ABSTRACT

A small number of previous cross-sectional studies have examined the relationship between endurance training status on recovery of performance and metabolites from sprinting. However, no longitudinal studies have been undertaken. In addition, there is a dearth of information on female subjects and on running exercise which prompted the need, in the present thesis, to address the effect of menstrual cycle phase on performance and metabolic responses during a repeated sprint run. Thus, the overall purpose of the present thesis was to examine the effect of short-term endurance training on a repeated sprint in female subjects.

A number of methodological studies (for which 25 subjects volunteered) were undertaken as preparation for the main experimental chapters of the thesis (Chapter 3). The first methodological study examined the reliability of performance during a 30-s sprint on the non-motorised treadmill. Performance was reproducible as indicated by the 95% limits of agreement for PPO (5 ± 42 W) and by the ratio limits of agreement for MPO (1.01 *±1.06) during the 30 s sprint. In the second methodological study it was found that capillary lactate concentrations were significantly higher than venous blood lactate after a 30 s sprint (P < 0.05). The third methodological study revealed that a repeated sprint run caused an additional plasma volume loss when compared with the loss caused by a change in posture alone (12.7 % vs 7.5 % for sprint and posture change, respectively, P < 0.05). Finally, it seems that prolonged freezing (up to 13 months) does not have a detrimental influence on whole blood lactate concentration, but repeated defrosting may result in errors in the determination of blood lactate at high lactate concentrations (methodological study 4).

The first main experiment examined the effects of menstrual cycle phase on performance and metabolic responses during a repeated sprint run (2x30 s, with a 2 min passive recovery) in 8 volunteers (chapter 4). Performance was unaltered during the follicular, mid-cycle and luteal phase of the menstrual cycle as reflected by PPO (461 ± 51 and 395 ± 48, 443 ± 43 and 359 ± 44, 449 ± 52 and 397 ± 48 W, for the first and second sprint, during the follicular, mid-cycle and luteal phase, respectively, P > 0.05) and MPO (302 ± 41 and 252 ± 29, 298 ± 37 and 248 ± 29, 298 ± 39 and 252
± 35 W, for the first and second sprint, at follicular, mid-cycle and luteal phases, respectively, $P > 0.05$). Similarly, blood metabolic responses were unaffected by menstrual cycle phase as reflected by the unchanged metabolic profile of blood lactate, plasma ammonia, blood pH and % changes in plasma volume across menstrual cycle. These results suggest that the hormonal fluctuations of 17-β-estradiol (estradiol) and progesterone, due to menstrual cycle phase, have no effect on repeated sprint performance and possibly on the metabolic responses as reflected by the blood metabolic responses.

The second main experiment examined the effects of short-term endurance training on power output recovery and metabolic responses to a repeated sprint run (2x30 s with a 2 min passive recovery) (chapter 5, n = 16). Six weeks of endurance training resulted in a 3 % increase ($P < 0.05$) in $\dot{V}O_{2\text{ max}}$ (from 48.7 ± 4.4 before training to 50.17 ± 5.1 mL·kg$^{-1}$·min$^{-1}$ after training) in the training group (n = 8) in comparison with 1.9 % decrease (from 50.4 ± 1.3 to 49.4 ± 1.2 mL·kg$^{-1}$·min$^{-1}$ post-trial) in the control group (n = 7). In addition, % $\dot{V}O_{2\text{ max}}$ @ 4 mmol·L$^{-1}$ [the relative intensity (% $\dot{V}O_{2\text{ max}}$) corresponding to blood lactate concentration of 4 mmol·L$^{-1}$] was 3 % higher (from 82 to 84 %) in the training group as compared with the 1 % decrease in the control group (from 81 to 80 %) ($P < 0.05$). These endurance adaptations were accompanied by a 7 % improvement in MPO recovery (in the second of two 30 s sprints) in the training group in comparison with 2 % increases in the control group after training ($P < 0.05$). Metabolic responses to sprints were unaltered after training, but there was a tendency for higher pH immediately after sprint 1 in the training group in comparison with the control group (7.12 ± 0.07 vs 7.19 ± 0.06 and 7.09 ± 0.07 vs 7.10 ±0.06, before and after training, in the training and control group, respectively, $P = 0.082$). These findings suggest that endurance training can be beneficial in terms of quicker recovery of performance during a repeated sprint run.

The third main experiment examined the effects of endurance training on performance recovery and muscle metabolites (chapter 6, n=14). Endurance training resulted in a tendency towards lower blood lactate concentrations during sub-maximal exercise in the training group in comparison with the control group ($P = 0.063$) whilst time to exhaustion for the incremental $\dot{V}O_{2\text{ max}}$ test was 12.7 % longer for the training group.
ABSTRACT

in comparison with 4.1 % decrease in the control group \( (P = 0.095) \). These endurance training adaptations were accompanied by a 7 % improvement \((77 \pm 7 \text{ to } 84 \pm 5 \text{ W})\) in MPO recovery in the second of two 30 s sprints in the training group while in the control group MPO recovery improved by just 2 % \((87 \pm 8 \text{ to } 89 \pm 8 \%)\) \((P < 0.05)\). In addition, similar increases in the recovery of peak speed \((3.4 \% \text{ vs } 1 \%, P < 0.05)\), and mean speed \((5 \% \text{ vs } 0.9 \%, P < 0.05)\) were also evident in the training in comparison with control group. Endurance training resulted in 5.6 % decrease in ATP provision from PCr degradation \( \approx 14 \text{ s post-sprint 1} \) \((P < 0.05)\) while glycogen degradation was 10 % lower \((P = 0.063)\). The latter alterations, in turn, resulted in a tendency towards less reliance on anaerobic energy resources for energy supply after training in the training group \((11 \%, P = 0.098)\). These results corroborate the findings of chapter 5, but it is still unclear which physiological mechanisms were instrumental in enhancing recovery of performance. It is possible that a faster initial PCr resynthesis or an improved mechanical efficiency or an increased reliance on aerobic metabolism, independently, all together, or in any combination, could have contributed to these improvements in performance recovery.

In conclusion the present thesis has shown that: the non-motorised treadmill is a reliable tool for the examination of sprint running performance in the laboratory; that performance and metabolic responses during a repeated sprint run are unaffected by menstrual cycle phase and; that endurance training enhances the recovery of power in female subjects during a repeated sprint run of \(2 \times 30 \text{ s duration with a 2 min passive recovery}\). The mechanisms underlying the performance improvement following endurance training are unknown, but it is possible that faster PCr resynthesis during the initial phase of recovery \(<1 \text{ min}) after the sprint is the dominant factor, while greater reliance on aerobic metabolism and improved mechanical efficiency can not be excluded.

**Key words:** repeated sprints, recovery, performance, metabolism, menstrual cycle phase, endurance training.
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Dedication

This thesis is dedicated to my Mum, Dad, Sister and Brother for their absolute great support during this hard work.
ΜΗ ΛΥΠΑΣΑΙ ΤΟΝ ΑΕΤΟ ΠΟΥ ΟΤΑΝ ΠΕΤΑ ΕΧΕΙ ΠΑΝΤΑ ΚΑΤΑΙΓΙΔΑ
ΝΑ ΛΥΠΑΣΑΙ ΤΟ ΠΟΥΛΙ ΠΟΥ ΔΕΝ ΜΠΟΡΕΙ ΝΑ ΠΕΤΑΞΕΙ

ELEUTHERIUS VENIZELOS 1930
PREFACE

Some of the findings from the experiments presented in the present thesis have been published as follows:


# Table of Contents

<table>
<thead>
<tr>
<th>Chapter 1</th>
<th>Introduction</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chapter 2</td>
<td>Review of the Literature</td>
<td>5</td>
</tr>
<tr>
<td>2.1.</td>
<td>Introduction</td>
<td>5</td>
</tr>
<tr>
<td>2.1.1.</td>
<td>Definition of terms</td>
<td>5</td>
</tr>
<tr>
<td>2.2.</td>
<td>Effects of short-term endurance training on recovery after sprinting</td>
<td>6</td>
</tr>
<tr>
<td>2.2.1.</td>
<td>Introduction</td>
<td>6</td>
</tr>
<tr>
<td>2.2.2.</td>
<td>Physiological and biochemical adaptations to short-term endurance training</td>
<td>7</td>
</tr>
<tr>
<td>2.2.3.</td>
<td>The effect of endurance training on force, speed and power recovery</td>
<td>9</td>
</tr>
<tr>
<td>2.2.4.</td>
<td>Resynthesis of PCr</td>
<td>12</td>
</tr>
<tr>
<td>2.2.5.</td>
<td>Effect of short-term endurance training on oxygen uptake during sprinting</td>
<td>16</td>
</tr>
<tr>
<td>2.2.6.</td>
<td>Oxygen consumption during recovery from sprinting</td>
<td>19</td>
</tr>
<tr>
<td>2.2.7.</td>
<td>Lactate removal from muscle and blood</td>
<td>21</td>
</tr>
<tr>
<td>2.2.8.</td>
<td>Summary: hypothetical mechanisms which link endurance training with improvements in power output recovery after sprinting</td>
<td>24</td>
</tr>
<tr>
<td>2.3.</td>
<td>Effects of menstrual cycle phase upon metabolism and performance</td>
<td>25</td>
</tr>
<tr>
<td>2.3.1.</td>
<td>Definitions</td>
<td>25</td>
</tr>
<tr>
<td>2.3.2.</td>
<td>Physiology and regulation of menstrual cycle</td>
<td>28</td>
</tr>
<tr>
<td>2.3.3.</td>
<td>The normal menstrual cycle</td>
<td>31</td>
</tr>
<tr>
<td>2.3.4.</td>
<td>Early perceptions</td>
<td>33</td>
</tr>
<tr>
<td>2.3.5.</td>
<td>Relationships between estradiol and alterations in performance and exercise physiological responses</td>
<td>34</td>
</tr>
<tr>
<td>2.3.6.</td>
<td>Relationship between estradiol and alterations in</td>
<td>34</td>
</tr>
</tbody>
</table>
performance and exercise physiological parameters 38

2.3.7. Effects of menstrual cycle phase on strength 39

2.3.8. Effects of menstrual cycle phase upon maximal intensity exercise 45
  2.3.8.1. Effects on performance 45
  2.3.8.2. Effects on physiological responses 48

2.3.9. Effects of menstrual cycle phase upon sub-maximal intensity exercise 48
  2.3.9.1. Introduction 48
  2.3.9.2. Effects on sub-maximum physiological responses 49
  2.3.9.3. Effects on maximum oxygen uptake 52
  2.3.9.4. Effects on $\% \dot{V}O_2 \max \at 4 \text{mmol}L^{-1}$ 53

2.3.10. Oral contraceptives and exercise 53
  2.3.10.1. Introduction 53
  2.3.10.2. Oral contraceptives and performance 54
  2.3.10.3. Oral contraceptives and physiological responses 55
  2.3.10.4. Summary 55

2.3.11. Summary 55

2.4. General summary 56

Chapter 3 General Methods 60

3.1. Introduction 60

3.2. Subjects 61

3.3. Familiarisation 62
  3.3.1. Nonmotorised treadmill 62
  3.3.2. Motorised treadmill 63

3.4. Ergometers 64
  3.4.1. Nonmotorised treadmill 64
    3.4.1.1. Introduction 64
    3.4.1.2. Speed measurement and calibration 65
3.4.1.3. Propulsive force measurement and calibration 65
3.4.1.4. Treadmill warm-up 66
3.4.2. Motorised treadmill 66
3.5. Protocols 67
3.5.1. Single 30 s protocol 67
3.5.1.1. Rationale 67
3.5.1.2. Procedures 69
3.5.1.3. Variables measured 70
3.5.2. Repeated 30 s protocol 71
3.5.2.1. Rationale 71
3.5.2.2. Procedures 71
3.5.2.3. Variables measured 72
3.5.3. Speed-\( \dot{V} O_2 \) protocol 72
3.5.3.1. Rationale 72
3.5.3.2. Procedures 72
3.5.3.3. Variables measured 73
3.5.4. Maximum oxygen uptake protocol 73
3.5.4.1. Rationale 73
3.5.4.2. Procedures 73
3.5.4.3. Variables measured 76
3.5.5. Speed-lactate test protocol 76
3.5.5.1. Rationale 76
3.5.5.2. Procedures 78
3.5.5.3. Variables measured 79
3.5.6. Training protocol 79
3.5.6.1. A brief review of terminology and training principles 79
3.5.6.2. Rationale 80
3.5.6.3. Procedures 82
3.5.6.4. Monitoring of training 83
3.6. Anthropometric measurements 85
3.6.1. Stature 85
3.6.2. Body mass
3.7. Clear Plan Home Ovulation Test
3.8. Heart rate monitoring
3.9. Perceived Exertion
3.10. Environmental conditions
  3.10.1. Temperature/humidity
  3.10.2. Barometric pressure
  3.10.3. Time of testing
3.11. Diet Control
3.12. Collection and treatment of gas
3.13. Collection, treatment, storage and analysis of blood samples
  3.13.1. Collection of the blood samples
  3.13.2. Treatment and analysis of blood pH
  3.13.3. Treatment, storage and analysis of blood metabolites
  3.13.4. Treatment and analysis of haematocrit and haemoglobin
  3.13.5. Treatment, storage and analysis of serum hormones
3.14. Collection, Treatment, storage and analysis of muscle samples
  3.14.1. Muscle biopsy procedure
  3.14.2. Initial treatment and storage of muscle samples
  3.14.3. Analytical methods
    3.14.3.1. Extraction procedure
    3.14.3.2. Mixed muscle metabolites assays
3.15. Metabolic calculations
  3.15.1. Metabolic calculations for gas samples
  3.15.2. Metabolic calculations for blood samples
  3.15.3. Metabolic calculations for muscle samples
3.16. Information technology
3.17. Statistical analysis
3.18. Methodological study 1: Test-retest reliability of the
      Performance variables and blood lactate responses to a 30 s
      Treadmill sprint protocol
  3.18.1. Introduction
  3.18.2. Methods
    3.18.2.1. Subjects
3.18.2.2. Ergometer 107
3.18.2.3. Familiarisation 107
3.18.2.4. Protocol and experimental design 107
3.18.2.5. Statistical analysis 108

3.18.3. Results 109
3.18.3.1. Performance variables 109
3.18.3.2. Blood lactate 111

3.18.4. Discussion 113

3.19. Methodological study 2: Effects of different sampling sites and analyses on blood lactate concentration 119
3.19.1. Introduction 119
3.19.2. Methods 121
3.19.2.1. Subjects 121
3.19.2.2. Ergometer 121
3.19.2.3. Familiarisation 121
3.19.2.4. Protocol and experimental design 121
3.19.2.5. Collection, Treatment and storage of the blood samples 122
3.19.2.6. Statistical analysis 124

3.19.3. Results 125
3.19.3.1. Performance variables 125
3.19.3.2. Venous lactate vs capillary lactate 125
3.19.3.3. Venous lactate vs YSI venous lactate 127
3.19.3.4. YSI plasma lactate vs Cobas Mira Plus plasma lactate 128

3.19.4. Discussion 129

3.20. Methodological study 3: Separate and combined influence of posture and sprinting upon plasma volume changes 137
3.20.1. Introduction 137
3.20.2. Methods 140
3.20.2.1. Subjects 140
3.20.2.2. Ergometer 141
3.20.2.3. Familiarisation 141
3.20.2.4. Protocol and experimental design 141
3.20.2.5. Collection and treatment of the blood samples 143
3.20.2.6. Statistical analysis 144
3.20.3. Results 145
  3.20.3.1. Body mass 145
  3.20.3.2. Environmental conditions 145
  3.20.3.3. Performance variables 145
  3.20.3.4. Plasma volume changes 145
  3.20.3.5. Blood volume changes 148
  3.20.3.6. Cell volume changes 148
3.20.4. Discussion 150

3.21. Methodological study 4: Blood lactate storage 155
  3.21.1. Introduction 155
  3.21.2. Methods 155
  3.21.3. Results 156
  3.21.4. Discussion 157

Chapter 4

The effects of menstrual cycle phase upon sprint running 159

4.1. Introduction 159
4.2. Methods 162
  4.2.1. Subjects 162
  4.2.2. Ergometer 163
  4.2.3. Familiarisations 163
  4.2.4. Menstrual cycle information 163
  4.2.5. Protocol and experimental design 164
  4.2.6. Clinical biochemistry 165
  4.2.6. Maximum oxygen uptake and speed lactate test 165
  4.2.7. Dietary analysis 165
  4.2.9. Statistical analyses 166
4.3. Results 166
  4.3.1. Hormonal documentation of menstrual cycle 166
  4.3.2. Performance profile 168
  4.3.3. Blood metabolites 171
  4.3.4. Maximum oxygen uptake and the
percentage of maximum oxygen uptake
at a blood lactate concentration of 4 mmol·L⁻¹
\((\% \dot{V}O_2 \text{ max} @ 4 \text{ mmol·L}^{-1})\)

4.3.5. Dietary analysis 175
4.4. Discussion 175

Chapter 5
The effects of endurance training on power output recovery and blood metabolic responses after sprinting 180
5.1. Introduction 180
5.2. Methods 182
5.2.1. Subjects 182
5.2.2. Ergometers 182
5.2.3. Familiarisations 182
5.2.4. \(\dot{V}O_2\text{max} \) and speed-lactate test 182
5.2.5. Protocol and experimental design 182
5.2.6. Menstrual cycle information 184
5.2.7. Clinical biochemistry 184
5.3.8. Statistical analysis 184
5.3. Results 185
5.3.1. Endurance training adaptations 186
5.3.2. Sprint performance responses 190
5.3.3. Blood metabolic responses 191
5.3.4. Menstrual cycle information 195
5.3.5. Correlations 196
5.4. Discussion 197

Chapter 6
Effects of short-term endurance training on performance and muscle metabolic recovery after sprinting 209
6.1. Introduction 209
6.2. Methods 210
6.2.1. Subjects 210
6.2.2. Ergometers 211
6.2.3. Familiarisations 211
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.2.4.</td>
<td>$\dot{V}O_{2max}$ and speed-lactate test</td>
<td>211</td>
</tr>
<tr>
<td>6.2.5.</td>
<td>Protocol and experimental design</td>
<td>211</td>
</tr>
<tr>
<td>6.2.6.</td>
<td>Menstrual cycle information</td>
<td>214</td>
</tr>
<tr>
<td>6.2.7.</td>
<td>Clinical biochemistry</td>
<td>214</td>
</tr>
<tr>
<td>6.3.8.</td>
<td>Statistical analysis</td>
<td>214</td>
</tr>
<tr>
<td>6.3.</td>
<td>Results</td>
<td></td>
</tr>
<tr>
<td>6.3.1.</td>
<td>Endurance training adaptations</td>
<td>215</td>
</tr>
<tr>
<td>6.3.2.</td>
<td>Sprint performance responses</td>
<td>219</td>
</tr>
<tr>
<td>6.3.3.</td>
<td>Muscle metabolic responses to the sprint and recovery</td>
<td>221</td>
</tr>
<tr>
<td>6.3.4.</td>
<td>Blood metabolic responses to sprint</td>
<td>225</td>
</tr>
<tr>
<td>6.3.5.</td>
<td>Physiological responses to sprint</td>
<td>228</td>
</tr>
<tr>
<td>5.3.5.</td>
<td>Menstrual cycle information</td>
<td>229</td>
</tr>
<tr>
<td>5.3.6.</td>
<td>Correlations</td>
<td>231</td>
</tr>
<tr>
<td>6.4.</td>
<td>Discussion</td>
<td>232</td>
</tr>
<tr>
<td>Chapter 7</td>
<td>General Discussion</td>
<td>246</td>
</tr>
<tr>
<td>7.1.</td>
<td>Introduction</td>
<td>246</td>
</tr>
<tr>
<td>7.2.</td>
<td>Main findings</td>
<td>246</td>
</tr>
<tr>
<td>7.3.</td>
<td>Effects of short-term endurance training on performance and muscle metabolism after a repeated treadmill sprint in females</td>
<td>247</td>
</tr>
<tr>
<td>7.5.</td>
<td>Methodological studies</td>
<td>251</td>
</tr>
<tr>
<td>7.6.</td>
<td>Limitations</td>
<td>252</td>
</tr>
<tr>
<td>7.7.</td>
<td>Directions for future research</td>
<td>256</td>
</tr>
<tr>
<td>7.8.</td>
<td>Practical recommendations</td>
<td>257</td>
</tr>
<tr>
<td>References</td>
<td></td>
<td>260</td>
</tr>
<tr>
<td>Appendices</td>
<td></td>
<td>315</td>
</tr>
</tbody>
</table>
List of figures

Figure 2.1. Schematic representation of menstrual cycle phase (from Vander et al. 2001). 26

Figure 2.2. A more detailed schematic representation of menstrual cycle phase (from Vander et al. 2001). 27

Figure 2.3. Stages of normal follicle during the process of its maturation (from Vander et al. 2001). 28

Figure 2.4. Schematic representation of the hormonal changes just prior to and at ovulation (from Speroff, 1999). 30

Figure 2.5. The structural formula of Progesterone and estradiol (17β-estradiol). 35

Figure 2.6. Hormonal fluctuations of estradiol, progesterone, LH and FSH during one menstrual cycle (from Vander et al. 2001). 37

Figure 2.7. A model of the potential effects of endurance training on recovery of power output after a repeated/multiple sprints. Where CKE: creatine kinase equilibrium. 59

Figure 3.1. The schematic representation of the single 30 s sprint test protocol. 68

Figure 3.2. The schematic representation of the repeated sprint test protocol. 71

Figure 3.3. Speed-\( \dot{V}O_2 \) test protocol. Shaded areas indicate the last min of each stage (4 min stages) in which collection of expired air, heart rate and perceived exertion took place. 73

Figure 3.4. Maximum oxygen uptake (\( \dot{V}O_2 max \)) test protocol. Shaded areas indicate collection of expired air, heart rate and perceived exertion readings for 1 min in each 3 min stage (whole blocks). Numbers on the figure denote the exact timing of the data collections. 75
Figure 3.5. Speed-lactate test protocol. Shaded areas from each block indicate the last min of each stage (4 min stages) in which collection of expired air, heart rate and perceived exertion readings took place. Bold lines at the end of each block denote capillary blood sampling.

Figure 3.6. The schematic representation of the 30 min training protocol.

Figure 3.7. Flow diagram of blood collection and treatment. Numbers in the boxes indicate order of each process.

Figure 3.8. Time plan for the present experimental design. Where F indicates familiarisations as described in paragraph 3.3.1.

Figure 3.9. A Bland and Altman plot for PPO (n = 8).

Figure 3.10. The histogram of the ratios for the MPO.

Figure 3.11. Time plan for the present experimental design. Where F indicates familiarisations as described in paragraph 3.3.1. Shaded areas denote days that do not require the presence of the participants in the lab.

Figure 3.12. A flow diagram that portrays the treatment of the Samples.

Figure 3.13. Time course of venous and capillary whole blood lactate concentration at rest, post warm-up (PWP) and at 1, 5 and 10 min post-sprint (1'PS, 5'PS and 10'PS) following a single 30 s treadmill sprint (mean ± SD, n = 8).

Figure 3.14. A Bland and Altman plot for venous vs capillary lactate (n = 8).

Figure 3.15. Time course of venous and capillary whole blood lactate concentration (mean ± SD, n = 7) analysed enzymatically (VL) and with YSI (YSI-VL) at rest, post warm-up (PWP) and at 1, 5 and 10 min post-sprint (1'PS, 5'PS and 10'PS).
Figure 3.16. Time course of plasma lactate with two automatic analysers, YSI (PL-YSI) and Cobas Mira Plus (PL-COBAS) at rest, post warm-up (PWP) and at 1, 5 and 10 min post-sprint (1'PS, 5'PS and 10'PS) (mean ± SD, n = 5) at rest following a single 30 s treadmill sprint.

Figure 3.17. The schematic representation of the protocol.

Figure 3.18. Time plan for the present experimental design. Where F indicates familiarisations as described in paragraph 3.3.1.

Figure 3.19. % Estimated relative changes in plasma volume (PV) at post warm-up and following a single 30 s treadmill sprint between the sprint and control trial (mean ± SD, n = 8). PWP 1: 1st sample post warm-up, PWP 2: 2nd sample post warm-up, PWP 3: 3rd sample post warm-up, and PS1 to PS6 are the corresponding sampling after the sprint.

Figure 3.20. % Estimated absolute changes in plasma volume (PV) between sprint and control trial at rest, post warm-up and following a single 30 s treadmill sprint between the sprint and control trial (mean ± SD, n = 8). PWP 1: 1st sample post warm-up, PWP 2: 2nd sample post warm-up, PWP 3: 3rd sample post warm-up, and PS1 to PS6 are the corresponding sampling after the sprint.

Figure 3.21. % Estimated relative changes in blood volume (BV) between sprint and control trial at post warm-up and following a single 30 s treadmill sprint between the sprint and control trial (mean ± SD, n = 8). PWP 1: 1st sample post warm-up, PWP 2: 2nd sample post warm-up, PWP 3: 3rd sample post warm-up, and PS1 to PS6 are the corresponding sampling after the sprint.

Figure 3.22. % Estimated relative changes in cell volume (CV) between the sprint and control trial at post warm-up and following a single 30 s treadmill sprint between the sprint and control trial (mean ± SD, n = 8). PWP 1: 1st sample post warm-up, PWP 2: 2nd sample post warm-up, PWP 3: 3rd sample post warm-up, and PS1 to PS6
are the corresponding sampling after the sprint.

**Figure 4.1.** A hypothetical 28 d ovulatory cycle. Dark blue arrows indicate testing. In favour of clarity the scale is hypothetical.

**Figure 4.2.** The schematic representation of the protocol.

**Figure 4.3.** Resting Progesterone concentrations during follicular, mid-cycle and luteal phase of the menstrual cycle (mean ± SD, n = 8).

**Figure 4.4.** Resting estradiol concentrations during follicular, mid-cycle and luteal phase of the menstrual cycle (mean ± SD, n = 8).

**Figure 4.5.** Venous whole blood lactate concentrations at rest, post warm-up (PWP), post-sprint 1 (PS1), post-sprint 2 (PS2) and at 5 (5), 10 (10), 15 (15), 20 (20) and 30 min (30) of recovery after the second sprint at follicular, mid-cycle and luteal phases of the menstrual cycle (mean ± SD, n = 8).

**Figure 4.6.** Venous plasma ammonia concentrations at rest, post warm-up (PWP), post-sprint 1 (PS1), post-sprint 2 (PS2) and at 5 (5), 10 (10), 15 (15), 20 (20) and 30 min (30) of recovery after the second sprint at follicular, mid-cycle and luteal phases of the menstrual cycle (mean ± SD, n = 8).

**Figure 4.7.** Venous whole blood pH at rest, post warm-up (PWP), post-sprint 1 (PS1), post-sprint 2 (PS2) and at 5 (5), 10 (10), 15 (15), 20 (20) and 30 min (30) of recovery after the second sprint at follicular, mid-cycle and luteal phases of the menstrual cycle (mean ± SD, n = 8).

**Figure 4.8.** % Estimated plasma volume changes at rest, post warm-up (PWP), post-sprint 1 (PS1), post-sprint 2 (PS2) and at 5 (5), 10 (10), 15 (15), 20 (20) and 30 min (30) of recovery after the second sprint at follicular, mid-cycle and luteal phases of the menstrual cycle (mean ± SD, n = 8).

**Figure 4.9.** Percentage of fats, carbohydrates (CHO) and proteins (PRO) in habitual diet (n = 7).

**Figure 5.1.** The schematic representation of the protocol.
Figure 5.2. Time plan for the present experimental design. Where F indicates familiarisations as described in paragraph 3.3.1. VO2max-1: \( \dot{V}O_2 \) max test before training; VO2max-2: \( \dot{V}O_2 \) max test after training; SPLA-T 1: speed-lactate test before training; SPLA-T 2: speed-lactate test after training.

Figure 5.3. Treadmill running time (min) for each week of training for the training group (means ± SD, n = 8).

Figure 5.4. Distance run (m) for each week of training for the training group (means ± SD, n = 8).

Figure 5.5. \( \dot{V}O_2 \) max before and after six weeks of experimental period in both groups (means ± SD, n = 8 for training group, n = 7 for the control group). Where PRE (pre-training); POST (post-training); CG (control group); TG (training group).

Figure 5.6. The speed-lactate test with an example of the speeds that correspond to 4 mmol· L\(^{-1}\) before (PRE) and after (POST) training in the training group (means ± SD, n = 8).

Figure 5.7. The speed-lactate test with an example of the speeds that correspond to 4 mmol· L\(^{-1}\) before (PRE) and after (POST) training in the control group (means ± SD, n = 8).

Figure 5.8. Whole blood lactate responses after each sprint and recovery from the sprints, before (PRE) and after (POST) six weeks of experimental period in training (TG,) and control (CG) group; PWP = post warm-up; PS1 = post-sprint 1; PS2 = post-sprint 2; 5, 10, 15, 20 and 30 = min during recovery from sprints in which blood sampling took place. (mean ± SD, n = 8 for TG, n = 7 for CG).

Fig. 5.9. Blood pH responses after each sprint and recovery from the sprints, before (PRE) and after (POST) six weeks of experimental period in training (TG) and control (CG) group; PWP = post warm-up; PS1 = post-sprint 1; PS2 = post-sprint 2; 5, 10, 15, 20 and 30 = min during recovery from sprints in which
blood sampling took place.
(mean ± SD, n = 8 for TG, n = 7 for CG).

Figure 5.10. Plasma ammonia responses after each sprint and recovery from the sprints, before (PRE) and after (POST) six weeks of experimental period in training (TG) and control (CG) group; PWP = post warm-up; PS1 = post-sprint 1; PS2 = post-sprint 2; 5, 10, 15, 20 and 30 = min during recovery from sprints in which blood sampling took place. (mean ± SD, n = 8 for TG, n = 7 for CG).

Figure 5.11. Estimated percentage changes of plasma volume responses after each sprint and recovery from the sprints, before (PRE) and after (POST) six weeks of experimental period in training (TG) and control (CG) group; PWP = post warm-up; PS1 = post-sprint 1; PS2 = post-sprint 2; 5, 10, 15, 20 and 30 = min during recovery from sprints in which blood sampling took place.
(mean ± SD, n = 8 for TG, n = 7 for CG).

Figure 6.1. The schematic representation of the protocol.

Figure 6.2. The schematic representation of the sprint test 2 (biopsy test).

Figure 6.3. Time plan for the present experimental design. Where F indicates familiarisations as described in paragraph 3.3.1. VO2max-1: \( \dot{V}O_2 \) max test before training; VO2max-2: \( \dot{V}O_2 \) max test after training; SPLA-T 1: speed-lactate test before training; SPLA-T 2: speed-lactate test after training.

Figure 6.4. Treadmill running time (min) for each week of training for the training group (mean ± SD, n = 9).

Figure 6.5. Distance run (m) for each week of training for the training group (mean ± SD, n = 9).

Figure 6.6. \( \dot{V}O_2 \) max test, before (pre) and after (post) training in training (TG, n = 9) and control (CG, n = 5) group. (mean ± SD).

Figure 6.7. Time to exhaustion during the \( \dot{V}O_2 \) max test,
before (pre) and after (post) training in training (TG, n = 9) and control (CG, n = 5) group. (mean ± SD).

Figure 6.8. The speed-lactate test with an example of the speeds that correspond to 4 mmol·L⁻¹ before (PRE) and after (POST) training in the training group (TG, mean ± SD, n = 9).

Figure 6.9. The speed-lactate test with an example of the speeds that correspond to 4 mmol·L⁻¹ before (PRE) and after (POST) training in the control group (CG, mean ± SD, n = 5).

Figure 6.10. PCr recovery before and after training for concentrations corrected for total creatine Where REC = recovery; Pre = pre-training; Post = post-training (means ± SD, n = 9).

Figure 6.11. PCr recovery before and after training for concentrations not corrected for total creatine (n = 9). Where REC = recovery; Pre = pre-training; Post = post-training (means ± SD, n = 9).

Figure 6.12. Muscle lactate recovery before and after training (n = 9). Where REC = recovery; Pre = pre-training; Post = post-training (means ± SD, n = 9).

Figure 6.13. Blood lactate concentrations at rest, immediately post warm-up (PWP), immediately post-sprint 1 (PS1) and 2 (PS2) and during the recovery from the sprints (numbers indicate minutes of blood sampling after the 2nd sprint) before (pre) and after (post) training in training (TG, n = 9) and control (CG, n = 5) group, respectively (mean ± SD).

Figure 6.14. Blood pH at rest, immediately post warm-up (PWP), immediately post-sprint 1 (PS1) and 2 (PS2) and during the recovery from the sprints (numbers indicate minutes of blood sampling after the 2nd sprint) before (pre) and after (post) training in training (TG, n = 9) and control (CG, n = 5) group, respectively (mean ± SD).

Figure 6.15. Plasma ammonia concentrations at rest, immediately post warm-up (PWP), immediately post-sprint 1 (PS1) and 2 (PS2) and during the
recovery from the sprints (numbers indicate minutes of blood sampling after the 2nd sprint) before (pre) and after (post) training in training (TG, n = 9) and control (CG, n = 5) group, respectively. Where, b = main effect: time, $P < 0.01$. (mean ± SD).

Figure 6.16. Estimated plasma volume changes at rest, immediately post warm-up (PWP), immediately post-sprint 1 (PS1) and 2 (PS2) and during the recovery from the sprints (numbers indicate minutes of blood sampling after the 2nd sprint) before (pre) and after (post) training in training (TG, n = 9) and control (CG, n = 5) group, respectively (mean ± SD).
**List of plates**

| Plate 3.1 | The collection of a capillary blood sample on the motorised treadmill during the speed-lactate test. | Page 63 |
| Plate 3.2 | The sprint treadmill (non-motorised). | Page 64 |
| Plate 3.3 | Muscle biopsy procedure during the very exact moment when the needle is getting inside the subject’s vastus lateralis by the surgeon Mr Leslie Boobis. | Page 94 |
| Plate 3.4 | Injection of the anaesthetic prior to the biopsy by the surgeon. | Page 96 |
List of tables

Table 3.1. Treadmill calibration data (with subject on). 67
Table 3.2. Treadmill calibration data (without subject on). 67
Table 3.3. Intraassay coefficient of variations (CV) for blood sample assays. Where *: from Morris (1999). 91
Table 3.4. Intraassay coefficient of variations (CV) for muscle sample assays. Data from Bogdanis (1994). Values are mmol·kg⁻¹·dry muscle⁻¹. 99
Table 3.5. Performance data for a single 30 s treadmill sprint (mean ± SD, n = 8). Statistical analyses refer to paired t-test, 95 % limits of agreement (absolute limits), correlation coefficient between trial 1 and trial 2 (r), correlation coefficient between the absolute differences and the mean (R1), Ratio Limits of agreement (ratio limits), coefficient of variation (CV), correlation coefficient between the absolute differences and the mean (R2) after the log transformation, and, sample size estimation for a future study involving test-treatment-test design. (10 % change, statistical power: 0.90). Where FIPO = fatigue index for power; FISP = fatigue index for speed. 110
Table 3.6. Blood lactate concentrations at rest, post warm-up and during 10 min recovery from a single 30 s treadmill sprint (mean ± SD, n = 8). Statistical analyses refer to paired t-test, 95 % limits of agreement (absolute limits), correlation coefficient between trial 1 and trial 2 (r),
correlation coefficient between the absolute differences and the mean (R1), Ratio limits of agreement (ratio limits), coefficient of variation (CV), correlation coefficient between the absolute differences and the mean (R2) after the log transformation, and, sample size estimation for a future study involving test-treatment-test design. (10 % change, statistical power: 0.90).

Table 3.7. The different analytical methods, sampling sites and blood portions utilised are tabulated below.

Table 3.8. Between assay means and coefficient of variations for the methods utilised (Asterisks indicate that these data are from Bruce et al. unpublished observations).

Table 3.9. Venous (VL) vs Capillary (CL) lactate (mean ± SD, n = 8). Statistical analyses refer to paired t-test, 95 % limits of agreement (absolute limits), correlation coefficient between trial 1 and trial 2 (r), correlation coefficient between the absolute differences and the mean (R1), Ratio limits of agreement (ratio limits), coefficient of variation (CV), correlation coefficient between the absolute differences and the mean (R2) after the log transformation, and, sample size estimation for a future study involving test-treatment-test design. (10 % change, statistical power: 0.90).

Table 3.10. Venous blood lactate analysed enzymatically (VL) vs YSI venous (YSI-L) blood lactate at rest, post warm-up and following a single 30 s treadmill sprint (mean ± SD, n = 7). Statistical analyses refer to paired t-test, 95 % limits of agreement...
(absolute limits), correlation coefficient between trial 1 and trial 2 \((r)\), correlation coefficient between the absolute differences and the mean \((R1)\), Ratio limits of agreement \((\text{ratio limits})\), coefficient of variation \((\text{CV})\), correlation coefficient between the absolute differences and the mean \((R2)\) after the log transformation, and, sample size estimation for a future study involving test-treatment-test design. \((10\ \% \text{ change, statistical power: 0.90})\).

Table 3.11. YSI plasma \((\text{YSI-PL})\) vs Cobas Mira Plus plasma \((\text{COBAS-PL})\) lactate at rest, post warm-up and following a single 30 s treadmill sprint \((\text{mean} \pm \text{SD}, n = 5)\). Statistical analyses refer to paired t-test, 95\% limits of agreement \((\text{absolute limits})\), correlation coefficient between trial 1 and trial 2 \((r)\), correlation coefficient between the absolute differences and the mean \((R1)\), Ratio Limits of agreement \((\text{ratio limits})\), coefficient of variation \((\text{CV})\), correlation coefficient between the absolute differences and the mean \((R2)\) after the log transformation, and, sample size estimation for a future study involving test-treatment-test design. \((10\ \% \text{ change, statistical power: 0.90})\).

Table 3.12. Studies that examined finger \((\text{FC})\) or ear-lobe \((\text{ELC})\) capillary lactate vs venous \((V)\). Where \(\text{IT} = \text{incremental test}\).

Table 3.13. A summary of studies completed to date and reported plasma volume changes after treadmill sprinting.

Table 3.14. Raw values for Hct and Hb at rest, post warm-up
and during 10 min recovery from a single 30 s treadmill sprint between sprint and control trial (mean ± SD, n = 8). “PS” indicates post-sprint.

Table 3.15. % Estimated relative changes in plasma volume (PV) at rest, post warm-up and following a single 30 s treadmill sprint between the sprint and control trial (mean ± SD, n = 8). “PS” indicates post-sprint.

Table 3.16. % Relative changes in Blood Volume between sprint and control trial at rest, post warm-up and following a single 30 s treadmill sprint between the sprint and control trial (mean ± SD, n = 8). “PS” indicates post-sprint.

Table 3.17. % Relative changes in cell volume between sprint and control trial at rest, post warm-up and following a single 30 s treadmill sprint between the sprint and control trial (mean ± SD, n = 8). “PS” indicates post-sprint.

Table 3.18. Time schedule for the lactate assays. Where, RV: resting concentration; PWP: post warm-up; 5 PS2: 5 min post-sprint 2; 1st, 2nd and 3rd DEF: 1st, 2nd and 3rd that the samples defrosted (to be assayed).

Table 3.19. Time schedule for the lactate assays. Concentrations are in mmol· L⁻¹. Where, RV: resting concentration; PWP: post warm-up; 5 PS2: 5 min post-sprint 2; 1st, 2nd and 3rd DEF: 1st, 2nd and 3rd that the samples defrosted (to be assayed).

Table 4.1. Hormonal profile for resting 17β-estradiol and progesterone during the follicular, mid-cycle and luteal phase of the menstrual cycle (mean ± SD, n = 8).

Table 4.2. Power output profile [PPO, MPO and fatigue index for power (Flpo)] during two 30 s sprints interspersed with 2 min passive recovery during the follicular, mid-cycle and luteal phase of the menstrual cycle (mean ± SD, n = 8).

Table 4.3. Recovery of power output profile
[PPO, MPO and fatigue index for power (Fl\textsubscript{po})] during two 30 s sprints interspersed with 2 min passive recovery during follicular, mid-cycle and luteal phase of the menstrual cycle (mean ± SD, n = 8).

Table 4.4. Speed profile
[(peak speed, mean speed and fatigue index for speed F\textsubscript{sp})] during two 30 s sprints interspersed with 2 min passive recovery during follicular, mid-cycle and luteal phases of the menstrual cycle (mean ± SD, n = 8).

Table 4.5. Recovery of speed profile
[(peak speed, mean speed and fatigue index for speed F\textsubscript{sp})] during two 30 s sprints interspersed with 2 min passive recovery during follicular, mid-cycle and luteal phases of the menstrual cycle (mean ± SD, n = 8).

Table 4.6. Force profile (peak force and mean force) during two 30 s sprints interspersed with 2 min passive recovery during follicular, mid-cycle and luteal phases of the menstrual cycle (mean ± SD, n = 8).

Table 4.7. Recovery of force profile (peak force and mean force) during two 30 s sprints interspersed with 2 min passive recovery during follicular, mid-cycle and luteal phases of the menstrual cycle (mean ± SD, n = 8).

Table 4.8. \(\dot{V}O_2\text{max}, \% \dot{V}O_2\text{max} @ 4 \text{ mmol L}^{-1}\) and training background of the subjects.

Figure 4.9. Percentage of fats, carbohydrates (CHO) and proteins (PRO) in habitual diet (n = 7).

Table 5.1. \(\dot{V}O_2\text{max}(\text{mL kg}^{-1} \text{ min}^{-1})\) test physiological profile in training group (TG) and control group (CG), pre (PRE) and post (POST) training. Where "\(\text{tg}\)" training by group interaction, (\(P < 0.05\)). (mean ± SD, n = 8 for TG, n = 7 for CG).

Table 5.2. Metabolic and psychological profile of the training group (n = 8) and control group (n = 7)
before (PRE) and after training (POST). Where, 4, 8, 12 and 16 indicate collection min during the 16 min speed-lactate test. $\dot{V}O_2$, $\dot{V}E$, $\dot{V}CO_2$, $\dot{V}O_2/\dot{V}E$, is in mL·kg$^{-1}$·min$^{-1}$, while LA in mmol·L$^{-1}$ (mean±SD).

Table 5.3. Sprint Performance parameters for the training group (TG, mean ± SD) and control group (CG) before and after training. Where "e" = training by group interaction (P < 0.05); FIPo = fatigue index for power; FISP = fatigue index for speed. (mean ± SD, n = 8 for TG, n = 7 for CG).

Table 5.4. Individual values for progesterone and estradiol before (PRE) and after (POST) training in training (n = 8) and control group (n = 7).

Table 5.5. Oral contraceptive details for individual oral contraceptive users.

Table 6.1. $\dot{V}O_2 max$ (mL·kg$^{-1}$·min$^{-1}$) test physiological profile in training group (TG) and control group (CG), pre and post training (mean ± SD, n = 9 for TG, n = 5 for CG).

Table 6.2. Speed-lactate data for the training group (n = 9) and control group (n = 5) before (PRE) and after training (POST). Where, 4, 8, 12 and 16 indicate collection min during the 16 min speed-lactate test. $\dot{V}O_2$, $\dot{V}E$, $\dot{V}CO_2$, $\dot{V}O_2/\dot{V}E$, is in mL·kg$^{-1}$·min$^{-1}$, while LA in mmol·L$^{-1}$ (mean±SD).

Table 6.3. Sprint Performance parameters (2 x 30 s sprints) for training group (mean ± SD, n = 9) and control group (mean ± SD, n = 5) before (pre) and after (post) training. (mean ± SD, n = 9 for TG, n = 5 for CG).

Table 6.4. Sprint Performance variables for the training group (mean ± SD, n = 9) for the single (biopsy) trial.

Table 6.5. Muscle metabolites (mmol·kg dry muscle$^{-1}$) in vastus lateralis before and after training for the training group (mean ± SD, n = 9). Where,
PS = post-sprint; REC = 2 min recovery; 
U = uncorrected concentrations for TCr; 
C = corrected for TCr; N/A = not applicable; 
G6P = glucose 6 phosphate.

Table 6.6. ATP provision (mmol·kg dry muscle\(^{-1}\)) from the anaerobic resources before and after training in the training group (means ± SD, n = 9).

Table 6.7. Rates of ATP provision from the various anaerobic resources before and after training in the training group (means ± SD, n = 9).

Table 6.8. Oxygen uptake (mL·kg\(^{-1}\).min\(^{-1}\)) at rest, during sprint 1, during the initial 50 s (REC 1) of the 2 min recovery after the sprint 1, during the second 50 s (REC 2), during the total recovery period (TOTAL REC) and during sprint 2 before (PRE) and after (POST) training in training and control groups (mean ± SD, n = 9 and n = 5 for training and control group, respectively).

Table 6.9. Individual values for progesterone and estradiol before (PRE) and after (POST) training in training (n = 9) and control (n = 5) group. Where +: individuals with perimenstrual problems; *: individuals using oral contraceptives.
CHAPTER 1: INTRODUCTION

The processes occurring during recovery from exercise are just as important as those occurring during exercise itself (Bowers and Fox, 1992). The metabolic responses at the cessation of exercise are of major significance as they determine the time that an athlete's physiological mechanisms need to return to the pre-exercise conditions (Tsampoukos, 1997). The ability to recover quickly is an important determinant of performance in those sports where repeated bouts of all-out activity are required (Tomlin and Wenger, 2001). In addition, in training sessions a fast recovery is important for the quantity and quality of work which can be completed in one session (Bogdanis, 1996b). However, the pioneer of the importance of recovery from maximal exercise was the Ancient Greek Philosopher Flavius Philostratus who first described this issue in his book “Handbook for the Athletics Coach and Gymnasticus” (Philostratus, A.D. 170-240).

Exercise training interspersed with periods of recovery (interval training) first appeared thanks to the German 800 m record holder Rudolf Harbig in the 1930s while the originality of the method is attributed to coach Gerschler and cardiologist Reindell (Snell, 1990). Nevertheless, scientific data concerning the physiological significance of this method, and, thus the study of recovery, have been determined and described later (Astrand et al., 1960; Christensen et al., 1960).

These early attempts involved experimental perturbations that focused on sub-maximal intensity exercise and it was not until the early/mid-eighties that maximal intensity exercise attracted interest from the scientific community (Wootton and Williams, 1983; McCartney et al., 1983). This delay was thought to be due to the lack of appropriate methods and protocols to study such exercise (Cheetham et al., 1986). This problem was partially resolved with the development of a maximal cycle ergometer test by Bar-Or and Cumming in the mid/end-seventies (Cumming, 1975; Bar-Or, 1978). This test, subsequently described as the Wingate Test or Wingate Anaerobic Test (WAnT), allowed the measurement of power output during sprinting in the laboratory (Inbar et al., 1996). The reintroduction, by Bergstrom (1962), of the needle biopsy technique was another methodological breakthrough which allowed the collection of muscle metabolite data. As a result of further methodological
developments it is now possible to examine sprint running in addition to sprint
cycling in the laboratory (Lakomy, 1987) and to identify metabolic responses at the
single fibre level (e.g. Greenhaff et al., 1994). Furthermore, both maximal dynamic
exercise involving a small muscle mass and electrically induced contractions (Spriet
et al., 1987) have also received attention, as the exercising muscle is easily isolated
and invasive techniques such as arterial (Lindinger et al., 1992) and venous (Nevill et
al., 1996a) cannulation can be employed, thus contributing to knowledge concerning
the physiology of sprinting (Nevill et al., 1996b).

These methodological advances have expanded understanding of the physiological
mechanisms underlying sprint performance despite the fact that the aetiology of
fatigue is as yet not clear (Nevill et al., 1996b). Similarly, the mechanisms operating
during the recovery are not yet fully understood, although it is widely accepted that
the following processes are occurring: the redistribution of potassium between
intracellular and extracellular space (Cherry et al., 1998); the restoration of
phosphocreatine (PCr) levels (Bogdanis et al., 1996a); and the recovery of acid-base
balance (Maughan et al., 1997). There are a number of ways that an individual can
improve their potential for a faster recovery and thus better performance. One
approach is the use of nutritional supplements such as creatine which can, under some
circumstances, improve recovery of maximal exercise (Bogdanis et al., 1996b;
Peyrebrune et al., 1998). It has also been shown that endurance training has the
potentials to improve the recovery of power (Bogdanis, 1996), possibly because of a
link between PCr resynthesis, power output recovery and endurance fitness (Bogdanis
et al., 1996a). However, except for a number of cross-sectional studies (Hakkinen and
Myllyla, 1990; Casey, 1991; Hamilton et al., 1991; Graham et al., 1995; Hoffman,
1997; Tomlin and Wenger, 2002) this issue has not been addressed directly, using a,
longitudinal study. Finally, anabolic steroids may also play a dominant role in
recovery; but this it is not a topic that will be discussed further in this thesis due to the
ethical unacceptability of such practices. Thus, the purpose of the present thesis is to
examine the effect of short-term endurance training on the recovery from maximal
intensity exercise (sprinting). Sprinting is defined as exercise performed at a maximal
rate from the onset of exercise, normally for 30-s or less.
The aforementioned methodological advances have been accompanied by social and cultural breakthroughs which have enhanced the participation of women in training and competition. For example, the restriction of the Olympic Games to males as with its ancient counterpart was lifted during the Amsterdam Games of 1928 and since then the range of opportunities for women within major competitions such as Olympic Games and World Cups has been vastly increased (i.e. in 1980s team sports with body contact were first included in the Olympic Games programme, Pfister 2000). As a consequence, this increased involvement of women in sports has prompted exercise physiologists to pay closer attention to physiological issues which specifically relate to women (i.e. Drinkwater, 2000).

It is now recognised that the interplay of the many hormones associated with a woman’s physiological profile illustrate a complex and highly differentiated endocrine system (Norman and Litwack, 1997). One major area of interest is the potential effect of hormonal fluctuations due to female sexual cycle (term introduced by Guyton and Hall, 1996), upon metabolism and performance. A number of studies have appeared in the last two decades examining the effects of the menstrual cycle on performance and metabolism during maximal (e.g. Miscek et al., 1997; Masterson, 1999; Giacomoni et al., 2000) or sub-maximal (e.g. Nicklas et al., 1989; Lebrun et al., 1995) exercise. However, in terms of sprinting, findings to date are equivocal (e.g. Miscek et al., 1997; Masterson, 1999; Giacomoni et al., 2000) and apart from some field studies (Giacomoni et al., 2000) and a few studies where there has been some limited control of cycle phase (Parish and Jakeman, 1987; Miscek et al., 1997) there is lack of data examining the recovery of power output and metabolic responses in female subjects.

Thus, the purpose of the present thesis is to examine the effects of endurance training on the recovery of power output, as well as blood and muscle metabolites, after maximal sprint running in female athletes. The overall hypothesis to be tested is that the recovery of power output in female subjects following a maximal 30 s sprint is enhanced by short-term endurance training via improvement of PCr resynthesis and more rapid recovery of acid-base balance. A secondary but equally important purpose is to test the hypothesis that menstrual cycle phase will affect power output and recovery of power output during two repeated 30 s sprints.
This thesis is divided into seven chapters. Following the introduction (CHAPTER 1: INTRODUCTION) the second chapter (CHAPTER 2: REVIEW OF THE LITERATURE) consists of a thorough review of the literature examining the recovery from exercise and the effects of endurance training. The third chapter (CHAPTER 3: GENERAL METHODS) will deal with the methods used for the completion of the experimental work. In this chapter four methodological experiments are described: the reliability of power output during a 30 s protocol; the effects of sampling site, analytical methods and blood portions on blood lactate concentration will be assessed, the confounding effects of posture on plasma volume as associated with sprint running; and the stability of whole blood lactate during prolonged freezing. The fourth chapter (CHAPTER 4: MAIN STUDY 1, “THE EFFECTS OF MENSTRUAL CYCLE UPON SPRINT RUNNING PERFORMANCE”) will examine the effects of menstrual cycle on performance and blood metabolites. The fifth chapter (CHAPTER 5: MAIN STUDY 2, “THE EFFECTS OF ENDURANCE TRAINING ON POWER OUTPUT RECOVERY AND BLOOD METABOLIC RESPONSES AFTER SPRINTING”) will investigate the effects of endurance training on power output recovery and selective blood metabolites after sprinting. In chapter six (CHAPTER 6: MAIN STUDY 3, “THE EFFECTS OF SHORT-TERM ENDURANCE TRAINING ON PERFORMANCE AND MUSCLE METABOLIC RECOVERY AFTER SPRINTING”) the effects of short-term endurance training upon power output recovery and the recovery of muscle metabolites will be examined. Finally, in chapter seven (CHAPTER 7: GENERAL DISCUSSION) an attempt will be made to draw together the findings of the thesis and to come to some conclusions regarding the effect of endurance training on the recovery of performance and metabolism after sprinting.
CHAPTER 2: REVIEW OF THE LITERATURE

2.1. INTRODUCTION

Published research concerning maximal intensity exercise expanded rapidly during the last two decades of the 20th century. Scientists now have available to them a plethora of tools and experimental models which, with appropriate manipulation, are able to contribute to a better understanding of the physiological mechanisms underlying intense exercise of short duration. The experimental evidence to date has highlighted the difficulties of conducting such studies since maximal intensity exercise (sprint) taxes the human body near to its maximum and a 30-s sprint has been estimated to represent an exercise intensity of approximately 300 % $\dot{V}O_2$ max (Wootton, 1984).

In spite of this increase in the number of studies related to high-intensity exercise there are still unresolved issues such as the aetiology of fatigue during sprinting and factors affecting recovery from maximal exercise. Maximal (sprint) exercise is defined for the purpose of this review as exercise conducted with maximal leg and/or arm speed from the onset of exercise of 30-s duration or less. The aim of this chapter is to highlight the literature to date relating to the effect of endurance training on performance, recovery of performance and metabolism during sprint exercise. The focus will be on human skeletal muscle studies and where necessary data from animal studies will be also included.

The review is organised under two main sections: ‘Effects of short-term endurance training on sprint performance and recovery after sprinting’ (section 2.2) and: ‘The effects of menstrual cycle phase on performance and metabolism’ (section 2.3).

2.1.1. DEFINITION OF TERMS

For the present thesis the following terminology will be used:

- Sprinting/maximal intensity exercise is defined as exercise performed at a maximal rate from the onset of exercise, normally for 30 s or less to permit
maximal exertion from the onset of exercise. The term “flat out” will be also used synonymously.

- High-intensity exercise is defined as exercise performed at exercise intensity above that required to elicit $\dot{V} O_2 max$. Other terms such as “short-term intense exercise” or “intense exercise of short-term duration” will be also used synonymously.

- Sub-maximal intensity is defined as the range of intensities below that require to elicit $\dot{V} O_2 max$.

- According to Jones and Round (1990) power is the rate of doing work and in sprinting events all competitors will do approximately the same amount of work since they are moving their own body weight in similar air and frictional resistances over the same distance (work = force x distance). Then, according to the same authors, the difference between the winner and loser will be the time taken to do this work or, alternatively, the power output. For this reason in the present thesis power output will be the single best determinant of performance measurement with respect to sprinting.

- Finally, performance capacity is referred to as the maximal or sub-maximal effort to exhaustion.

2.2. EFFECTS OF SHORT-ENDURANCE TRAINING ON RECOVERY AFTER SPRINTING

2.2.1. INTRODUCTION

In this section the physiological and biochemical adaptations to short-term endurance training (6-8 weeks) will be presented (section 2.2.2) followed by an examination of the potential link between endurance training status and metabolic and performance recovery from sprinting (section 2.2.2 to 2.2.7). Finally, in section 2.2.8 a summary of the physiological mechanisms which may underpin the enhanced recovery of sprint performance following endurance training are presented.
2.2.2. PHYSIOLOGICAL AND BIOCHEMICAL ADAPTATIONS TO SHORT-TERM ENDURANCE TRAINING

In order to evaluate the effects of a particular training regimen on performance recovery during repeated sprints, the endurance adaptations to the training programme, both peripheral and central, should be known. As discussed later in chapter 3 (section 3.5.6) the main criterion for the adoption of the training programme for the present thesis was the observation that six weeks of training at an intensity of 85% $\dot{V}O_2$ max for 30 min duration did not compromise sprint performance while both $\dot{V}O_2$ max and endurance capacity were enhanced (Hardman et al., 1986).

It seems that six weeks of continuous endurance training at approximately 85% $\dot{V}O_2$ max is an adequate stimulus to increase maximal aerobic power. However, different types of exercise result in different percentage improvements. Thus, continuous training on a cycle ergometer is usually the most beneficial with improvements from 14 to 27% (Andersen and Henriksson, 1977a; Andersen and Henriksson, 1977b; Gaesser and Rich, 1984; Hardman et al., 1986; Mayes et al., 1987). On the other hand the same duration, intensity and frequency of exercise but on a treadmill gave much smaller elevations in aerobic power, 4.6 to 7%, (Williams and Nute, 1986; Callister et al., 1988). At first glance it seems that cycling exercise results in greater elevations of $\dot{V}O_2$ max in comparison with running. However, endurance adaptations appear to be identical when the training is conducted either on a bike or on a treadmill as long as the training stimulus is the same (Pechar et al., 1974). The smaller improvements observed by Williams and Nute (1986) and Callister et al. (1988) experiments are most probably attributed to the already high initial fitness of their subjects in comparison with Andersen and Henriksson (1977a), Andersen and Henriksson (1977b), Gaesser and Rich (1984), Hardman et al. (1986) and Mayes et al., (1987). Support for this suggestion comes from direct experimental data, as there is an inverse relationship between initial $\dot{V}O_2$ max and percentage of change in $\dot{V}O_2$ max (Pollock, 1973; Mayes et al., 1987).

A more "sensitive" approach to assess endurance fitness is the use of certain blood lactate concentrations (2-4 mmol· L$^{-1}$) (Weltman, 1995). Using the latter method Yoshida and colleagues (1982b) conditioned their subjects 3 times/week for 8 weeks
at an intensity of 80-95 % $\dot{V}O_2$ max (the large range was due to the expression of the training programme intensity relative to a blood lactate concentration of 4 mmol·L⁻¹) and found a 14 % increase in aerobic power. The sensitivity of blood lactate as an index of endurance training at an intensity corresponding to 4 mmol·L⁻¹ was indeed shown with a 23 % decrease of its absolute concentration after training. The trend of reduced absolute blood lactate concentrations after short-term endurance training has appeared in all investigations to date (Williams and Nute, 1986; Mayes et al., 1987) and is consistent with observations in cross-sectional studies (e.g. Coggan et al., 1995).

It seems that training of 30 min duration and frequency 3 times/week is adequate to alter the capillary network of human skeletal muscle (Andersen and Henriksson, 1977a). These researchers investigated the effects of short-term endurance training on the capillary supply of the quadriceps femoris muscle. They found significant increases of capillary density, capillaries per fibre, mean numbers of capillaries in contact with fibres of each type (I, IIA, IIB) and capillary density to fibre-type area (non significant in IIB, but similar in magnitude changes with the other fibres). In the same period $\dot{V}O_2$ max values had increased by 16 % compared with pre-training values.

In a parallel study, Henriksson and Reitman (1977) also found that mitochondrial enzyme activities such as succinate dehydrogenase (marker of TCA cycle) and cytochrome oxidase (marker of respiratory chain) activities are enhanced within 3 weeks of exercise with either 20 min at 89 % $\dot{V}O_2$ max, or, with 40 min at 80 % $\dot{V}O_2$ max (4 weeks in both occasions). The different time course between $\dot{V}O_2$ max and enzymatic activity during detraining and to some extent during the training period led to the authors to conclude that $\dot{V}O_2$ max is associated with, but not dependent on, muscle oxidative capacity.

In a related study from the same research group, Andersen and Henriksson (1977b) demonstrated that short-term endurance training (8 wk, 30 min a day, 4 d a wk at 81% $\dot{V}O_2$ max) on a friction-loaded cycle ergometer can convert a small (10 %), but significant part of the IIB population into IIA type. These alterations in the mechanical
properties of the skeletal muscle were accompanied by an 18% increase in $\dot{V}O_2 \text{max}$ ($P < 0.001$).

The above findings indicate that short-term continuous endurance exercise provides an adequate stimulus for both central and peripheral adaptations. Whether or not, and to what extent these adaptations can be connected with recovery from sprinting is discussed in the next paragraphs.

### 2.2.3. THE EFFECT OF ENDURANCE TRAINING ON FORCE, SPEED AND POWER RECOVERY

The first study to report a positive link between endurance training and recovery of force was conducted by Hakkinen and Myllyla (1990), but it was Bogdanis and co-workers (1996a) who clearly demonstrated this association by high positive correlations between mean power output and mean pedalling speed recovery during the first 10 s of the second sprint and PCr resynthesis ($r = 0.84$, $P < 0.05$ and $r = 0.91$, $P < 0.01$, respectively), and between these two mechanical parameters and $\% \dot{V}O_2 \text{max} @ 4 \text{mmol·L}^{-1}$ ($r = 0.75-0.94$; $P < 0.05$ to $P < 0.01$). These findings came to verify earlier experimental observations from the same research group that PCr restoration parallels power output recovery (Bogdanis et al., 1995).

On the other hand Hakkinen and Myllyla (1990) comparing junior endurance, strength and power athletes in the recovery of maximum strength after isometric muscle action (leg extension as long as possible) at 60% MVC to exhaustion, did observe higher recovery rates after 3 min rest ($P < 0.01$) in endurance athletes as opposed to strength and power athletes. However, the authors did not measure $\dot{V}O_2 \text{max}$ or any other endurance fitness index so their conclusions were limited. In addition, the initial time to exhaustion (non-fatiguing trial) differed between groups (endurance athletes exerted less force, but they could continue for longer) making the comparisons difficult. It is very important to have a similar PPO, MPO, force and speed in the non-fatigued stage in both experimental groups since the percent recovery of those variables is strictly dependent upon the initial values [eg. in Bogdanis et al., 1996a, the recovery of MPO (10 s) is defined as the MPO (10 s) in the second sprint.
expressed as the a percentage of the MPO (10 s) in the first sprint]. The application of this experimental artefact can be seen clearly from the study of Caiga and Doherty (1995) where the first known attempt to directly address whether endurance training can be beneficial in the recovery of power output after 30-s sprints was made. Although the 9 weeks endurance training regimen (interval training, 3 min exercise at an intensity equivalent to a heart rate: < 90 % $\dot{V}O_2$ max, 3 times a week) significantly improved both relative (mL·kg$^{-1}$·min$^{-1}$) and absolute $\dot{V}O_2$ max (L·min$^{-1}$) as well as the MPO (5 s) and total work done (30 s) in the later bouts (4 x 30-s sprints with 3 min active recovery in between), it also enhanced the MPO (10 s) and total work done (30 s) in the initial sprints, and thus, made it impossible to distinguish any effect on recovery of MPO (5 s). Similarly, Edge et al. (2002) were unable to detect MPO (6 s) recovery improvements after either a continuous (20-35 min exercise at 85-95 % of undefined lactate threshold) or interval (6-10 x 2 min intervals at 130-140% of undefined lactate threshold) endurance training programme for 5 weeks. The pre- and post-training performance test was 5 x 6-s cycle sprints interspersed with 30 s passive recovery. The higher MPO (6 s) in the first sprints after training made any comparison impossible. The above discussion illustrates the necessity of the employment of the right training protocol: a protocol that elicits changes in endurance indexes (i.e. $\dot{V}O_2$ max, lactate threshold, % $\dot{V}O_2$ max @ 4 mmol·L$^{-1}$), but does not change the performance of the reference or first sprint. Judging from the limited data on the effects of endurance training on sprinting (see chapter 3, section 3.5.6.) such training protocols seem extremely difficult to design and implement.

Cross-sectional studies of sprint- in comparison with endurance-trained athletes are the alternative option to the time consuming and in some cases cumbersome, longitudinal studies. Furthermore, endurance/sprint-trained athletes represent extremes in terms of physiological adaptations and thus can give a clearer picture of the recovery of power output due to different training regimens. In contrast, a major drawback, apart the one already mentioned (power output mismatch between groups), can be the involvement of other factors such as genetic inheritance, habitual diet and body composition etc. Nevertheless, despite such limitations in the studies of Casey, (1991) and Graham et al. (1995) it was clearly shown that endurance-trained athletes were superior with regards to both PPO and MPO (30 s) recovery after 30-s cycle
sprinting in comparison with their sprint-trained counterparts. *Graham et al. (1995)*, however, made an intelligent effort to normalise the data concerning the disparity in MPO (30 s) by requesting that the sprinters perform an additional double sprint on a separate occasion with the work completed in the first sprint the same as that achieved by the endurance group. This sprint lasted 25 s on average, but the results did not change (endurance athletes still recovered significantly better) and, therefore confirmed the superior ability of endurance-trained individuals to recover quicker.

In shorter duration sprints *Dawson and colleagues (1993)* have also reported a relationship \( r = 0.62, P < 0.05 \) between \( \dot{V}O_2 \text{max} \) and percentage decrement (\% decrement between the first and last sprint) in speed during the course of 6 x 40 m track sprints. On the other hand there was not a statistically significant relationship between absolute \( \dot{V}O_2 \text{max} \) (L·min\(^{-1}\)) and percentage decrement in work done during the repeated sprint test on a cycle ergometer (6 x 6-s interspersed with 30 s rest) which suggests a differentiation in the underlying mechanisms for the recovery process between whole body (running) and smaller muscle mass (cycling) exercise. *Tomlin and Wenger (2002)* took into consideration the risk of data misinterpretations due to the unbalanced PPO and total work done (6 s) in the first maximal bout (10 x 6-s cycle sprints separated with 60 s passive rest) and selected a homogeneous group of female soccer players. The soccer players had a similar MPO (6 s), total work done (average in 10 x 6-s sprints) and percentage decrement [(the percent drop-off from highest MPO (6 s») and differed only in \( \dot{V}O_2 \text{max} \) (low-trained group: \( \dot{V}O_2 \text{max} < 38 \) moderately-trained group: \( \dot{V}O_2 \text{max} > 43 \) mL·kg\(^{-1}\)·min\(^{-1}\)). There was a statistically significant negative correlation between \( \dot{V}O_2 \text{max} \) and \% drop-off in MPO (6 s). Furthermore, the moderately trained group had better maintenance of MPO (6 s) during the final 4 bouts and a smaller decrement in percentage decrement in MPO (6 s) over the low-trained group.

In a slightly different experiment of 6 x 15-s maximal bouts with 90 s active recovery on a cycle ergometer, *McMahon and Wenger (1998)* confirmed the link between \( \dot{V}O_2 \text{max} \) (mL·kg\(^{-1}\)·min\(^{-1}\)) and fatigue index of MPO (15 s) and PPO (3 s) in bouts 5 and 6 (\( r = -.49, P = 0.03 \) and \( r = -.62, P = 0.02 \) for the \% drop-off in MPO and PPO, respectively). Furthermore, they also suggested that the peripheral component of
maximum oxygen uptake [as indicated by the significant positive correlation between arteriovenous difference in oxygen and the fatigue index in MPO (6 s) in bouts 5 and 6] is better associated with power output (MPO in this occasion) recovery.

To test the same hypothesis using a more applied approach, Hoffman (1997) required infantry soldiers to undertake a continuous 143 m sprint with several changes in direction (Semenick, 1994). Furthermore, in order to assess the recovery of the speed after this exercise model, the test was performed two more times and a fatigue index was developed (dividing the mean time of the three sprints by the fastest recorded time of the three sprints). It was found that the fitter soldiers (endurance fitness was determined with a 2000 m run) could recover their speed faster as compared with the less fit individuals ($r = 0.33$, $P < 0.05$ between endurance fitness and % drop-off). Nonetheless, the test required a certain level of skill (Tomlin and Wenger, 2001), an issue that it was not addressed by the author.

Finally, the same author attempted to examine whether endurance fitness has the same potential benefits in a smaller sample of basketball players (Hoffman et al., 1999). Performing the same test on a basketball pitch and assessing $\dot{V}O_2$ max (mL·kg$^{-1}$·min$^{-1}$) directly, the authors failed to establish a relationship between endurance fitness and recovery of speed. Again, it is not clear whether, and to what extent, the small sample size and skill level of participants influenced their results.

2.2.4. RESYNTHESIS OF PCr

There is a general consensus that PCr recovery is an oxygen based process as indicated by numerous protocols employing either circulation occlusion (Taylor et al., 1983; Quistorf et al., 1992; Yoshida and Watari, 1997; Hogan et al., 1999) or by comparing endurance athletes with relatively untrained individuals (McCully et al., 1989; Takahashi et al., 1995). This association between PCr recovery and oxidative potential suggests that endurance improvements through a training regimen could possibly have a positive impact on PCr restoration after exercise.
The majority of these investigations have generally used localised low or high intensity exercise and $^{31}$P-MRS to identify the above relationship, usually utilising a cross sectional design. One of the first experiments was undertaken by McCully and colleagues (1989) and wrist flexions were employed as experimental model. Subjects were divided according to their training background (4 rowers, 4 controls) and performed wrist flexions that resulted in mild alterations in metabolic profile (for example pH did not change). Rowers showed better PCr restoration after this type of exercise in comparison with the controls and it was concluded that this was due to their superior aerobic power (it was not measured). The same research group (McCully et al., 1991) using a similar design (calf plantar flexions, $^{31}$P-MRS), investigated the effects of a 14 d endurance training schedule (two leg raises for 1 h) on PCr restoration. The PCr half-time recovery was faster after training; however, only 4 individuals participated in this training regimen and no control group was employed. In addition, no $\dot{V}O_2\,max$ test or any other endurance fitness test was performed. Takahashi et al. (1995) used knee extensions in combination with $^{31}$P-MRS to assess post-exercise PCr resynthesis. Using endurance athletes and recreational controls the investigators employed 4 different exercise intensities which in turn resulted in a different metabolic profile at the end of each exercise. In the initial two low intensities no differences were seen in the metabolic responses (similar reductions in the PCr and calculated pH) and post-exercise metabolic kinetics. However, in the last two more intense stages end PCr and pH differed between groups as well as recovery of these metabolic responses. However, similar immediate post-exercise metabolic responses are necessary in order to provide the foundation to test the hypothesis that metabolic recovery rates differ between groups (Cooke et al., 1997). Thus, the metabolic responses to an exercise bout are important as recovery will to some extent be dependent on the metabolic response to exercise alone.

Slightly different results were reported by Petersen and Cooke (1994) where PCr kinetics during 2 min unilateral plantar flexion exercise and recovery were assessed in endurance ($\dot{V}O_2\,max$: 67 mL·kg$^{-1}$·min$^{-1}$) and sedentary controls ($\dot{V}O_2\,max$: 46.1 mL·kg$^{-1}$·min$^{-1}$) with $^{31}$P-MRS. PCr recovery did not differ between groups, but there was a disparity in pH kinetics where a faster elevation towards resting levels occurred in endurance athletes. The authors justified the discrepancy (PCr resynthesis was not
faster in the endurance group) between their findings and earlier studies by the relatively low pH post-exercise for both groups in their study in comparison with other studies where post-exercise pH was little changed from rest (e.g. McCully and colleagues, 1989 and 1991). The suggestion that PCr synthesis is not enhanced in endurance-trained individuals when the pH is low derives from the fact that high accumulation of $H^+$ (low pH) will drive forwards the creatine kinase equilibrium towards PCr breakdown thereby minimising faster PCr resynthesis due to higher endurance fitness (McMahon and Jenkins, 2002). In a later study Cooke et al. (1997), using the same exercise pattern, PCr recovery was examined in two groups of physical education students separated on the basis of their $V_O^2_{max}$. Although, $V_O^2_{max}$ differences were similar to their previous reported research (46.6 vs. 64.6 mL·kg$^{-1}$·min$^{-1}$, low aerobic vs. high aerobic power) neither PCr nor pH kinetics during recovery were different between groups. Again, end exercise pH was very low in this investigation. Among the explanations given by the authors for these findings was that grouping individuals based on their $V_O^2_{max}$ maybe not be the most appropriate method, because peripheral factors may contribute to a greater extent to the recovery process.

Bogdanis and co-workers (1996a) have shown that PCr recovery after 30-s cycle sprinting was correlated with % $V_O^2_{max}$ @ 4 mmol·L$^{-1}$ and not with $V_O^2_{max}$ suggesting a closer link between endurance capacity and PCr replenishment (for study details see previous section). This latter study was also the only true maximal intensity protocol reported in the scientific literature (that is, sprinting) to examine PCr restoration that employed to date. In other studies the exercise intensities could be expressed at best as high-intensity, but not sprinting (McCully et al., 1989; McCully et al., 1991; Petersen and Cooke, 1994; Takahashi et al., 1995; Yoshida and Watari, 1997). It is currently uncertain whether peripheral or central factors are responsible for regulating PCr kinetics despite the trend towards the former (Bogdanis et al., 1996a; McMahon and Jenkins, 2002). It is also unclear whether the relationship between peripheral or central factors and PCr kinetics is the same for whole body maximal intensity exercise (i.e. on a motorised treadmill) and for exercise with a smaller muscle mass such as cycling (e.g. Bogdanis et al., 1996a). The clarification of this matter is further complicated because the maximal oxygen that can be used per
unit time is the product of both oxygen transport and oxygen utilisation while there is a debate as to which of these factors predominates during maximal exercise. Moreover, some individuals may possess higher ability of the former or the latter factor and still produce the same $\dot{V}O_2\text{max}$ (McMahon and Wenger, 1998). This may also explain the large inter-subject variability in PCR recovery kinetics observed by Roussel et al. (2000). However, it still remains debatable which factors limit the maximal respiratory rate of muscle tissue although there is a tendency towards the oxygen supply (McArdle et al., 2001), as a result of observations that oxygen extraction in skeletal muscle remains near maximal in heavy exercise (Lash et al., 1995; Basset and Howley, 2000).

A recent review has emphasised the importance of ADP as a regulator of the rate of PCR resynthesis after exercise, either directly through its free cytosolic concentration or indirectly through its effect on the free energy of ATP hydrolysis (McMahon and Jenkins, 2002). However, despite the fact that the calculated ADP (equation 2.1. McMahon and Jenkins, 2002):

$$1.66 \times 10^9 \text{mol}^{-1} = [(\text{creatinine}) \times (\Sigma \text{ATP})] \times [(\text{PCR}) \times (\Sigma \text{ADP}) \times (\text{H}^+)]^{-1},$$

where $1.66 \times 10^9 \text{mol}^{-1}$ is the constant for the mass action ratio concentration in studies seems to be elevated after exercise using $^{31}\text{P}-\text{MRS}$ technique (Takahashi et al., 1995; Roussel et al., 2000) no such finding has been observed after maximal intensity exercise using the needle muscle biopsy technique (Nevill et al., 1989; Bogdanis et al., 1995). This disparity is probably the result of different methodologies used and more particularly the fact that $^{31}\text{P}-\text{MRS}$ detects those metabolites that are in free active equilibrium in the cytoplasm whereas the needle biopsy technique measures also bound compounds (Sapega et al., 1987). In addition, pH calculated through the $^{31}\text{P}-\text{MRS}$ method represents intracellular pH, while pH measurements via muscle biopsy technique represent an average pH value for the entire tissue, intracellular and extracellular (Wilson et al., 1988). On the other hand $^{31}\text{P}-\text{MRS}$ does not give absolute values, but only relative changes for the metabolites in question (Sapega et al., 1987). It is currently unclear to what extent these methodological differences can affect the findings of a maximal intensity exercise protocol.
2.2.5. EFFECT OF SHORT-TERM ENDURANCE TRAINING ON OXYGEN UPTAKE DURING SPRINTING

There is increasing evidence that one of the beneficial adaptations due to endurance training might be an enhanced oxygen uptake during repeated sprints (Hamilton et al., 1991; Tomlin and Wenger, 2002). This greater reliance on the aerobic energy system may reduce the hydrogen ion concentration due to relatively less activation of the glycolytic energy system and thus a lowered production of lactic acid. Subsequently, fatigue may be delayed as hydrogen ion accumulation will be less (Hermansen, 1982).

Hamilton et al., (1991) found that during the course of ten flat-out 6-s treadmill bouts (30 s passive rest in between) endurance-trained volunteers consumed more oxygen (measured for sprint-recovery efforts together) than their game player counterparts and accumulated less blood lactate. They also found a significant positive correlation between fatigue index (percentage changes between the first and the last sprint) for mean speed and oxygen uptake during the sprint-recovery bouts. Although there was a slight mismatch in PPO, game players had a greater decrement in MPO than endurance athletes. Moreover, in both groups oxygen uptake gradually increased with subsequent sprints and there was a significant positive relationship ($r = 0.83, P < 0.05$) between $\dot{V} O_2 \text{max}$ and oxygen uptake per sprint-recovery bout and significant negative relationship between $\dot{V} O_2 \text{max}$ and fatigue index for both PPO and mean speed ($r = -0.60$ and $r = -0.68$, respectively, $P < 0.05$). These authors interpreted the data in the context that endurance athletes rely less on the glycolytic energy system than game players as reflected by the lower lactate values and higher oxygen consumption.

Similar findings were reported by Tomlin and Wenger (2002) where 13 female soccer players, matched for their MPO (6 s) in the first sprint, but separated according to their differences in $\dot{V} O_2 \text{max}$, performed ten 6-s sprints (30 s passive rest) on a friction-braked cycle ergometer. As previously reported (Hamilton et al., 1991) $\dot{V} O_2$ increased with every subsequent sprint-recovery bout and in the Tomlin and Wenger (2002) investigation this elevation was accompanied by a significant positive correlation between $\dot{V} O_2 \text{max}$ and sprint-recovery $\dot{V} O_2$ ($r = 0.78, P = 0.02$). Furthermore, the % decrement in MPO (6 s) was more pronounced in the less well-
trained individuals and they also consumed less oxygen in the last two sprint-recovery bouts than the moderately trained individuals. Bogdanis and co-workers (1996a) also revealed a significant relationship between $\dot{V} O_2 max$ and the aerobic contribution in sprint 1 ($r = 0.79$, $P = 0.05$) and sprint 2 ($r = 0.87$, $P = 0.01$) during two 30-s cycle sprints, suggesting that $\dot{V} O_2 max$ determines the magnitude of oxygen uptake during intermittent maximal exercise. However, these authors also demonstrated high positive correlations between $\% \dot{V} O_2 max @ 4 \text{ mmol}$-$\text{L}^{-1}$ and both MPO and mean speed recovery during the first 10 s of the second sprint, while such a relationship did not appear between those two variables and $\dot{V} O_2 max$. Thus, aerobic power may determine the magnitude of the aerobic contribution during sprints, but endurance capacity may govern the recovery of performance variables. It seems that a debate emerges concerning this issue with some researchers supporting the view that $\dot{V} O_2 max$ is the dominant factor (Tomlin and Wenger, 2002) while others promote the idea that peripheral factors, as indicated by $\% \dot{V} O_2 max @ 4 \text{ mmol}$-$\text{L}^{-1}$, are the major determinant (Bogdanis et al., 1996a).

However, since maximal aerobic power is the product of oxygen transport and oxygen utilisation ($\dot{V} O_2 max = Q \times (a-v)O_2 max$) thereby both central (cardiac output) and peripheral (arteriovenous difference for oxygen) factors play a role, $\dot{V} O_2 max$ can not be excluded. In addition, this analysis suggests that despite the superficial disagreement between the research groups (i.e. Bogdanis et al., 1996a; Tomlin and Wenger, 2002) inter-individual differences may mask any apparent association between maximum oxygen consumption and recovery of power output. More particularly, in the Bogdanis and co-workers study their subjects may have had a more pronounced adaptation of the peripheral component of $\dot{V} O_2 max$ (that is, arteriovenous difference for oxygen) as reflected by the $\% \dot{V} O_2 max @ 4 \text{ mmol}$-$\text{L}^{-1}$ rather than central (that is, cardiac output). On the other hand in the investigation by Tomlin and Wenger (2002), their subjects may have possessed a more balanced or a higher adaptation in the central component. This notion is further supported by the experiment of McMahon and Wenger (1998) where a close relationship between $\dot{V} O_2 max$ (especially the peripheral component) and power output recovery appeared.
The advantage of a high $\dot{V}O_2 max$ for power recovery during intermittent maximal intensity exercise has also been demonstrated by Balsom and colleagues (1994b) who employed a slightly different experimental approach. Their subjects performed ten 6-s cycle sprints interspersed with a 30 s passive recovery under normoxic and hypoxic conditions. In the hypoxic trial participants produced lower speeds and as a consequence, the % decrement in speed (percentage decrement between the first and the last sprint) was higher in comparison with the normoxic conditions. In addition, $\dot{V}O_2$ was lower in hypoxic conditions due to lower oxygen availability, a finding that corroborates the view of the importance of oxygen consumption. In a parallel study, the same research group (Balsom et al., 1994a) reconfirmed the necessity of oxygen availability in an experimental design where erythropoietin (EPO) was administrated to a group of six physical education students. Six weeks of EPO administration resulted in an 8 % higher $\dot{V}O_2 max$ and significantly lower concentrations of lactate and hypoxanthine after fifteen 6-s bouts of high-intensity (5.5 m·s·l) treadmill running (10 % incline, 24 s rest between runs). Although there was no control group and oxygen uptake was not determined, the authors explained these findings by a greater aerobic contribution and faster PCr resynthesis after the treatment period.

It should be pointed out though, that during the aforementioned studies, expired air was collected without separating the sprint and recovery periods (probably due to the short period of the sprint) and therefore their findings do not represent the true $\dot{V}O_2$ during sprinting. Moreover, the Douglas bag method is not a very accurate technique when gas collections are only 30 s (Taylor et al., 1955; Howley et al., 1995). In addition, there are a number of cross sectional studies which have deployed either 30-s sprints (Calbet et al., 2003) or high-intensity exercise of fixed duration (Thomson and Garvie, 1981; Medbo and Sejersted, 1985) in sprint in comparison with endurance trained athletes where, even though the subjects had completely different training backgrounds, the oxygen consumption was the same. So, it remains to be seen whether endurance training can modify the contribution of the energy systems that are recruited during a single sprint.
2.2.6. OXYGEN CONSUMPTION DURING RECOVERY FROM SPRINTING

It has long been suggested that the initial $\dot{V}O_2$ during recovery from short-term intense exercise is associated with PCr and ATP degradation (Hultman et al., 1967; Piiper and Spiller, 1970). At the same percentage of $\dot{V}O_2$ max trained individuals consume more oxygen than their untrained counterparts resulting in higher $\dot{V}O_2$ during recovery (Hagberg et al., 1980). Therefore, during recovery from sprinting, if endurance training has induced a higher $\dot{V}O_2$ during the sprint then there should be a higher oxygen uptake immediately after the maximal bout. A higher oxygen uptake at the beginning of the recovery will promote faster restoration of phosphagens (Quistorf et al., 1992) and thereby a more rapid recovery of power output in endurance fitter individuals. The time course of the fast component of oxygen uptake during recovery of about 1-2 min (Piiper and Spiller, 1970) matches favourably with the half-time of PCr resynthesis calculated to occur within 1 min (Bogdanis et al., 1995) post-sprint. This in turn gives even more ground to the hypothesis of a tight link between ATP/PCr replenishment and immediate oxygen uptake after sprinting.

There are very few previously published papers on the topic of the relationship between oxygen uptake during recovery and recovery of phosphagens. Nevertheless Hamilton and colleagues (1991), comparing endurance athletes and game players’ power outputs during intermittent maximal intensity whole body exercise, reported data for $\dot{V}O_2$ during recovery. The authors observed a fast and slow component of this oxygen uptake during the recovery period similar to the traditional kinetics of “oxygen debt” while there was a tendency for oxygen uptake during recovery to be higher in game players (less endurance-fit individuals) throughout the 15 min post-exercise recovery period (ns). Close inspection of their data reveals a somewhat different view though, which was not discussed by the authors. In the first minute post-exercise (representing the fast component) endurance athletes had higher $\dot{V}O_2$ compared with the game players although there was no statistical comparison of these data reported in the paper. This observation of a higher initial recovery oxygen uptake for the endurance athletes together with their faster recovery during repeated sprints supports the suggestion that if endurance training can enhance recovery oxygen uptake then this may be beneficial in terms of phosphagen recovery and thereby power output recovery.
The connection between oxygen uptake during recovery and physical fitness has also been investigated by Merscier (2001, cited in Tomlin and Wenger). The author found moderate relationships between 3 min oxygen uptake during recovery and $\dot{V}O_2\text{ max}$ following 3 x 20- (r = 0.67, P = 0.002) and 6 x 10-s (r = 0.47, P = 0.03). However, for a shorter 12 x 5-s sprints protocol there was no significant correlation. It seems that in order to detect a link between $\dot{V}O_2$ after short-term intense exercise and endurance fitness that more severe exercise is needed in terms of duration (Tomlin and Wenger 2001). On the other hand recovery durations of 3 min may miss the opportunity for a higher correlation (Tomlin and Wenger 2001) since the most of the recovery $\dot{V}O_2$ occurs in the first 1-2 min (Piiper and Spiller, 1970), a time period that in turn is similar to the half-time of PCr recovery in this type of exercise (Bogdanis et al., 1995).

However, Bell et al. (1997) utilising a homogeneous group of endurance athletes failed to reveal any relationship between recovery $\dot{V}O_2$ and endurance fitness as indicated by $\dot{V}O_2\text{ max}$ or ventilatory threshold. Their exercise protocol consisted of 3 bouts of cycling at 125% $\dot{V}O_2\text{ max}$ for 1 min separated with 5 min rest. The authors claimed that in a homogeneous group of endurance athletes the metabolic recovery after the high-intensity exercise is determined by diverse factors compared with those in power/strength trained athletes. In addition, there is the theoretical possibility that a ceiling exists beyond which further improvements in endurance fitness do not have an impact on recovery from short-term intense exercise (Bell et al., 1997; Tomlin and Wenger, 2001). Moreover, the lack of association between recovery $\dot{V}O_2$ and fitness indexes may be the result of the use of half-time recovery $\dot{V}O_2$, which may not accurately reflects endurance adaptations in cross-sectional studies (Tomlin and Wenger, 2001). Lack of control of diet, time of the day for testing and for previous exercise may also have influenced the results of earlier studies given that all these factors impact upon resting oxygen uptake (Poehlman, 1989) and thus recovery $\dot{V}O_2$.

In conclusion, it should be emphasised that, even though recovery $\dot{V}O_2$ (its fast component) has been associated with endurance training adaptations, direct observations of recovery of phosphagens, recovery $\dot{V}O_2$ and endurance fitness have

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ANTONIS TSAMPOUKOS
Ph.D. THESIS
not, as yet, been reported. In addition, there is not general consensus with respects to the importance of oxygen uptake in the recovery processes (Banghso et al., 1990; Green and Dawson, 1993). Thus, it is currently unsettled as to whether and to what extent recovery $\dot{V}O_2$ is related to high energy phosphagen recovery after sprinting.

2.2.7. LACTATE REMOVAL FROM MUSCLE AND BLOOD

One of the potential contributors of fatigue in human skeletal muscle during repeated sprinting, as already mentioned in previous paragraphs, is the decrease of pH. Since the release of H$^+$ due to lactic acid production has been estimated to account for more than 85 % of this decrease in pH values (Sahlin, 1986), it can be claimed that enhanced lactate removal (and associated H$^+$ removal) from active muscle to blood and from blood to other body compartments may be beneficial in recovery of such activities, especially with MPO (Spriet et al., 1989; Gaitanos et al., 1993; Maughan et al., 1997). Endurance training adaptations have been long associated with reduced blood lactate concentrations after exercise at both the same absolute and relative exercise intensities (Hurley et al., 1984) despite the fact that there is ongoing controversy with respects to whether or not lower blood lactate concentrations are the result of abridged lactate production or an increase in lactate removal (Coggan and Williams, 1995). However, in terms of recovery from maximal exercise the beneficial effects of endurance training, if any, may be more focused on lactate removal. Although there is a complete gap in the scientific literature concerning maximal intensity exercise (including cross-sectional studies) an evaluation of the existing data from sub-maximal/intensity exercise will be presented below.

The first observations from Bonen and Belcastro (1976) showed that trained individuals may possess faster lactate disappearance rates (from blood) than their less trained counterparts. However, subsequent findings were not consistent and both supported (Oyono-Enguelle et al., 1990; Freund et al., 1992; Messonnier et al., 2001) and contested (Evans and Cureton, 1983; Bassett et al., 1991; Taoutaou et al., 1996; Oosthuse and Carter, 1999; Bret et al., 2003) the views of Bonen and Belcastro (1976). It seems that the variety of the protocols employed, subjects’ characteristics
and the mathematical equations performed to assess lactate removal from blood all contributed to these discrepancies.

For example, in the study of Evans and Cureton (1983) the failure to find any relationship between improved lactate clearance from blood and endurance training may be due to the mathematical model used (logarithmic transformations) for the analysis. In addition, the first sample was withdrawn in the 3rd min of the recovery and thereby the authors may have missed the peak blood lactate concentration which is an important factor when lactate removal rates are calculated. Moreover, improved lactate efflux from the muscle after endurance training (Basset et al., 1991; Taoutaou et al., 1996; Messonnier et al., 2001) would have probably further masked their findings and emphasises the need for frequent sampling when lactate recovery is to be studied.

However, the factors that mostly influence the findings of investigations examining whether or not endurance training may result in an enhanced clearance of lactate from the blood are the lack of equity of duration and intensity of the exercise protocol employed to elevate blood lactate concentrations between groups of different fitness status. This view comes from the findings of Freund et al. (1986) and Freund et al. (1990) who showed that lactate removal after exercise is dependent on the previous duration and work rate performed by individuals. This experimental artefact is shown in the findings of Freund et al. (1992), where, after an incremental cycle test to exhaustion, sickle cell patients (disease known to impair oxidative capacity), endurance-trained subjects and healthy untrained controls had similar arterial lactate recovery curves as evaluated with a biexponential mathematical model. However, the initial treatment of the data was made on the basis of their last bout where the endurance-trained subjects had performed significantly more bouts than the healthy subjects who had in turn performed more bouts than the sickle cell patients. Comparing the different groups on the basis of the highest exercise bout that all groups performed, it was clearly shown that the fitter individuals had faster lactate clearance from the blood. The same reasons may also account for the results of Basset et al. (1991) where although both endurance athletes and untrained individuals completed the same amount of exercise (3 min cycling) the intensity was somewhat
different (85 vs 80 % % $V_{O2\text{ max}}$ in endurance vs untrained, respectively). This resulted in significant higher peak lactate concentrations in trained individuals, which may influence the recovery curves as already mentioned above in the light of evidence of an inverse relationship between lactate clearance during recovery and intensity of prior exercise Freund et al., (1986).

The higher exercise intensity and the longer duration achieved by triathletes after a volitional test to exhaustion in comparison with sprinters (Taoutaou et al., 1996) are in line with the above suggestions. Similarly, Oosthuysse and Carter (1999), separating their groups based on % $V_{O2\text{ max}}$, found no differences in lactate disappearance curves after an incremental cycle test to exhaustion. Again, endurance athletes were able to sustain exercise for longer, thus achieving higher power outputs and higher (even though not statistically significant) venous blood lactate values thereby prohibiting clear conclusions.

The cumbersome process of matching different groups in terms of power output or work rate and duration of exercise, as is the case in cross-sectional studies, make comparisons difficult, and stable conclusions between individuals in terms of lactate removal complex. This condition is depicted more clearly in the maximal intensity exercise serial models (e.g. Nevill et al., 1996a) where sprinters and endurance athletes achieve quite different power outputs and lactate accumulations. Identical duration (Freund et al. 1990) and intensity (Freund et al., 1986) of prior exercise of the different groups involved in the assessment of lactate disappearance rates is required regardless of the mathematical model used to treat the results.

Finally, Messonnier and colleagues (2001) found that both lactate exchange and removal abilities improve after endurance training at the same sub-maximal (90 % $V_{O2\text{ max}}$) relative intensity. It seems that when the previous work performed is at relatively low intensity, and thereby lower accumulation of lactate in the blood occurs, endurance status can discriminate faster lactate dissipation. Apparently, the bouts adopted from Evans and Cureton (1983) and Bishop et al. (2003a) were above that "threshold" (> 110 % $V_{O2\text{ max}}$) which may explain, at least in part, why those authors did not find any better lactate removal after training.
Another issue, that has been extensively discussed in the previous paragraph concerning PCr restoration, is that grouping of subjects according to their $\dot{V} O_2\text{max}$, may not be the only option, since improved lactate clearance is also probably related to peripheral factors (Oyono-Enguelle et al., 1990; Freund et al., 1992; Taoutaou et al., 1996). Separating participants based on $\% \dot{V} O_2 \text{max} @ 4 \text{ mmol·L}^{-1}$ is an alternative attractive approach, or at least an additional measure, to supplement groupings on the basis of $\dot{V} O_2\text{max}$.

The above findings indicate the difficulty in studying the effect of training on blood lactate removal after exercise. Differences in post-exercise sampling, mathematical models, duration and intensity of prior exercise as well as training status of the subjects make this exceptionally difficult. It is also unclear to what extent the findings from sub-maximal/high-intensity exercise apply to sprinting. It is definitely a fertile area for further research.

2.2.8. SUMMARY: HYPOTHETICAL MECHANISMS WHICH LINK ENDURANCE TRAINING WITH IMPROVEMENTS IN POWER OUTPUT RECOVERY AFTER SPRINTING

It has been hypothesised that higher maximal aerobic power due to the training would lead to a greater aerobic contribution during a sprint (Bogdanis et al., 1996a) and thus lesser dependence on the glycolytic system for energy and thereby less accumulation of muscle lactate. The latter would contribute to a smaller amount of H⁺ accumulation into the muscle cell (Sahlin, 1986) and consequently the depression of pH would be smaller, a condition that adversely affects contractile properties and favours fatigue (Sahlin, 1992). During recovery, and in particular just after the end of the sprint an elevated pH in comparison with pre-training values could therefore facilitate performance recovery.

Larger initial oxygen uptake during the first part of recovery ($\approx 1 \text{ min}$) will ameliorate to some extent, the resynthesis of the phosphagens (ATP/PCr) since PCr resynthesis in the initial phase of recovery is positively influenced by high availability of oxygen (Hultman et al., 1967; Piiper and Spiller, 1970). Central blood flow will also
contribute to this process through an elevated cardiac output via increases in stroke volume (Ekblom and Hermansen, 1968) thereby enhancing oxygen delivery, as well as enhanced movement of nutrients into the recovering muscle, processes that are closely coupled with PCr resynthesis (Quistorf et al., 1992). This will be achieved by increased blood flow to the muscles resulting from improved capillarisation (Anersen and Hensiksson, 1977a), arterial vascular reactivity muscle vascular conductance, oxygen-haemoglobin affinity and venous compliance and reactivity (Snell et al., 1987). Within the muscle itself, better-conditioned muscle will exhibit increases in oxidative potential enzymes, expansions in mitochondria capacity (Holloszy and Coyle, 1984) and elevations in myoglobin concentrations (Saltin and Rowell, 1980). The latter adaptations will then result in improved oxygen extraction of the muscle (Rowell, 1993), and, thus will prepare the muscle cell to accelerate PCr replenishment since PCr recovery is oxygen dependent process. Augmented blood flow and improved oxygen extraction will contribute also to greater removal of lactate through a better efflux from the muscle and probably higher buffering capacity.

This theoretical framework suggests that a fitter sprinter and/or games player, in terms of endurance, possesses faster PCr anaplerosis and elevated pH, which are both associated with improved PPO and MPO, respectively, (Maughan et al., 1997) in a subsequent sprint. This notion has also been strongly supported by the sporting community. However, the above hypothesis remains untested.

2.3. EFFECTS OF MENSTRUAL CYCLE PHASE UPON METABOLISM AND PERFORMANCE

2.3.1. DEFINITIONS
The normal reproductive years of the female individual are characterised by a highly complicated and differential endocrine system. This differentiation is necessary due to the hormonal changes as governed by the ovaries and female sexual organs (Guyton and Hall, 1996; Norman and Litwack, 1997; Vander et al., 2001). The process of all these mechanisms has been termed menstrual cycle by many physiologists (Sherwood, 1989; Vander et al., 2001) and gynaecologists (Shangold, 1994; Speroff,
Nevertheless, Guyton and Hall, (1996) have questioned this term for its accuracy proposing the term “female sexual cycle” as more appropriate. To avoid confusion the first term, menstrual cycle, will be used in this thesis.

The menstrual cycle is usually divided into two phases, follicular or proliferative and luteal or secretory (Sherwood, 1989; Shangold, 1994; Guyton and Hall, 1996; Vander et al., 2001). However, due to the specific endocrinological events that take place at the time just prior to, during and immediately after ovulation some scientists (Speroff, 1999) have divided the menstrual cycle into three phases including the ovulation as a separate phase. Figure 2.1 gives a diagrammatic representation of these three phases having superimposed the two major female reproductive hormones 17-β estradiol (estradiol) and progesterone.

In terms of athletic performance and the general pattern that is going to be followed in this thesis, it is of practical importance to distinguish the following phases of the menstrual cycle taking as day 1 the first day of the menstrual bleeding in a hypothetical 28 days cycle (figure 2.2):
Menstruation: the days during the menstrual bleeding. Usually days 1-7 of a hypothetical 28 days menstrual cycle.

Follicular: day 1 of the menstrual cycle and until the initiation of estradiol surge (exclusive).

Mid-cycle: the days from the significant rise in estradiol concentration until the day of ovulation (inclusive).

Luteal: the days after ovulation till the next menstrual cycle.

Late luteal: the last 2-3 days of the cycle.

Figure 2.2. A more detailed schematic representation of menstrual cycle phase (from Vander et al. 2001).

When the two additional phases (menstruation and late luteal) are not being discussed it means that they are included in the respective phases (follicular and luteal). However, due to the high diversity of terms and definitions in the literature, where appropriate, the definition of menstrual cycle phase from each author for each particular study will be also provided.

The rationale of the inclusion of two or more phases in addition to the ones suggested by Speroff (1999) has its roots in the potential performance decrements associated
with perimenstrual discomfort present in some menstrual cycles at the end (late luteal) and/or the first (menstruation) days of the menstrual cycle (Giacomoni et al., 2000).

2.3.2. PHYSIOLOGY AND REGULATION OF MENSTRUAL CYCLE.

For the brief review of the events that occur during the time course of the human menstrual cycle the split as suggested by Speroff, (1999) will be used. Moreover, the emphasis will be placed on the actions/concentrations of estradiol and progesterone, which, as it will be revealed in later sections, are associated with alterations in performance and metabolism during exercise.

During the follicular phase the aim of the female reproductive system is to ensure, through an orderly sequence of events, that the proper number of follicles is ready for ovulation. There are four stages that a normal follicle passes during the process of its maturation (figure 2.3).

Figure 2.3. Stages of normal follicle during the process of its maturation (from Vander et al. 2001).
In its initial "appearance" the follicle is named primordial and consists of one primary oocyte surrounded by a single layer of spindle-shaped cells called granulosa cells (figure 2.3). In this period follicular growth is only influenced by factors derived from the oocyte (Speroff, 1999).

Further development of the primordial follicle initiates its entry in the preantral stage (figure 2.3). In this developmental period the oocyte enlarges and is surrounded by a new membrane the zona pellucida. It is in this stage that the hormones start to be involved in the follicle maturation with follicle-stimulate hormone (FSH) to propel follicles in this preantral stage (Yong et al., 1992). In this stage there is a small, but insignificant, rise in estradiol (Speroff, 1999) while the existence of low concentration of progesterone derives from the adrenal gland (Jude et al., 1992).

In the next stage, a series of events take place in which, with the synergistic influence of estradiol and FSH, a production of follicular fluid appears in the intracellular spaces of the granulosa forming a cavity while the preantral follicle passes in antral stage (Speroff, 1999). It is in this stage that the dominant follicle is selected and the first significant rise of estradiol occurs.

In the last stage, the antral follicle gradually transforms to a preovulatory follicle where the granulosa cells enlarge, there is acquisition of lipids and the follicle becomes richly vascular (Vander et al., 2001). In this stage there is an initially slow rise and after that an exponential rise in estradiol concentration with the peak at approximately 24-36 h prior to ovulation (Pauerestein et al., 1978). It should be emphasised here that even though this time course is the most often met, individual variations are large (World Health Organisation, 1980). This rise in estradiol must be significant enough in both concentration (> 200 pg·mL⁻¹) and dose (≈ 50 h) in order to have a positive feedback on Lutenising hormone (LH) concentration and thus for the event of ovulation to occur (Young and Jaffe, 1976; Speroff, 1999). Nevertheless, the latter is not always the case even though it is characteristic of 98.5 % of the cycles studied (Simon et al., 1987). In the same period there is a small but significant increase in progesterone (Chikasawa et al., 1986) concentration (< 2 ng·mL⁻¹) which
favourably affects the positive feedback response to estrogens in both a time and dose dependent manner (Speroff, 1999).

The above process usually last 10-14 days with large variation among women and between menstrual cycles in the same woman. It is the variation and the length of the follicular phase that determine the length and variation of menstrual cycle rather than the length of the luteal phase (Speroff, 1999).

The mid-cycle phase of the menstrual cycle includes the process of ovulation of the dominant follicle. Accurate estimations place (figure 2.4) the event of ovulation 10-12 h after the LH peak and 24-36 h after peak estradiol levels are attained (Pauerestein et al., 1978; World Health Organisation, 1980; Hoff et al., 1983; Speroff, 1999). Similar to estradiol LH has to attain and maintain a threshold (1262 ng·mL⁻¹) of about 14-27 h in order for full maturation of the oocyte to occur (Zelinski-Wooten et al., 1992). As was the case for the follicular, ovulation can vary considerably from cycle to cycle even with the same women (Pauerestein et al., 1978; World Health Organisation, 1980; Speroff, 1999). The aim of the LH in this stage is to stimulate continuation of reduction division in the oocyte, luteinisation of the granulosa and synthesis of progesterone within the follicle while the functional importance of progesterone associated with the enhanced activity of proteolytic enzymes for digestion and rupture of the follicular wall (Speroff, 1999).

![Figure 2.4. Schematic representation of the hormonal changes just prior to and at ovulation (from Speroff, 1999).](image-url)
The last phase of the menstrual cycle is the luteal phase where the creation of the corpus luteum from the remaining granulosa and theca cells from the dominant follicle is the major event (Vander et al., 2001). In non-pregnant women the corpus luteum secretes large quantities of progesterone and estradiol which reach their peak 8 or 9 days after ovulation, a period that coincides and is associated with peak vascularisation (Speroff, 1999). Again the exact timing of peak levels of the two main reproductive hormones is much dependent onto the individual’s normal variation. A number of studies have shown that a luteal lasting between 11-17 days can be considered normal (Lenton et al., 1984b), despite the fact that 26% of luteal phases lasting only 10 days can also be normal (Lenton et al., 1984b).

Finally, during the last few days of the luteal phase the corpus luteum starts to degenerate resulting in a plummeted decrease in the circulatory levels of estradiol and progesterone (figure 2.2).

2.3.3. THE NORMAL MENSTRUAL CYCLE.

Perhaps one of the most confusing issues that currently exist in the literature is the plethora of definitions as to what is a normal menstrual cycle. According to Shangold (1994) a normal menstrual cycle lasts 23 to 35 days while Norman and Litwack (1997) agree a range 25 to 35 days. Vander et al. (2001) gives a more general profile and states that menstrual cycle varies considerably from woman to woman and in any particular woman giving an average cycle length of about 28 days as normal. This 28-day average is also cited in Guyton and Hall (1996) even though these physiologists do report that a menstrual cycle can be normal with a cycle length of 20 to 45 days. However, what all these textbooks fail to report is why a particular cycle, with a particular length, is considered normal.

Starting the debate of the typically reported average cycle length of 28 days, it is clear that the authors of these textbooks have interpreted the research findings in a rather superficial manner. However, 28 days has been reported the most common mode (Treolar et al., 1967; Vollman, 1977), but not the average of a typical menstrual cycle. This perplexity has probably its roots in exactly this mistaken interpretation of the mode (i.e. the most common value) as average. To give an example, in the study
of Vollman (1977) only 12.4% of the menstrual cycle examined had a length of 28 days. Thus, when the menstrual cycle is considered it is more accurate to give a range of the cycle length, rather than average.

The solution of which menstrual cycle can be defined as normal becomes more complex since experimental evidence has highlighted the possibility that even though the cycle length of a particular menstrual cycle is within a predetermined range (for a hypothetical example of 25-35 days) there is still the chance to be anovulatory (Langren et al., 1980). It is now well recognised that indirect methods such as basal body temperature used in the studies of Treolar et al. (1967) and Vollman (1977) have limited capability to accurately represent the actual hormonal fluctuations of the normal menstrual cycle since basal body temperature can be influenced by other factors such as illness, alcohol, hot drinks and restless sleep (Bauman, 1981). In addition, as many as 22% of women with normal hormonal profiles do not show a rise in their basal body temperature during their menstrual cycle (Moghis, 1976; Bauman, 1981). Thus, the British Royal College of Obstetricians and Gynaecologists clearly states in page 4 of its guidelines that temperature charts are of limited use (The Royal College of Obstetricians and Gynaecologists, 1992). It has therefore, become popular to use radioimmunoassays to determine the exact concentrations of hormones in a particular phase of the menstrual cycle and to correlate these hormones with the event of ovulation (e.g. World Health Organisation, 1980). As can be seen from figure 2.2 (accepting that there are individual variations), by performing a clinical and histological examination of the ovary and corpus luteum at the same time as determining the hormonal profile via blood samples, it is possible to identify the temporal relationships of progesterone, estradiol, LH and ovulation as shown in figure 2.2 (Pauerestein et al., 1978; World Health Organisation, 1980).

There is now general consensus that hormonal documentation (usually with progesterone and/or estradiol) is the more appropriate way to judge whether a menstrual cycle is normal (e.g. Speroff, 1999) and in terms of exercise to relate these hormonal variations with performance and metabolism (Lebrun, 1993). A progesterone concentration of > 5 ng·mL⁻¹ is generally accepted as reflecting a normal ovulatory menstrual cycle (Johanson et al., 1971; Langren et al., 1980). As has already been mentioned, high estradiol circulatory levels are required to induce
positive feedback in the process of ovulation (mid-cycle phase) while in most cases luteal estradiol is lower than this value but higher than the follicular phase (Langren et al., 1980). Combining these findings it can be said that a normal menstrual cycle has the following characteristics:

• Luteal progesterone of > 5 ng·mL⁻¹ (Johanson et al., 1971; Langren et al., 1980).
• Luteal phase length of approximately (usually) 11-17 days (Lenton et al., 1984a; Lenton et al., 1984b; Smith et al., 1985).
• Large increase in (pre-ovulatory (24-36 h) circulatory levels estradiol in comparison with the early follicular phase (usually, but not always, above 200 pg·mL⁻¹) (Young and Jaffe, 1976; Simon et al., 1987; Speroff, 1999).
• Pre-ovulatory progesterone levels higher than follicular phase but below 2 ng·mL⁻¹ (Chikasawa et al., 1986).

Depending on the purpose of the study these indices can be taken individually or in combination to determine menstrual cycle phase. It should be mentioned here though, that menstrual cycle length longer than 40 days regardless of luteal phase length and hormonal profile is not usually accepted (World Health Organisation, 1980; Lenton et al., 1984a; Lenton et al., 1984b; Smith et al., 1985).

2.3.4. EARLY PERCEPTIONS.
Many superstitious beliefs about the menstrual cycle and performance (Lebrun, 1993), or even menstrual cycle per se (Speroff, 1999), have existed throughout history. The famous dictum of Fraenkel in 1911 (quoted in paper of Landgren et al., 1980) that “the only regular feature of the menstrual cycle is its lack of regularity” appears to be an overstatement as is the notion that “luteal phase is most frequently 14 days” claimed by Ober in 1957 (quoted in paper of Landgren et al., 1980). From the discussion in sections 2.3.1 to 2.3.3 it was clear that normal menstrual cycle has considerable variability, but also does follow a general pattern typical in most women. Nevertheless, these early points of view should not be overly criticised since the lack of technical knowledge for hormonal measurement in the blood (that is,
radioimmunoassays) did not allow these pioneers to gain any picture of the complex endocrinological events involved in the menstrual cycle.

Many misconceptions also appeared in the early attempts to identify the potential effects of menstrual cycle on performance. These first studies were qualitative in nature and the use of questionnaires was the main research tool. In one of these retrospective surveys Erdelyi (1962) studied 557 women from a variety of sports and 48.2% of them reported alterations in performance due to the menstrual cycle phase. Their results were very varied since some athletes mentioned better performance during menstruation (13%, for definitions see section 5.1) and 31% perceived that their performance was weakened during menstruation. However, in general these women perceived their performances to be better during the latter stages of the menstrual cycle as compared with the early days of the menstrual flow. These inconsistencies were characteristic of other studies of this type that followed. It is very difficult to interpret these studies since clear definition of cycle phase was impossible due to the lack of hormonal documentation and/or indirect methods such as basal body temperature, one of the main drawbacks of the retrospective survey research.

Although the survey based studies are of very limited value for monitoring the physiological events of the menstrual cycle and their potential effects on performance, they do give some stimulus for future research (Lebrun, 1993).

In the next paragraphs research based on direct (hormonal profile) or indirect basal body temperature methods will be predominantly reviewed. However, wherever needed, supplementary evidence of less controlled studies will be also discussed.

2.3.5. RELATIONSHIP BETWEEN ESTRADIOL AND ALTERATIONS ON PERFORMANCE AND EXERCISE PHYSIOLOGICAL RESPONSES.

Naturally secreted estradiol is an 18-carbon steroid with an aromatic A ring and a phenolic hydroxyl (figure 2.5). The main resource of estradiol is the ovarian follicular cell (Norman and Litwack, 1997; Speroff, 1999). The secretion of estradiol has different rates during different phases of the menstrual cycle (figure 2.6) being higher while approaching ovulation (Hoff et al., 1983). Metabolic clearance rate (the
estimation of the rate at which the steroid is irreversibly removed from the plasma by inactivation) at the late follicular (mid-cycle) is 1300 liters-days$^{-1}$ (Norman and Litwack, 1997).

Figure 2.5. The structural formula of Progesterone and estradiol (17β-estradiol).

Estradiol has been associated with alterations in performance and exercise metabolism. The most often mentioned hypothesis is that estradiol alters substrate metabolism by enhancing fat utilisation and sparing glycogen during sub-maximal exercise (Bunt, 1990; Ashley et al., 2000). Although the definite mechanisms of such metabolic actions have not been clearly illustrated yet, it is believed that they are related to basal levels of other hormones (Bunt, 1990) and alterations in the activities of specific enzymes such as lipoprotein lipase (Kendrick et al., 1987). The bulk of data of these observations come mainly from animal studies (rats) with usually exogenous doses of estradiol ranging from physiological to pharmacological levels (Kendrick et al., 1987; Kendrick et al., 1991). These beneficial increases in circulatory estradiol are linked with better performance (using an animal model) through modified carbohydrate utilisation during exercise (Kendrick et al., 1987; Kendrick et al., 1991). There is limited direct information with regards to the effects of estradiol on human modified carbohydrate utilisation during exercise. The experimental model used is the effect of different phases of the menstrual cycle (e.g. Nicklas et al., 1989; McCracken et al., 1994; Lebrun et al., 1995) on indirect indices of carbohydrate metabolism such as the respiratory exchange ratio (RER) and blood lactate. Only a few studies have examined changes (if any) in muscle glycogen concentration during different phases of menstrual cycle (e.g. Nicklas et al., 1989;
Hackney, 1999). These investigations verified the estradiol dependent glycogen sparing, however, the exact mechanisms were impossible to be ascertained due to the lack of the measurement of other metabolites, enzymes such as lipoprotein lipase and hormones. Estradiol has also been associated with the recovery of PCr after plantar flexion exercise linking the ovarian hormones with the recovery of power output (Harber et al., 1998). These researchers reached the above conclusion by comparing eumenorrheic and amenorrheic athletes and interpreting the higher values of estradiol (and progesterone) of eumenorrheic athletes as the cause of these differences. Their findings have been recently confirmed by a study in gonadectomised female rats (Ramanani et al., 1999). These investigators revealed that the presence of estradiol enhances the activity of myokinase and creatine phosphokinase enzymes and increases the rate of regeneration of ATP.

In addition, estradiol has been found to delay reductions in absolute resting plasma volume and thus exercise performance as long as is accompanied by low levels of progesterone (Fortney et al., 1988). The mechanism of such influences is not certain but is believed to act at the level of kidneys with an alteration or release of the antidiuretic hormone (Fortney et al., 1988). The antagonistic effects of progesterone upon estradiol metabolic reactions have been postulated (Crook et al., 1988; Bunt, 1990) with the most likely mechanism being progesterone-mediated reduction of estrogen receptors in the muscle (Vander et al., 2001). It is important to note though, that progesterone has a positive and synergistic role during the ovulation period and in concentrations below 2 ng·mL$^{-1}$ (see also section 2.3.2), while its negative effects on estradiol begins in the luteal phase (Speroff, 1999).
Finally, fluctuations of estradiol have been linked with susceptibility of an individual to sports injury (Liu et al., 1997; Kendal and Eston, 2002) and with perimenstrual pain (Giacomoni et al., 2000). Thus, it is possible that estradiol, may affect high-intensity eccentric exercise during the late luteal phase and at during menstruation phase (Giacomoni et al., 2000).
2.3.6. RELATIONSHIP BETWEEN PROGESTERONE AND ALTERATIONS IN PERFORMANCE AND EXERCISE PHYSIOLOGICAL PARAMETERS.

Progesterone is the principal progestin secreted by the corpus luteum (the other three are 20β-hydroxyprogesterone, 10α-hydroxyprogesterone, and 17α-hydroxyprogesterone which are all secreted in very small quantities) and its structure (figure 2.5) contains 21 carbons with oxo functionality (the prefix oxo denotes possession of the carbonyl group, C=O, in a structure in which other two bonds to carbon are attached to hydrocarbon moieties) on both C-3 and C-20 (Norman and Litwack, 1997). Although the corpus luteum is the main source of progesterone during the luteal phase, during the follicular phase the main contributor is the adrenal gland (Judd et al., 1992). Its secretion rates are episodic (Fillicori et al., 1984) and determined from adrenocorticotropic (ACTH) during follicular phase (Judd et al., 1992) and LH during luteal phase (Norman and Litwack, 1997). The metabolic clearance rate for progesterone is 2200 L·day\(^{-1}\) (Norman and Litwack, 1997).

Similar to estradiol, progesterone has also been implicated with performance alterations mainly via its effect on the respiratory system (Lebrun, 1993). Progesterone influences on the respiratory system were first postulated during the luteal phase of the menstrual cycle (England and Farhi, 1976) and during pregnancy (Artal et al., 1986). This progesterone-mediated hyperventilation has been confirmed during exercise during the luteal phase (e.g. Dombovy et al., 1987; Schoene et al., 1981). The finding that only eumennorrheic athletes (high concentrations of progesterone) and not amennorrheic athletes (low concentrations of progesterone) experience hyperventilation (Schoene et al., 1981) further links progesterone with alterations in the respiratory system. Pharmaceutical use of medroxyprogesterone acetate (a synthetic progestin) in patients with central hypoventilation syndrome to stimulate ventilation (Sulton et al., 1975) supports the above findings. It has also been shown that progesterone has stimulatory effects on the hypercapnic (response to high carbon dioxide in the blood) and hypoxic (response to low arterial oxygen) response at rest during the luteal phase (Schoene et al., 1981; Williams and Kranhebull, 1997). This stimulatory effect is also present in exercise during the luteal phase (Schoene et al., 1981; Dombovy et al., 1987). The mechanism by which progesterone acts upon
the respiratory system is thought to be centrally located (Bayliss and Millhorn, 1992). It has been suggested that the progesterone response is mediated in the hypothalamus via an estrogen dependent progesterone receptor mechanism and requires RNA and protein synthesis (Bayliss and Millhorn, 1992). These progesterone adjustments in the respiratory system have been implicated in performance variations during the luteal phase in comparison with the follicular phase of the menstrual cycle (Schoene et al., 1981; Dombovy et al., 1987) due to an increase in the ventilatory response to hypoxia and hypercapnia probably by increasing the subjective sensation of dyspnoea which has been speculated to adversely affect performance (Lebrun, 1993). The detrimental effects of dyspnoea on performance due to increased sensitivity to hypoxia and hypercapnia have been observed by comparing endurance athletes (decreased ventilatory response to hypoxia and hypercapnia) and nonathletes (Byrne-Quinn et al., 1971; Martin et al., 1979).

It has also been suggested that progesterone may play a role in increasing basal body temperature, although the primary reason for the rise is the increase in the production and secretion of norepinephrine, which is considered the thermogenic neural hormone (Moghissi, 1980). Progesterone has also been implicated by some authors in nonpregnant women in contributing to glycogen sparing as manifested by lower R and blood lactate concentrations (Dombovy et al., 1987).

Finally, progesterone does change body fluid distribution by its effect on the kidney to block the action of aldosterone resulting in loss of sodium and water and thus reductions in plasma volume (Gaebelein and Senay, 1982).

2.3.7. EFFECTS OF MENSTRUAL CYCLE PHASE ON STRENGTH.
It is well established that the steroid hormones such as estradiol and testosterone are major contributors in muscle building and strength (Catlin, 1995). This fact combined with the well documented natural fluctuations of women's sex hormones during a normal menstrual cycle gave ground to the opinion that strength can also vary. Initial evidence of this claim derives predominantly from cross-sectional studies comparing pre- and post-menopausal women. The rationale for possible variations in strength across the menstrual cycle is based on the finding that the force generating capacity
(e.g. force per unit cross-sectional area) in adductor poillicis muscle (Philips et al., 1993) or quadriceps (Rutherford and Jones, 1992) is decreased in post-menopausal women (lack of estrogens and progestins) in comparison with the pre-menopausal women (presence of estrogens and progestins) linking the absence of estradiol and/or progesterone with strength decrements. In addition, it has been demonstrated that hormone replacement therapy can prevent this decline in force, which further supports the association between sex hormones and force (Rutherford and Jones, 1992). Finally, estradiol has been shown to have a positive impact in rat skeletal muscle concerning the anaerobic regeneration of ATP (Ramanani et al., 1999), which is the main energy source for maximum effort.

A number of studies have attempted to investigate these possible effects of menstrual cycle phase on strength. In one of the earliest investigations Wearing and colleagues (1972) tested an unknown number of female volunteers with a battery of physical fitness tests including hip strength flexion and extension with a cable potentiometer device. They found poorest performances during the menstrual flow while the best performances occurred during the premenstrual phase (which according to the authors corresponded to the last seven days of the menstrual cycle). Unfortunately, learning effects and the lack of hormonal verification of the menstrual cycle limit the validity of their results. Petrofsky et al. (1976) using only 3 subjects came to the conclusion that maximum voluntary contraction (MVC, or, in other words maximum isometric strength) is not affected by menstrual cycle, but isometric endurance at 40 % of MVC is better at the mid-cycle phase in comparison with luteal phase. Similar results are reported by Wirth and Lohman (1982) who studied handgrip endurance time and force output at 50 % MVC and revealed that in the follicular phase strength endurance is increased. The findings of Sarwar and co-workers (1996) supported the view that strength could be higher at the mid-cycle as revealed by the 11 % significant increase in quadriceps and handgrip strength during this phase in sedentary women. However, Davies et al. (1991) found better handgrip and standing long jump performances during the menstrual bleeding (days 1-4) as compared with the follicular phase (defined as days 12 to 14) and the luteal phase (defined as days 19-21). However, inappropriate statistical analyses (paired t-test instead of ANOVA for repeated measures) and methodological limitations (no progesterone and estradiol values were reported) may have influenced the findings of Davies et al. (1991).
On the other hand, a number of studies have not shown any effect of the menstrual cycle on strength measurements. Knee extension and handgrip strength MVC were unaltered due to the menstrual cycle in 12 active female volunteers during menstruation, or during the follicular, mid-cycle or late luteal phases (Higgs and Robertson, 1981). Dibrezzo et al., (1991) examined isokinetic knee extension and flexion at various speeds ($60^\circ$, $180^\circ$ and $240^\circ$-s$^{-1}$) in 21 women of unknown physical fitness. No variations in peak torque or strength endurance appeared in their investigation. Similar, conclusions were drawn by Quadagno et al. (1991) who examined 12 recreational weight lifters at three different phases of their menstrual cycle (premenstrual: days 13-28, menstrual: days 1-5 and postmenstrual: days 10-12).

Is very difficult to draw valid conclusions from the above published data since all these studies have failed to verify their results with hormonal documentation of the menstrual cycle phase in question. As already mentioned from the discussion in sections 2.3.1 to 2.3.3, day of the menstrual cycle is not a reliable guide to hormonal levels.

Nevertheless, a small number of investigations that have measured the hormonal profile although sparse do exist in the literature. These efforts only appeared in the last 5-6 years and their results seem to be fairly consistent towards a lack of differentiation of muscle strength across the menstrual cycle. Lebrun and co-workers (1995) found no phase difference between follicular and luteal phases in isokinetic strength flexion and extension and the cycle phase was confirmed with both progesterone and estradiol levels. Gur et al. (1999) attempted to naturally isolate the potential inotropic effects of estradiol by testing their 20 sedentary subjects in using a battery of isokinetic tests at mid-cycle (high levels of estradiol-low levels of progesterone) and at menstruation (days 1-5). They found no difference between these two phases in any variable tested and concluded that estradiol does not have any apparent influence on muscle power. However, close inspection of their data indicates that estradiol values averaged 166 pg·mL$^{-1}$ which is below the 200 pg·mL$^{-1}$ level for positive feedback from estradiol to occur (Young and Jaffe, 1976, and discussion in sections 2.3.2-2.3.3). Moreover, progesterone values were unchanged between the two phases ($0.7 \pm 0.3$ and $0.8 \pm 0.2$ ng·mL$^{-1}$), another sign that their subjects were not
actually in the period just prior to ovulation where estradiol has its highest values and hence any potential influence will be easier to detect. It was stated earlier that increases of circulatory progesterone prior to ovulation, but below 2 ng·mL\(^{-1}\), not only indicates that the particular woman is close to ovulation, but also exerts positive feedback on this process (Chikasawa et al., 1986). Thus, in the Gur et al. (1999) study estradiol values may have not reached physiological significance. Finally, Giacomoni et al. (2000) using subjects “on the pill” or not “on the pill” and with or without perimenstrual problems examined performance on a number of “anaerobic tests” (force-velocity, multi-jump test and squatting jump test). They found that the menstrual cycle influence on strength was negligible in all of the tests. It should emphasised here though, that women with perimenstrual problems had force decrements in the multi-jump test at menstruation and late luteal phase in comparison with the follicular phase. The authors concluded that the physiological mechanism responsible for the decrements in performance was the sharp fall of estradiol during the last few days of the cycle (late luteal) and at the menstruation through an action on the stretch-shortening cycle of tendons and ligaments. Estradiol has been suspected (Kendall and Eston, 2002) and experimentally shown in both humans (Liu et al., 1997) and animals (Tiidus and Borbardier, 1999) to interfere with muscle function when large fluctuations in the circulation occur, which may partly explain these decrements in strength performance. Findings of Giacomoni et al. (2000) may also explain why Wearing and colleagues (1972) found that force output diminishes at menstruation. However, these authors did not report any measurements of perimenstrual problems so clear conclusions can not be drawn. Similarly, the findings of Petrofsky et al. (1976) and Wirth and Lohman (1982) would be easier to interpret if information for menstrual dysfunction was given. Furthermore, the disagreement of the results between those of Sarwar et al. (1996) and Gur et al. (1999) or Higgs and Robertson (1981) could be due to the fact that estradiol values were not high enough to induce any physiological effect and thus strength fluctuations in the last two studies. Finally, the above speculations justify early observations from survey studies (Erdelyi, 1962) that women perceived that their performances could change during menstruation.
The literature is not only divided concerning the effects of the menstrual cycle on strength *per se*, but also with the indirect evidence that supports these results. For example, even though some research findings support the view that strength decreases in post-menopausal women (*Rutherford and Jones, 1992; Philips et al., 1993; Kent-Braun and Alexander, 1999*) and this is prevented with hormone replacement therapy (*Philips et al., 1993*) there are data that support the view that strength does not increase with hormone replacement therapy (*Jubrias et al., 1997*). It should be noted here though, that the latter authors did mention that the lack of differences of strength during MVC of the dorsiflexor muscle may be due to the phenomenon that a considerable number of the young women failed to fully activate their muscles (condition that occurred after superimposing a train of stimuli during the third MVC performed by the subject) and thus underestimated their strength output.

The association of estradiol with strength has also been recently challenged (*Rice, 1988; Bassey et al., 1995; Greeves et al., 1997; Bassey et al., 1998*). These investigators reported a lack of correlation between muscle strength and estradiol under various experimental perturbations such as testing subjects during a normal menstrual cycle (*Rice, 1988; Bassey et al., 1995*), or, by supplementing with exogenous estradiol for in vitro fertilisation and hormone replacement therapy (*Greeves et al., 1997; Bassey et al., 1998*). However, others do confirm such a relationship (*Reis et al., 1995*). *Reis et al. (1995)* found that strength training was more effective when the training units were incorporated when the natural concentrations of estradiol were high. Moreover, there was a significant relationship between estradiol and the gains in strength ($r = 0.91, P = 0.002$). It is not clear what effect supraphysiological estradiol levels have upon strength (*Greeves et al., 1997*) or to what extent the exogenous estradiol can influence the force output (*Bassey et al., 1998*). As previously mentioned the full effects of estradiol can only be established when the progesterone levels are known. Finally, *Rice (1988)* surprisingly, tested their subjects at the beginning of the menstrual cycle (days 2-5) when it is known (sections 2.3.2-2.3.3) that estradiol has low circulatory levels. Subsequently, the low concentrations of estradiol reported in their study ($\approx$124 to 143 pg·mL$^{-1}$) may explain the lack of positive correlation in their study between estradiol and strength.
A recent investigation (Janse de Jonge et al., 2001) examined the effects of various phases of menstrual cycle on strength at menstruation (days 1-3), during a period of three days prior to ovulation and during the luteal phase. No changes in strength appeared. However, the authors monitored the menstrual cycle with basal body temperature and a calendar and assumed that the estradiol peak lasts approximately 3 days as suggested by earlier research (Langren et al., 1980). Nevertheless, the bulk of the literature suggests a much shorten time course for this estradiol peak (Pauerestein et al., 1978; World Health Organisation, 1980; Hoff et al., 1983; Speroff, 1999) and thus it remains possible that Janse de Jonge et al. (2001) may have missed this peak.

It has been suggested that it may actually be progesterone that is the hormone responsible for alterations on strength (Greeves et al., 1997). However, such speculations do not agree with the current views of the relationship between progesterone and strength (Kraemer, 1992). Nonetheless, it is impossible to isolate progesterone naturally concentration since it always coincides with elevated levels of estradiol (sections 2.3.2-2.3.3).

The solution in all these debates would be the knowledge of the underlying mechanisms that account for the influence, if any, of the menstrual cycle on strength. Only Sarwar et al. (1996) have made some suggestions regarding mechanisms. These authors suggested that estradiol has a positive influence upon muscle Pi which is known to maintain the stretch force (number of attached cross-bridges and level of activation) during contraction (Pate and Cooke, 1989). However, how estradiol effects this positive impact on Pi is not explained.

Thus, the current knowledge is equivocal with respect to the possible influence of the two main reproductive hormones on strength. Further research is needed to elucidate these issues.
2.3.8. EFFECTS OF MENSTRUAL CYCLE PHASE UPON MAXIMAL INTENSITY EXERCISE.

2.3.8.1. Effects on performance

It is known that (Jones and Round, 1990):

\[ \text{power output} = \text{force} \times \text{velocity (speed)} \]

Assuming that force can be altered due to the menstrual cycle (see previous section) then power output can also change. Moreover, it has been postulated that exercise involving maximal eccentric muscle action can also be affected by hormonal variations of menstrual cycle, possibly due to the sharp fall of estradiol at the end of the cycle (Giacomoni et al., 2000). Sprint running, but not sprint cycling does include an eccentric component (Lakomy, 1988). Thus, from a theoretical point of view sprinting running could be affected by menstrual cycle.

Unfortunately, very few studies have used sprint running as the exercise model, so studies utilizing other types of exercise must be reviewed. Swimming performance expressed as the fastest time to complete 100 m free style was not altered significantly in the premenstrual (days 13-28 from the beginning of the menses), menstrual (days 1-5 from the beginning of the menses) and postmenstrual phase (10-12 days from the beginning of the menses) in well-trained varsity level swimmers (Quadagno et al., 1991). The lack of impact of menstrual cycle phase upon sprinting has been advocated by Miskec et al. (1997) who employed intermittent maximal exercise (15 x 15-s sprints interspersed with 2 min passive rest) on a cycle ergometer during menstruation (first 2 d of the cycle) and nonmenstruation (days 19-21 from the onset of the menses), by De Bruyn-Prevost et al. (1984) who employed a cycle test to exhaustion (about 36 s) during menstruation (day 1-2 of the cycle), ovulation (day 14 of the cycle) and at the end of the cycle (1-2 days before menstruation). The Giacomoni et al. (2000) findings are in agreement with previous observations using sprint cycling as the exercise mode (4 x 8-s sprints against increasing breaking forces) with no alteration in power output during menstruation (day 1-4), mid-follicular (day 7-9) and mid-luteal phases (day 19-21 of the cycle, verified with progesterone measurements). Finally, one of the few projects that used hormonal documentation of cycle phase (Lynch and Nimmo, 1998) examined the effects of the menstrual cycle upon high-
intensity capacity (repeated 20 s “sprints” with 100 s passive rest, to exhaustion on an inclined treadmill). High-intensity capacity did not change during mid-follicular (defined as day 7-10 from the beginning of the menses) and late luteal (defined as 12 d after ovulation and 1-3 days preceding the next cycle and assuming a 28 d cycle) phase of the menstrual cycle. Opposingly, there are other reports that corroborate the opinion of improved performance in the very few days of the cycle (days 1-4) as shown by a better swimming time in 100 m free style (Brooks-Gunn et al., 1986), or, in the follicular phase (day 8 from the onset of the menses) as revealed with 50 m free style swimming (Bale and Nelson, 1985) and repeated 30-s sprints (6 x 30-s) on a cycle ergometer (Parish and Jakeman, 1987). The literature becomes even more complicated with reports of better performance in the luteal phase (14-15 d after the offset of the menstrual bleeding) as compared with the follicular phase (day 2 of the cycle) as suggested by higher speed during a 30-s cycle sprint (Masterson, 1999).

The majority of these studies have the major drawback of reporting day of the menstrual cycle, which does not accurately reflect the cyclic variations of the ovarian hormones (see discussion in previous sections). In addition, the interpretation of such findings can be perplexing due to the various exercise protocols, training status of the subjects and timing of the tests with respects to menstrual cycle. Moreover, the majority of these studies are not hypothesis based and descriptive in nature. For example, apart from the study of Giacomoni et al. (2000), no studies to date have given a solid rationale for their studies other than “to assess the effects of the menstrual cycle on high-intensity exercise”. Thus, any conclusions should be drawn with caution. However, on balance it seems that performance during maximal intensity exercise does not vary due to cyclic fluctuations of the reproductive hormones. The majority of studies, with (Lynch and Nimmo, 1998; Giacomoni et al., 2000) or without (De Bruyn-Prevost et al., 1984; Quadagno et al., 1991; Miskec et al., 1997) hormonal documentation, suggest that the menstrual cycle does not influence sprinting performance. One exception to this general conclusion is the findings of Masterson (1999) which can be explained as consequence of the menstrual syndrome that perhaps their subjects suffered (although the author mentions this possibility, there was no attempt to measure symptoms). Lack of perimenstrual pain may also explain the failure to observe any deleterious effect of the menstrual cycle during menstruation upon performance (De Bruyn-Prevost et al., 1984; Quadagno et
al., 1991; Miskec et al., 1997). Nonetheless, there is no scientific explanation concerning the results of Bale and Nelson (1985), Brooks-Gunn et al. (1986) and Parish and Jakeman (1987). Careful inspection of their papers results in the feeling that poor design (absence of familiarisation or control of previous exercise) and the lack of hormonal documentation of cycle phase may have resulted in spurious data. Moreover, the study of Parish and Jakeman (1987) used a protocol of six 30-s flat out sprints interspersed with 2.5 min rest. Experience in our laboratory and the opinions of others in the international literature (Giacomoni et al., 2000) would suggest that subjects would be unable to complete six 30-s sprints if the exercise was maximal from the onset of exercise. Alternatively, in the Parish and Jakeman (1987) study the better performances (PPO) during the mid-follicular period (days 7-9) may simply reflect that their subjects were close to ovulation (high levels of estradiol) and thus had better performances, although this does not explain the better MPO at day 26. This explanation does apply for the study of Brooks-Gunn et al. (1986) where mid-follicular (day 10 of the cycle) performance was higher than during the premenstrual period (last 4 d of the cycle). Again, in the Bale and Nelson (1985) investigation the order of better performances for 50 m swimming was day 8 > day 15 > day 21 > day 1 (menstrual discomfort reported from the subjects in the last two trials). Perimenstrual discomfort can appear even 7 days prior to the onset of the menses, depending on the subject, and can persist usually for a few days at the onset of the new menstrual cycle (Speroff, 1999). This time course matches well with the testing timing of the above studies and perhaps explains their “anecdotal results”. These potential effects of estradiol will be discussed below.

It seems that the bulk of research has missed the opportunity (by not testing just prior to ovulation) to assess the potential effects of estradiol on performance. This avoidance of testing just prior to ovulation is justifiable due to the extreme difficulty of naturally isolating the effects of estradiol in in vivo experiments, especially when involving human subjects (Bunt, 1990). However, since progesterone has antiestradiol effects studies that did examine performance just prior to ovulation would be of great interest. Initial evidence corroborates this view but derives from studies that lack hormonal documentation of menstrual cycle phase (Bale and Nelson, 1985; Brooks-Gunn et al., 1986; Parish and Jakeman, 1987).
With the exception of one study, published only in abstract form (Liu Shuhui, 1992) progesterone has not been associated with performance improvements. It should be noted though, that "sprinters motor ability" (according to the author) was related to the density of progesterone secretion, negatively to estradiol secretion, with better performance in the latter phase of ovulation (definition according to the author) and weakest performance in the initial and intermediate phases of menstruation.

Finally, although there is indirect evidence that the menstrual cycle can influence PCr recovery rates (Harber et al., 1998) as indicated by slower rates of PCr resynthesis in amenorrheic (high levels of estradiol and progesterone) in comparison with eumenorrheic (high levels of estradiol and progesterone) endurance athletes there are no data in the literature to support the idea that menstrual cycle phase can affect PCr recovery.

### 2.3.8.2. Effects on physiological responses

The impact of the menstrual cycle on physiological variables after maximal exercise has only been addressed by two studies (Miskec et al., 1997; Lynch and Nimmo, 1998). No alterations in blood lactate or plasma ammonia concentration and in estimated percentage change in plasma volume were reported for follicular in comparison with luteal phases. However, data from animal studies suggests that it is possible that menstrual cycle phase could affect metabolic responses (Ramamani et al., 1999). To our knowledge no biopsy studies involving maximal intensity exercise and the menstrual cycle in humans have been undertaken. Further research is required to elucidate these issues.

### 2.3.9. EFFECTS OF MENSTRUAL CYCLE PHASE UPON SUB-MAXIMAL INTENSITY EXERCISE

#### 2.3.9.1. Introduction

In these sections the possible effects of the menstrual cycle upon physiological responses during sub-maximal exercise will be reviewed. The potential mechanisms by which the two highly biologically active hormones progesterone and estradiol might affect exercise are discussed in sections 2.3.5-2.3.6. This review includes...
papers with hormonal documentation of cycle phase at a range of sub-maximal intensities (exercise protocols corresponding to intensities below those that \( \dot{V}O_2 \text{ max} \), section 2.3.9.2) and investigations that examine the effects of menstrual cycle phase upon \( \dot{V}O_2 \text{ max} \) (section 2.3.9.3). This review is also limited to protocols that do not exceed 20 min in duration since the sub-maximal protocols used in the present thesis were below 20 min duration (as described in chapter 3). The metabolic responses reviewed are muscle glycogen (two studies) and blood lactate, respiratory (\( \dot{V}O_2, CO_2, R, \dot{V}E \)) and cardiovascular (heart rate) responses. Psychological responses (that is, RPE) are also reported.

2.3.9.2. Effects on sub-maximum physiological responses

One of the pioneers regarding the effects of the menstrual cycle on respiratory responses was Jurkowski et al. (1981) who investigated the effects of the menstrual cycle phase (follicular vs luteal) upon oxygen uptake, carbon dioxide production and minute ventilation during 20 min cycling exercise corresponding to an intensity of 40 % \( \dot{V}O_2 \text{ max} \). No differences were found in any of these respiratory parameters due to the menstrual cycle. Similarly, other investigators employing either cycling (Lamont, 1986; Nicklas et al., 1989; Hackney et al., 1991; Bailey et al. 2000; Campbell et al., 2001) or treadmill exercise (Beidleman et al., 1999) at 70 % \( \dot{V}O_2 \text{ max} \), did not find any differences in respiratory responses. Likewise, Kanaley et al. (1992) and Suh et al. (2002) did not reveal any variations in respiratory variables during treadmill exercise at 60 % \( \dot{V}O_2 \text{ max} \) or cycling at 45 and 65 % \( \dot{V}O_2 \text{ max} \), respectively.

In spite of the fact that Nicklas et al. (1989) and Hackney et al. (1991) mentioned no alterations in oxygen uptake at rest and during exercise, R was significantly higher at rest (during the follicular phase) at Nicklas et al. (1989) and lower in the luteal in comparison with the follicular phase (but not statistically significant during exercise), while in the Hackney et al. (1991) experiment, the overall R was lower during the mid-cycle phase (where peak estradiol concentrations occur) in comparison with the follicular phase and also was lower in the luteal phase (ns) in comparison with the follicular phase. Considering that R is an indirect index of substrate utilisation in the
muscle (McArdle et al., 1996) these were the first findings of metabolic alterations during the course of a menstrual cycle. The conclusions of Nicklas et al. (1989) were supported by higher muscle glycogen at rest during the luteal phase. These findings replicated later by the same research group (Hackney, 1999) with the additional finding of a higher utilisation of glycogen and higher R in the follicular in comparison with the luteal phase, which once more suggested a glycogen sparing effect during luteal. These results are in agreement with Dombovy et al. (1987) who also found a higher R during the follicular in comparison with the luteal phase during cycling exercise at various exercise intensities below 75 % $\dot{V}O_2$ max. Bearing in mind the findings from animal research (section 2.3.5) these results seem to verify the view of an alteration in substrate use during the luteal phase and an enhanced reliance on fat in eumennorrheic women at intensities below 70 % $\dot{V}O_2$ max. Nevertheless, the body of the literature supports the view that exercise intensity overcomes the effects of ovarian hormones, if any, in promoting lipid oxidation (Lamont, 1986; Nicklas et al., 1989; Hackney et al., 1991; Kanaley et al., 1992; Beidleman et al., 1999; Bailey et al. 2000; Cambell et al., 2001; Suh et al., 2002). Unfortunately though, the above findings were not always accompanied by additional information of other muscle metabolites such as lipoprotein lipase activity or circulatory hormonal responses such as growth hormone, all of which promote lipolysis (Bunt, 1990; Kendrick and Ellis, 1991).

There is a general consensus in the literature that the blood lactate response to exercise intensities above 70 % $\dot{V}O_2$ max is unaffected by menstrual cycle phase. Moreover, several lines of evidence support the view that menstrual cycle phase does not influence lactate concentration during sub-maximal exercise duration < 20 min regardless of the intensity of exercise (Jurkowski et al., 1981; Hessemuer and Bruck, 1985; Lamont, 1986; Nicklas et al., 1989; Kanaley et al., 1992; Cambell et al., 2001; Suh et al, 2002), even though exercise of longer duration has resulted in equivocal results (Jurkowski et al., 1981; McCracken et al., 1994). Underlying mechanisms of the effects of reproductive hormones on circulatory lactate as opposed to muscle glycogen are difficult to determine since lactate concentration in the blood is the difference between its accumulation in the blood and its disappearance from the blood (Brooks, 1985).
Another point of concern that attracted investigators was the potential progesterone-mediated increases in resting and exercise ventilation. As with other physiological factors concerning the menstrual cycle, opinions vary. Probably the best-controlled study was that conducted by Williams and Kranhenbuhl (1997) where 8 moderately trained volunteers were tested every third day during the course of their menstrual cycle. Trials (6 min at 55 % $\dot{V}O_2$ max and 6 min at 80 % $\dot{V}O_2$ max on a treadmill) were accompanied by hormonal documentation of cycle phase with both progesterone and estradiol. Both at rest and during exercise at 80 % $\dot{V}O_2$ max, higher oxygen uptake occurred during the mid-luteal in comparison with early follicular phase (low concentrations of progesterone and estradiol), elevations which were accompanied by higher perception of effort as indicated from greater RPE during the mid-luteal phase. The authors concluded that running economy is affected due to menstrual cycle at this intensity. The findings of Dombovy et al. (1987) partially corroborate the Williams and Kranhenbuhl (1997) study (higher ventilation at rest, but not in exercise and non-significant increases in $\dot{V}O_2$, but their subjects were tested at workloads on the bike lower than 75 % $\dot{V}O_2$ max). Hessember and Bruck (1985) found that both resting and exercise $\dot{V}O_2$ (cycling at 75 % $\dot{V}O_2$ max) were elevated in the luteal in comparison with the follicular phase. In contrast, there are a considerable number of studies that have showed no change in oxygen consumption during cycling or running exercise and at various intensities during different phases of the menstrual cycle (Jurkowski et al., 1981; Lamont, 1986; Nicklas et al., 1989; Hackney et al., 1991; Kanaley et al., 1992; Beidleman et al., 1999; Bailey et al. 2000; Cambell et al., 2001; Suh et al., 2002). It is very difficult to interpret this disparity in the data but it can be postulated that differences in hormonal concentrations of estradiol and progesterone, independently or in their ratios, as well as individual responsiveness to a given ovarian hormone level (Beidleman et al., 1999), and the relatively large within subjects and between days variability inherent in measures of ventilatory chemosensitivity (Sahn et al., 1977) may have contributed to these findings. For example, in the experiment of Williams and Kranhenbuhl (1997) the progesterone values were considerably higher in comparison with other studies where no variations in oxygen consumption were found. Different estradiol values between studies may also contribute to these discrepancies since, as already mentioned in section 2.3.6, the progesterone response is mediated in the hypothalamous via an estrogen dependent
progesterone receptor (Bayliss and Millhorn, 1992). In order to elucidate these issues future researchers should measure the concentrations of both hormones. Moreover, by stating individual values, even in formal communications (e.g. research papers), readers would be able to better scrutinise these data.

Finally, apart from one study (Hessember and Bruck, 1985) the bulk of the literature is consistent with the view that exercise heart rate is not affected by menstrual cycle (Nicklas et al., 1989; Hackney et al., 1991; Beidleman et al., 1999; Bailey et al. 2000; Cambell et al., 2001). The different values observed by Hessember and Bruck, (1985) may be attributed to the higher body temperature values at 2.45-3.15 a.m. time where the highest differences in body temperature occur between follicular and luteal.

2.3.9.3. Effects on maximum oxygen uptake
Judging from the information provided in the previous sections it does not seem that fluctuations of the ovarian hormones could build a hypothetical physiological mechanism that can influence maximum oxygen consumption. At exercise intensities approaching 100% $V\text{O}_2$ max carbohydrate oxidation is the dominant factor of energy substrate (Romijn et al., 1993; Greenhaff et al., 1993) thereby minimising any influence of estradiol. A number of studies have assessed the potential influence of menstrual cycle phase on aerobic power. The majority of them did not find any confounding effects of the menstrual cycle on maximum oxygen consumption utilising either bike (Dombovy et al., 1987; Schoene et al., 1981; Bemben et al., 1995; Beidleman et al., 1999) or treadmill (De Souza et al., 1990; Suh et al., 2002) as exercise protocols. All these experiments compared follicular and luteal phases, which were verified with progesterone, and in some cases (De Souza et al., 1990; Beidleman et al., 1999), with estradiol. There is one exception to the consensus in the literature when maximum oxygen uptake was found to be higher ($P = 0.06$) in the follicular in comparison with the luteal phase (Lebrun and co-workers, 1995). It is very difficult to ascertain the factors that led to these discrepancies. However, the overall difference was only 0.9 mL·kg$^{-1}$·min$^{-1}$ (53.7 ± 0.9 vs 52.8 ± 0.8 mL·kg$^{-1}$·min$^{-1}$ in the follicular and luteal phases, respectively), a value that is within the range of experimental error (Lebrun et al., 1995). In addition, Bemben et al. (1995) utilising a similar protocol did
not find any differences. Careful inspection of the data mentioned by Bemben et al. (1995) reveals that the continuous protocol they employed to determine aerobic power was horizontal (speed instead of gradient increments) procedure that leads to underestimation of $\dot{V}O_2 \text{max}$ (Hermansen and Saltin, 1969; Mayew and Gross, 1975; Taylor et al., 1955). An alternative hypothesis could be that discomfort during the luteal in comparison with follicular phase caused the difference (Speroff, 1999). Nevertheless, Lebrun and co-workers (1995) did not report information concerning menstrual discomfort and RPE was not measured.

In summary, based on the evidence in the published literature, $\dot{V}O_2 \text{max}$ is not influenced by the menstrual cycle. There is also a lack of underlying mechanisms linking estradiol with aerobic power.

2.3.9.4. Effects on $\% \dot{V}O_2 \text{max} @ 4 \text{ mmol·L}^{-1}$

The $\% \dot{V}O_2 \text{max} @ 4 \text{ mmol·L}^{-1}$ is the relative exercise intensity ($\%\dot{V}O_2 \text{max}$) corresponding to blood lactate concentration of $4 \text{ mmol·L}^{-1}$. Therefore, this index can be affected by both maximum and sub-maximum oxygen consumption and the blood lactate concentrations at this intensity. Judging from the review of the literature in the last two paragraphs blood lactate concentration and $\dot{V}O_2 \text{max}$ are not compromised due to menstrual cycle phase. Likewise, sub-maximum oxygen consumption, apart from one study (Williams and Kranhenbuhl, 1997), does not seem to be affected by the menstrual cycle. Nevertheless, no study to date, has directly addressed the potential effects of menstrual cycle upon $\% \dot{V}O_2 \text{max} @ 4 \text{ mmol·L}^{-1}$.

2.3.10. ORAL CONTRACEPTIVES AND EXERCISE

2.3.10.1. Introduction

Oral contraceptives are well widespread especially in western societies as a means for birth control. This increasing popularity has also appeared among athletic women in whom, except oral contraceptives lead to an elimination or diminution of dysmenorrhoea (Lebrun, 1994). Since oral contraceptives prevent the elevation of the two major reproductive hormones in women over the course of a menstrual cycle,
then no fluctuations in these hormones will occur and subsequently no affects on performance and physiological responses will be revealed. What this simplified statement does not take into consideration though, is the fact that oral contraceptives, can be monophasic where equal doses of the estrogen and progestin components exist over the pill cycle or multiphasic where the doses are varied in order to mimic the physiological variation of the endogenous ovarian hormones. In addition, multiphasic oral contraceptives contain lower progestin doses, which may also have its affects (Bemben, 1993). Finally, high and low dose oral contraceptives as well as differences in the biochemical structure of the components may also be important since the biochemical structure determines receptor mediated and direct cellular effects (Bemben, 1993).

2.3.10.2. Oral contraceptives and performance
A recent study using sprinting exercise mode (Giacomoni et al., 2000) involved subjects taking oral contraceptives (monophasic oral contraceptives, that is, constant estrogen and progestin levels during 21 d) and their data showed no alterations in performance during four 8 s sprints in a cycle ergometer. However, when the two groups were divided into those with perimenstrual symptoms and without symptoms their performance in maximal jump power (five maximal vertical jumps) was reduced regardless as to whether they were “on the pill” or not. In addition, the presence or absence of symptoms did not correlate with oral contraceptives use (taken for at least 18 months prior to commence of the study according to the authors).

Lynch and Nimmo (1998) and Lynch et al. (2001) examined the effects of low dose monophasic oral contraceptives (taken for at least 12 months) on high-intensity intermittent treadmill running (20-s runs interspersed with 100 s passive rest to exhaustion, and, six 20-s runs with the same pattern where the last one was performed to exhaustion, respectively) during one menstrual cycle. No differences in high-intensity capacity were revealed.

Aerobic power seems to follow the same trends with respects to oral contraceptives use. Studies performed to date have failed to detect any influence of oral
contraceptives on $\dot{V}O_2$ max during short-term oral contraceptives use (21 d, Bryner et al., 1996). However, long-term oral contraceptives (6 mo) use has interfered negatively with aerobic power as indicated with the significant reduction (7 and 11%, respectively) in women taking either low dose monophasic (Notelovitz et al., 1987) or triphasic oral contraceptives (Casazza et al., 2002). Certainly more research in the area needs to be conducted to clarify these issues.

Investigations concerning oral contraceptives and strength parameters are also sparse in the literature. The few studies to date employing electrical stimulation techniques and handgrip force measurements suggest that strength is quite stable across pill cycle (Philips et al., 1996; Sarwar and co-workers 1996). Nevertheless, the long term effects of oral contraceptives use have not been assessed yet.

2.3.10.3. Oral contraceptives and physiological responses

The very few studies to date that have examined the effects of oral contraceptives use (at least 12 months) on metabolic responses after high-intensity exercise have not found any variations (Lynch and Nimmo 1998; Lynch et al., 2001) in metabolic response within one pill cycle (oxygen consumption, lactate, glycerol, free fatty acids, glucose and ammonia, protocols mentioned in the previous section). It is very difficult to generalise the limited data currently available and draw conclusions for other protocols. Further research is required to clarify these issues.

2.3.10.4. Summary

Initial evidence suggests that within one or two pill cycles performance (including sprint performance) is not affected. However, the long term effects of oral contraceptives use have not yet been fully described. In addition, it is not clear whether termination of pill consumption could have adverse effects on performance or metabolism and how long this process could take. Moreover, no information exists with respects to accommodation of oral contraceptives, if any. Thus, experimental findings to date should be treated with caution due to limited amount of information available as yet.
2.3.11. SUMMARY

The female individuals, during their reproductive years possess a highly complex endocrinological system making the study of their performance and physiological responses difficult. These difficulties become even more complicated when investigations lack hormonal documentation of menstrual cycle phase. Investigations to date have shown a tendency for increased fat metabolism during low intensity sub-maximal exercise (<65% \( \dot{V}O_2 \text{ max} \)) during the luteal phase, however, this is not universal finding. In terms of maximal intensity exercise or maximal strength the menstrual cycle phase does not seem to affect performance, even though studies with a solid methodological approach are sparse. Future studies should focus on naturally isolating the concentrations of estradiol and more closely assessing any effects of menstrual cycle on the maximal exercise eccentric component. Initial evidence suggests that oral contraceptives cannot influence performance within one pill cycle as opposed to long term effects but the evidence to date is very limited.

2.4. GENERAL SUMMARY

From a theoretical point of view, this review leads to the conclusion that short-term endurance training is capable of facilitating recovery during a repeated sprint or multiple sprints. A model that summarises the mechanisms that the current literature suggests is shown in figure 2.7. According to this model, endurance training will enhance power output restoration during a subsequent sprint(s) via improved PCR resynthesis (Bogdanis et al., 1996a) as a result of increased oxidative capacity (Walter et al., 1997) and oxygen delivery in the muscle (Quistorf et al., 1992). Better muscle and blood lactate (H+) removal after endurance training (Freund et al., 1992) will have a positive impact on power output recovery from a sprint both directly (Gaitanos et al., 1993; Maughan et al., 1997; Spriet et al., 1989) and indirectly via the creatine kinase equilibrium (equation 2) by blunting its forward reaction due to less H+ accumulation and facilitating PCR resynthesis (Walter et al., 1997).

\[
\text{PCr} + \text{MgADP}^- + H^+ \leftrightarrow \text{MgATP}^2^- + \text{creatine} \quad \text{[equation 2]}
\]
A higher aerobic contribution to the sprint(s) (Tomlin and Wenger, 2002) after endurance training will result in less reliance on the glycolytic energy system, thereby reducing muscle and blood lactate accumulation at the end of the sprint(s) (Hamilton et al., 1991). A reduction in muscle and blood lactate accumulation will be beneficial directly to power output recovery in the subsequent sprint(s) (Gaitanos et al., 1993; Maughan et al., 1997; Spriet et al., 1989), will increase lactate removal as a result of lessened lactate accumulation (Freund et al., 1986), and will augment PCr anaplerosis via the creatine kinase equilibrium as suggested above (Walter et al., 1997). In addition, a higher aerobic contribution to the sprint will result in a higher initial \( \dot{V}O_2 \) post-sprint (Hagberg et al., 1980) and thus better initial PCr resynthesis (Hultman et al., 1967) which in turn will lead to faster power output recovery in subsequent sprint(s). Finally, endurance training will increase the oxygen consumption during recovery from the sprint(s) (Balsom et al., 1994b), a condition that will be advantageous in terms of both lactate removal (Freund et al., 1992) and PCr resynthesis (Piiper and Spiller, 1970) thereby facilitating power output recovery in the following sprint(s) (Bogdanis et al., 1996a). However, all these suggestions are theoretical in nature and based on cross-sectional studies which have not always used maximal intensity exercise as the experimental model. Thus the main purpose of the present series of studies is to test the hypothesis that six weeks of endurance training is beneficial to non-endurance athletes in improving acid-base balance and PCr resynthesis after a single 30-s sprint and thus will increase the power output in a subsequent sprint.

Secondly, the present review of the literature highlighted the absence of investigations with respects to whole body maximal intensity exercise, apart from some studies involving swimming that lacked hormonal documentation of menstrual cycle phase and resulted in equivocal results (Bale and Nelson, 1985; Brooks-Gunn et al., 1986; Quadagno et al., 1991). In addition, there is sparse of information with regards to recovery from sprinting and a complete absence of studies (including sub-maximal investigations or other types of research) during which subjects tested prior to ovulation when only estradiol has high concentrations, while progesterone concentrations are still low. Thus, the other purpose of this series of studies is to investigate the effects of menstrual cycle phase on sprinting and recovery from
sprinting by implementing the novel approach of testing the participants prior to ovulation when only estradiol is high while progesterone levels remain low, during the luteal phase when both progesterone and estradiol levels are high and during the follicular phase when both progesterone and estradiol levels are low.

As already mentioned in chapter one, the main reason for inadequate data concerning maximal intensity exercise was the lack of appropriate methods (Nevill et al., 1996b) and in terms of sprint running the absence, until the mid-eighties, of a nonmotorised treadmill (Lakomy, 1987). However, the large number of subject familiarisations required (Lakomy, 1987; Gamble et al., 1988; Greenhaff and Nevill, 1999) may limit the reliability of the data unless thorough practice does take place. Thus, prior to the main studies it is necessary to undertake a reliability study to establish test-retest repeatability of power output (methodological study 1, chapter 3). In addition, it has been shown for sub-maximal exercise that lactate concentrations may vary when different sampling sites, blood portions and analysers are used (Foxdal et al., 1990). During maximal intensity exercise various sampling sites have been utilised such as finger capillary (Ohkawa et al., 1984), arteries (Oyono-Enguelle et al., 1989) and veins (Nevill et al., 1996a) and various blood portions such as plasma (Ahmaidi et al., 1996) and whole blood have been used (Nevill et al., 1996a). Thus, it was also necessary to examine the alternative sites and techniques in the series of studies presented in this thesis (methodological study 2, chapter 3). Moreover, many little is available concerning changes in plasma volume during sprinting which may lead to inadequate conclusions concerning various metabolic responses, especially those with a marginal change, due to sprinting. Thus, it was necessary to examine the separate and combined effects of posture and sprinting on plasma volume changes (methodological study 3, chapter 3). Finally, due to inherent nature of training studies blood samples for lactate are stored for varying periods of time prior to analysis. Therefore, it was considered very useful to establish whether or not storage had an impact on the concentrations reported in this thesis (methodological study 4, chapter 3).
Figure 2.7. A model of the potential effects of endurance training on recovery of power output after a repeated/multiple sprints. Where CKE: creatine kinase equilibrium.
CHAPTER 3: GENERAL METHODS

3.1. INTRODUCTION

This chapter describes the methodological procedures for the experimental work in the thesis, as well as the rationale for their use wherever appropriate. In addition, this chapter includes four novel methodological investigations specific to the methodology of this thesis. All testing was conducted in the Laboratories of the School of Sport and Exercise Sciences or in the Radiochemistry Laboratory of the Chemistry Department of Loughborough University.

Prior to the commencement of this experimental work the author of this thesis had been successfully trained in first aid, in drawing blood from a cannula [Appendix A1], in the venipuncture technique [Appendix A2] and in undertaking work in a radiochemistry lab [Appendix A3]. Venous cannulations were undertaken by Dr Mary E. Nevill or Dr David Stensel, both of Loughborough University. All staff were immunised against Hepatitis b prior to the work.

This chapter is organised in two parts. The first part deals with the general methods (sections 3.2 to 3.17) and covers subjects, ergometers (nonmotorised and motorised treadmills), the protocols employed (single and repeated 30-s sprint protocol, speed-$\dot{V}O_2$, $\dot{V}O_2$max, speed-lactate test, and training protocol), anthropometry (stature and body mass), the use of Clearplan Home Ovulation Test, heart rate monitoring, perceived exertion, environmental conditions (temperature, humidity, time of testing, barometric pressure and shoes and clothing), diet control, collection and treatment of expired air, treatment, storage and analysis of blood and muscle samples, metabolic calculations (for gas, blood and muscle samples), information technology and finally the statistical analyses performed. In the second part of the chapter, the four methodological experiments are described: a) Test-retest reliability of power during a 30-s sprint running, b) Effects of different sampling sites and analysis on blood lactate concentration, c) Effects of sprint or posture change on plasma volume responses after a 30-s sprint, and, d) Influence of storage and defrosting on venous whole blood lactate concentrations.
3.2. SUBJECTS

More than a hundred (including the "drop outs") healthy female subjects volunteered to participate in the experiments reported in this thesis. No subject was on any kind of medication that could interfere with the experimental procedures. Most of the subjects were Loughborough University students and members of various athletic clubs. The level of performance varied among individuals covering the whole spectrum of different performance abilities. Some competed at only varsity level, others were members of English University teams and, finally, some volunteers were national calibre athletes.

The criteria for participation and recruitment varied depending on the requirements of the particular study (further detail is provided in the methods section of each experimental chapter). The recruitment procedure began only after the experimental procedures had been approved by Loughborough University Ethical Committee [Appendix B1].

For the recruitment campaign, posters [Appendix C] were placed in "key" positions on the Loughborough University campus and on Internet sites. In addition, electronic mail was sent to all Loughborough University Sport and Exercise Science students including the same information as that in the posters. Dr Eleni Theodoraki from the School of Sport and Exercise Sciences, Loughborough University assisted in the development of the poster. In addition, general announcements to groups of athletes were made at club training sessions.

Once a volunteer came forward a full description of the experimental procedures, the benefits and potential risks of their participation in the study were given in both an oral and written form [Appendix D]. An informed consent form was signed [Appendix E], which clearly stated that subjects could withdraw from the study at any point without giving an explanation, should they desire to do so. Subsequently a pre-participation health status questionnaire [Appendix F1] was completed prior to final acceptance as a subject in the study. Also, a menstrual cycle information questionnaire [Appendix G] was completed. Information about the subjects’
performance level and current training status was obtained by means of a training status questionnaire [Appendix H].

Finally, it was emphasized to all the volunteers that their personal information and results would be strictly confidential which is in compliance with the Data Protection Act [Appendix B2, paragraph XIV].

3.3. FAMILIARISATION

3.3.1. NON-MOTORISED TREADMILL

One of the most demanding tasks of the experimental procedures for both the experimenter and the subjects was the familiarisation process. The sprint treadmill is a valuable tool for the laboratory analysis of the physiological demands of sprinting (Lakomy, 1987), but considerable amounts of practice time are required. The importance of substantial periods of habituation before valid data can be obtained has been reported by various users (Gamble et al., 1988; Greenhaff and Nevill, 1999).

Lakomy (1988) explains that the change in treadmill belt speed per stride is greater than would be expected for the whole body during free sprinting on the track, due to the low inertia of the treadmill belt. This requires a much greater effort from the participant in terms of movement co-ordination and, as a result, many compensating stride adjustments (Lakomy, 1988).

Thus, each subject was asked to attend 5 familiarisation sessions. In the first visit the familiarisation started with a demonstration of how to run on the treadmill. Tips for how to run on the sprint treadmill were also given to the participants as part of the subject information sheets [Appendix D]. When the participants fully understood the procedures they started practising by walking and jogging. Afterwards, and until the end of the session they started to increase their running speed until they were able to sprint maximally for a few seconds (2-3 s). During the second session one or two 10 s “flat out” sprints were performed. The third visit involved a 20-s sprint, while in the fourth and fifth visit subjects were asked to sprint the same distance as for the main trial (one or two 30-s sprints). If the volunteer had achieved the desirable performance
(that is, similar PPO/MPO/peak speed/mean speed in the last two familiarisation sessions) she was considered ready to participate in the main trials. A number of volunteers were asked to do more practice sessions when they showed inadequate skills. It should be emphasised, though, that the large number of familiarisations was one of the main reasons for "subject mortality" in these series of experiments.

3.3.2. MOTORISED TREADMILL

Before the performance of the $\dot{V}O_{2\text{max}}$ protocol each participant completed one familiarisation session with the motorised treadmill (Woodway, USA, plate 3.1.). This procedure aimed to habituate the volunteers with running on the motorised treadmill and the procedures of the protocols to be performed on it.

Subjects with no experience on this type of ergometer were requested to attend a session of at least 45 min duration before they were allowed to perform the main tests (Cavanagh and Williams, 1982; Schieb, 1986). The procedures involved running on the treadmill with zero elevation at various speeds, with or without the mouthpiece, emergency stops, signalling while running, and running uphill at various grades as for the $\dot{V}O_{2\text{max}}$ test.

Plate 3.1. The collection of a capillary blood sample on the motorised treadmill during the speed-lactate test.
3.4. ERGOMETERS

3.4.1. NON-MOTORISED TREADMILL

3.4.1.1. Introduction

The main ergometer used in this study was a non-motorised treadmill (plate 3.2) instrumented from a commercially available treadmill (Woodway model AB) by Lakomy (1987). In brief, the treadmill was levelled by placing the rear feet on supports while at the same time all four feet were secured to a base-board to prevent lateral movement during running. The handrails were removed and new brackets were made with the rails re-attached to the treadmill in such a position that the rails would not affect arms movement during sprinted.

Plate 3.2. The sprint treadmill (non-motorised).

Even though the sprint treadmill can not evaluate power output with the same precision as a friction-loaded cycle ergometer (Lakomy, 1988) it has some advantages over the cycle ergometer for examining sprint running performance: a) the exercise
CHAPTER 3 GENERAL METHODS

mode is running! b) leg speed (i.e. number of strides) is similar to that achieved in free running, c) body mass is unsupported as in free sprinting and d) the metabolic response as reflected by blood lactate concentration is similar to that of track sprinting. Thus, for the purpose of this thesis the sprint treadmill was preferred to the cycle ergometer.

3.4.1.2. Speed measurement and calibration

Treadmill speed was monitoring by the aid of a drive system, which was mounted to the front rolling drum of the treadmill to drive a high precision D.C. generator. During the sprints, outputs from the generator were continuously monitored by a microcomputer (BBC model B) via a single channel A-to-D converter (R.S. Components 574, Northants, U.K.) multiplexed using analogue switches. By connecting the multiplexer to the BBC microcomputer via a switch using 1 MHz port, the output of the A-to-D was latched and read by the User Port of this microcomputer. Immediate feedback for the volunteers running on the sprint treadmill was provided by a large lmA meter, which was positioned in front of the ergometer and interfaced with the generator so that the output could be processed to drive the dial. The dial displayed belt speed in m·s⁻¹.

Prior to every test a calibration routine was performed in order to assure the optimal operation of the ergometer. An electric motor was coupled permanently with the sprint treadmill to facilitate driving of the treadmill belt when required. While the motor was driving the belt at a near constant speed, 100 revolutions of the treadmill belt were counted. Logging the BBC microcomputer and counting belt revolutions were initiated simultaneously. Termination of the counting and logging was also at the same time. The mean output voltage corresponding to the number of revolutions was then used to calculate the calibration factor by the microcomputer and was then retrieved by the test programs.

3.4.1.3. Propulsive force measurement and calibration

The other component of external power output, restraint force, was measured with the aid of a force transducer (RS components, Northants, U.K.) mounted on the rear crossbar of the treadmill, which was attached to a non-elastic belt that passed around
the subjects’ waist. The rationale for force measurement in that way has its roots in Newton’s third Law of Motion, which states that for every action there is an equal and opposite reaction. This action can be measured as long as the individual is not moving relative to the ground and the opposite force that applies to the tether belt can be obtained. The latter is true with the nonmotorised treadmill (for more information see Lakomy, 1988).

Nonetheless, a modification was made to mount the force transducer on the wall behind the treadmill instead of on a flat crossbar between vertical rails as previously described (Lakomy, 1988). This position was permanent, fixed and safe, but did not allow for adjustments according to the volunteers’ height as originally suggested (Lakomy, 1988). Nevertheless, the error introduced by this modification is considered negligible (Lakomy, 1988).

The second calibration routine involved the output from the force transducer. Calibration was performed in the morning of each experimental day and had two phases: calibration of the zero force (output when no load was attached to the transducer), and, secondly, a known weight was attached to the transducer so as to provide a known force (for more detailed information see Lakomy, 1988).

3.4.1.4. Treadmill warm-up

Prior to each main test the 750-W electric motor was switched on in order to revolve the belt. This process lasted 50 s and was employed in order to standardise the treadmill internal resistance as previously described (Lakomy, 1988).

3.4.2. MOTORISED TREADMILL

Calibration of the treadmill speed (Woodway, USA) was performed at regular intervals with and without a subject on (British Association of Sports Sciences, 1987). The speeds chosen were 2.5, 3.0, 3.5 and 4.0 m·s⁻¹, respectively, and represented the range of speeds used by the subjects during all the tests and training protocols reported in this study (tables 3.1 and 3.2)
By placing a marker on the belt, which is clearly visible when the treadmill is running, it was possible to record the time taken for 30 complete revolutions by means of a stopwatch. Then, the length of the belt (5.22 m) was multiplied by 30 (revolutions) and the product was divided by the time took to complete 30 revolutions.

Table 3.1. Treadmill calibration data (with subject on).

<table>
<thead>
<tr>
<th>Treadmill speed, display reading, (m·s⁻¹)</th>
<th>30 belt revolutions x belt length (5.222 m) and divided by the time took to complete 30 revs at each speed</th>
<th>Error (%) of digital display</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.49/2.50</td>
<td>156.6/62.74</td>
<td>0.0/0.16</td>
</tr>
<tr>
<td>3.01</td>
<td>156.6/52.14</td>
<td>1.35</td>
</tr>
<tr>
<td>2.49/3.50</td>
<td>156.6/44.84</td>
<td>0.0/0.21</td>
</tr>
<tr>
<td>3.59</td>
<td>156.6/39.36</td>
<td>0.28</td>
</tr>
</tbody>
</table>

Table 3.2. Treadmill calibration data (without subject on).

<table>
<thead>
<tr>
<th>Treadmill speed, display reading, (m·s⁻¹)</th>
<th>30 belt revolutions x belt length (5.222 m) and divided by the time took to complete 30 revs at each speed</th>
<th>Error (%) of digital display</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.47/2.48</td>
<td>156.6/63.00</td>
<td>0.51/0.40</td>
</tr>
<tr>
<td>2.99/3.00</td>
<td>156.6/51.90</td>
<td>1.00/0.67</td>
</tr>
<tr>
<td>3.49/3.50</td>
<td>156.6/44.79</td>
<td>0.39/0.0</td>
</tr>
<tr>
<td>3.99/4.00</td>
<td>156.6/39.10</td>
<td>0.50/0.25</td>
</tr>
</tbody>
</table>

3.5. PROTOCOLS

3.5.1. SINGLE 30 s PROTOCOL

3.5.1.1. Rationale

The value and rationale for a single 30-s sprint protocol has been previously discussed (e.g. Inbar et al., 1996). In brief, the prevailing lack of acceptable protocols to assess maximal intensity exercise of short duration was the main reason for inadequate information concerning the metabolic responses to sprinting (Cheetham et al., 1986). However, the introduction of the Wingate Anaerobic Test (30-s “flat out” sprint) in
the mid seventies by Ayalon, Inbar and Bar-Or (1974) heralded a new era in maximal intensity exercise and many studies examining the metabolic responses to sprinting (mainly cycling) followed. It has been shown that cellular ATP supply for a 30-s flat out sprint is not provided exclusively by anaerobic energy resources, nonetheless, it is now well documented that PCr hydrolysis and substrate-level phosphorylation predominate (e.g. Bogdanis et al., 1996a).

Furthermore, the instrumentation by Lakomy (1987) of the nonmotorised treadmill brought about another breakthrough. It was the first time that sprint running could be evaluated in any detail in the laboratory.

The combination of these two advances has been used successfully in studying the physiology of sprint running (Cheetham et al., 1986; Nevill et al., 1989). Thus, to facilitate comparison with earlier studies and to add to the dearth of information for female subjects undertaking sprint running exercise, the 30-s sprint running protocol was selected as the test protocol for this study (fig. 3.1).

![Diagram of the single 30 s sprint test protocol]

WHERE: 10 min rest on the couch.
- black numbers indicate minutes.
- standardised warm-up.
- indicates blood sample.

Figure 3.1. The schematic representation of the single 30 s sprint test protocol.
3.5.1.2. Procedures

A standardised warm-up consisted of 3 min jogging at 2 m·s$^{-1}$ followed by 5 min stretching and two 30-s sub-maximal runs at 3.0 m·s$^{-1}$ and 3.5 m·s$^{-1}$, respectively, interspersed with 30 s rest. A similar warm-up for this type of exercise has been employed before (Cheetham et al., 1986; Nevill et al., 1989).

There is a scarcity of published papers examining the effects of different types of warm-up upon sprinting performance. Unpublished data from our laboratory and a preliminary report (Sani et al., 2003) suggests that changing the mode of the warm-up can alter the subsequent power output. For this reason the same pattern and intensity of warm-up was always followed.

Five minutes after the completion of the standardised warm-up a 30-s “flat out” sprint from a rolling start was performed by the participants on the “GO” command (fig.3.1). Participants were instructed to sprint maximally from the start of the sprint. Participants received strong verbal encouragement throughout the sprint. At the end of the sprint participants were directed to lie down in a semi-reclined position on the experimental couch where blood samples were drawn. Blood samples were taken at rest (same position as post-sprint), post warm-up (same position as post-sprint) and at 1, 5 and 10 min post-sprint (fig.3.1).

When more than one trial was performed, the time of the day was the same or very similar (Bernard et al., 1998). In addition, 24 h prior to the main trials subjects were instructed to follow the same diet on each occasion. For this reason a diet record sheet was given to them so that they could replicate their diet as closely as possible [Appendix I]. Drinking water was provided ad libitum and fans or air conditioning (main study 3) was available for all the trials.

Finally, for the second and third main studies the ethical committee considered that each subject should complete a health status questionnaire prior to every test that demanded maximal effort [Appendix F2]. This was in addition to the general health status questionnaire provided in advance of the commencement of experiments.
3.5.1.3. Variables measured

The variables measured during the 30-s sprint were:

- **Peak Power Output (PPO);** expressed in watts (W), which represents the highest power output achieved during the 30-s sprint (1 s averaging period).

- **Time to PPO;** expressed in seconds (s), denotes the time taken to achieve this PPO during the initial stages of the 30-s sprint.

- **Mean Power Output (MPO);** expressed in watts (W), refers to the average power output during the 30-s sprint.

- **Fatigue Index for power output (FI_{PPO});** characterises the difference between the peak and the end power output and is expressed as the percentage of the peak power.

- **Peak power output divided by the whole body mass (PPO/BM) was expressed in watts·kg^{-1}.**

- **Mean power output divided by the whole body mass (MPO/BM) is expressed in watts·kg^{-1}.**

- **Peak Speed;** expressed in m·s^{-1}, indicates the highest speed recorded during the 30-s sprint.

- **Mean Speed;** expressed in m·s^{-1}, corresponds to the average speed during the 30-s sprint.

- **Fatigue Index for Speed (FI_{SP});** signifies the difference between the peak and the end Speed and is expressed as the percentage of the peak speed.

- **The horizontal component of Work Done (Work) was expressed in Joules (j).**

- **Work was also expressed relative to the whole body mass (Work/BM) expressed in joules·kg^{-1}.**

- **Distance Run;** expressed in meters (m), refers to the distance covered during the 30-s sprint.

At a later stage all values were entered into an excel spreadsheet for further analysis [Appendix J].
3.5.2. REPEATED 30 s PROTOCOL

3.5.2.1. Rationale
The repeated 30 s protocol consisted of two 30-s sprints interspersed with 2 min of passive rest (fig.3.2). The short recovery period was selected to ensure poorer performance in the second sprint, thus facilitating the examination of the physiological mechanisms underlying performance recovery. Recovery of performance was expressed as the percentage of the value achieved in sprint 1 (Bogdanis et al., 1996a).

3.5.2.2. Procedures
The repeated 30 s protocol consisted of two 30-s sprints (fig.3.2). The procedures followed were the same as in the single 30 s protocol with the only difference that subjects repeated the test after a 2 min passive recovery period. Blood samples were taken at rest, post warm-up, post sprint 1, post sprint 2 and at 5, 10, 20 and 30 min during recovery from the sprints and while subjects were positioned on the experimental couch as for the single 30-s sprint (fig.3.2).

Test conditions (e.g. time of testing, diet) were standardised as described for the 30-s sprint (section 3.5.1.2).

**Figure 3.2. The schematic representation of the repeated sprint test protocol.**
3.5.2.3. Variables measured
The same variables reported for the single 30 s protocol were recorded here with the addition of recovery measurements as described in paragraph 3.5.2.1.

3.5.3. SPEED-\(\dot{V}O_2\) PROTOCOL

3.5.3.1. Rationale
Following familiarisation and a full recovery period subjects undertook a 16 min sub-maximal test (Williams and Nute, 1983), so that the relationship between oxygen uptake and treadmill speed could be identified (Farrell et al., 1979; Hagan et al., 1980; Conley and Kranhenbuhl, 1980).

3.5.3.2. Procedures
The protocol (fig.3.3) consisted of a 16 min continuous run with the grade at zero degrees and the speed increased every 4 min by 0.5 m·s\(^{-1}\) (Hagan et al., 1980; Williams and Nute, 1983). Heart rate recordings, Rates of Perceived Exertion (RPE) and expired air samples were collected in the last minute of each period. The speeds used were selected by taking into consideration the fitness status of each individual (Ramsbottom et al., 1987). Sub-maximal, but challenging speeds between approximately 60 and 90 % \(\dot{V}O_2\) max were selected.

The equation derived from the linear regression equation between treadmill speed and oxygen uptake for each participant was used to calculate the predicted running speeds for the speed-lactate test (section 5.3.5).
3.5.3.3. Variables measured

The following variables were recorded or calculated from the primary measurements (Appendix K): ventilation ($\dot{V} E$, expressed in mL·kg$^{-1}$·min$^{-1}$), oxygen uptake ($\dot{V} O_2$, expressed in mL·kg$^{-1}$·min$^{-1}$), carbon dioxide production (CO$_2$, expressed in mL·kg$^{-1}$·min$^{-1}$), respiratory exchange ratio (R, carbon dioxide production+oxygen uptake), ventilatory equivalent for oxygen ($\dot{V} E$·$\dot{V} O_2^{-1}$, ventilation+oxygen uptake expressed in mL·kg$^{-1}$·min$^{-1}$).

3.5.4. MAXIMUM OXYGEN UPTAKE PROTOCOL

3.5.4.1. Rationale

Maximum oxygen uptake has been long agreed as the international reference standard of cardiorespiratory fitness (Shephard et al., 1968; Astrand and Rodahl, 1986) and has been used for this purpose in numerous endurance training studies.

3.5.4.2. Procedures

On a separate occasion and after the completion (after at least 24 h) of the habituation period and the speed-$\dot{V} O_2$ test as described above, volunteers were asked to perform an exercise protocol designed to determine their maximal aerobic power (fig.3.4).
This protocol was based on the original investigation of Taylor et al. (1955), modified for the requirements of the present study (Williams and Nute, 1983). Since an interrupted test extending over multiple days is inconvenient and impractical (Froelicher et al., 1974; McConnell, 1988), a continuous version of this test was adopted. It has been previously reported that using a continuous in comparison with a discontinuous test does not change the $\dot{V}O_2$ max achieved (McArdle et al., 1973; Stamford, 1976; Harrison et al., 1980). The speed of the belt remained constant (Taylor et al., 1955; Hermansen and Saltin, 1969; Mayew and Gross, 1975) with the intensity being increased every 3 min by elevating the grade by 2.5% (Taylor et al., 1955; Buchfuhrer et al., 1983) from an initial inclination of 3.5% (Williams and Nute, 1983; Ramsbottom et al., 1989). Familiarisation runs predetermined the speed used maximal test itself. This speed was individualised for each subject and selected to cause exhaustion in 10 ± 2 min (Buchfuhrer et al., 1983). Expired air samples were collected for 1 min (Taylor et al, 1955; Howley et al., 1995) during every stage between 1.45 to 2.45 min as suggested originally by Taylor et al. (1955). This procedure was followed until the volunteer signalled that she could only maintain the exercise intensity for a further one minute during which another sample of expired air was collected. Heart rate was monitored every 15 s and at the same time as the expired air was collected. Treadmill handrails were not used for support at any time. Termination of the test was volitional. Subjects were encouraged to give maximal effort and their physical appearance was consistent with this. Volunteers were then cooled-down at a self-selected pace (Bonen and Belkastro, 1976; Tsampoukos, 1997).

Before the test each volunteer performed a 3-5 min warm-up (plus stretching) at a speed equivalent to ≈ 50-70% of their $\dot{V}O_2$ max as recommended by Shephard (1984), McConnell (1988) and Howley et al. (1995). Warm-up prior to $\dot{V}O_2$ max protocol yields slightly larger $\dot{V}O_2$ max readings (Taylor et al., 1955) and minimises musculoskeletal injuries and eletrocardiographic abnormalities (Shephard, 1984).

Drinking water was available to the participants before, during and after the test ad libitum. Fans were always available in order to enhance the evaporative cooling process of the subjects (McConnell, 1988). Nonetheless, no air conditioning was available to standardise the ambient temperature when repeated measurements were
performed. Despite this, the atmospheric temperature was within the acceptable limits for this type of measurements (17-27 °C, Taylor et al., 1955) and only varied by 2-3 °C. Subjects were asked to refrain from alcohol (24 h), large meals (4 h) and caffeine (12 h) preceding the tests (McConnell, 1988). Furthermore, participants were asked to avoid hard exercise for the day prior to the experiments (McArdle et al., 1973; McConnell, 1988), although there is much controversy in the literature on this topic (Stamford et al., 1978).

Figure 3.4. Maximum oxygen uptake ($\dot{V} O_2 max$) test protocol. Shaded areas indicate collection of expired air, heart rate and perceived exertion readings for 1 min in each 3 min stage (whole blocks). Numbers on the figure denote the exact timing of the data collections.

The main criterion for establishing that maximum oxygen uptake had been achieved was a plateau in the oxygen/exercise intensity relationship. This was defined as an increase in oxygen uptake of less than 2 mL·kg$^{-1}$·min$^{-1}$ with an increase in exercise intensity (Taylor et al., 1955). When this was not achieved the following additional criteria were considered:

a) A final respiratory exchange ratio of $\geq 1.15$ (Issekutz et al., 1962)

b) A final heart rate of within 10 beats·min$^{-1}$ of the age-related maximum (Shephard et al., 1968; British Association of Sport and Exercise Sciences, 1997).
c) A score on the completion of the test equal to or greater than 19 on the 15 point (5-20) Borg scale (Hammond and Froelicher, 1984).

d) Finally, when the female participant showed subjective fatigue, exhaustion and inability to continue, their value was also taken as representative of $\dot{V} O_2 \text{max}$ (Gibson et al., 1979).

Since maximum oxygen uptake requires maximal effort from the participant a health status questionnaire was completed prior to every test and was identical to that for 30 s protocol [Appendix F2].

3.5.4.3. Variables measured

The same variables were recorded as described previously for the speed-$\dot{V} O_2$ test.

3.5.5. SPEED-LACTATE TEST PROTOCOL

3.5.5.1. Rationale

Although maximum oxygen consumption is considered the gold standard for measuring cardiorespiratory fitness modest changes in $\dot{V} O_2 \text{max}$ do not always reflect the large improvements in endurance capacity and performance after training (Conley and Krahnenbuhl, 1980; Williams and Nute, 1986). In addition, the % $\dot{V} O_2 \text{max}$ at a given blood lactate concentration has been shown to increase with endurance training (Hurley et al., 1984; Williams and Nute, 1986) and to be related to endurance capacity, as reflected by the cycling or running time to exhaustion at 90 and % 88 % $\dot{V} O_2 \text{max}$, respectively (Williams and Nute, 1986; Coyle et al., 1988). The % $\dot{V} O_2 \text{max}$ at a given blood lactate concentration has been shown to be determined by different underlying physiological mechanisms to those that determine $\dot{V} O_2 \text{max}$ as indicated from lack of any relationship between the two (Hurley et al., 1984). Thus, the relative intensity (% $\dot{V} O_2 \text{max}$) corresponding to a blood lactate concentration of 4 mmol·L⁻¹ (% $\dot{V} O_2 \text{max} @ 4 \text{ mmol·L}^{-1}$) was used, in addition to $\dot{V} O_2 \text{max}$ itself, to monitor the effect of training in the present study (fig.2.5).

The notion that % $\dot{V} O_2 \text{max} @ 4 \text{ mmol·L}^{-1}$ can be used for other populations apart from endurance athletes is strengthened by the fact that running velocity
corresponding to 4 mmol·L\(^{-1}\) can also predict running performance in non-endurance athletes (Duggan and Tebbutt, 1990).

![Speed-lactate test protocol diagram](image)

**Figure 3.5.** Speed-lactate test protocol. Shaded areas from each block indicate the last min of each stage (4 min stages) in which collection of expired air, heart rate and perceived exertion readings took place. Bold lines at the end of each block denote capillary blood sampling.

It has been suggested in some studies that blood lactate concentrations can be altered due to different phases of menstrual cycle during sub-maximal exercise below 70 % \(\dot{V}O_2\) max (Kanaley et al., 1992; McCracken et al., 1994) implying that the speed lactate test employed here could be influenced too. However, the % \(\dot{V}O_2\) max @ 4 mmol·L\(^{-1}\) usually represents intensities higher than 70 % \(\dot{V}O_2\) max (Ramsbottom et al., 1989; Yoshida, 1990) and thus eliminates any confounding effect of menstrual cycle phase upon speed lactate test. Furthermore, Williams and Kranhenbuhl (1997) demonstrated that oxygen consumption is affected during the mid-luteal phase of the menstrual cycle at rest and at speeds equivalent to 85 % \(\dot{V}O_2\) max. Nonetheless, the weight of available evidence in the literature is opposed to this possibility and it seems that rather high progesterone levels are required for this metabolic response to occur (Beidleman et al., 1999).
3.5.5.2. Procedures

One day after the completion of the $\dot{V}O_2$ max test volunteers reported to the laboratory to perform a 16 min run on a level treadmill (fig.3.5). The test was continuous and the speed was increased every 4 min. The determination of the speeds was based on the equation derived from the linear regression of the relationship between running speed and oxygen consumption during the speed-$\dot{V}O_2$ max test. These speeds were calculated to represent 60, 70, 80 and 90 per cent of the subject’s $\dot{V}O_2$ max as previously described (Williams and Nute, 1983; Rambsbottom et al., 1989).

During the last minute of each stage, expired air samples were collected. Immediately afterwards and while the subject was still running on the treadmill a blood sample was drawn from the thumb (plate 3.1). When the sample had been taken (~30 s) the speed was increased. All the blood samples, with the exception of the resting sample, were taken while the participant was standing up. Heart rate was monitored every 15 s during the last min of each stage. Finally, at each stage perceived exertion was recorded using the Borg Scale.

The speed-lactate test was always scheduled 24 h after the $\dot{V}O_2$ max test. In the intervening 24 h subjects were asked not to undertake any exercise other than normal daily activities (Hartley et al., 1970). Moreover, as for the $\dot{V}O_2$ max test, no alcohol (24 h) and caffeine (12 h) were permitted prior to the trials (Weltman, 1995; Jones and Doust, 2001). Volunteers were also instructed to wear the same clothing and shoes (Cavanagh and Williams, 1982; Frederick et al., 1986) and to follow a similar diet (Yoshida, 1984) when the speed-lactate test was to be performed again. Fans and drinking water were available all the times and the environmental conditions were similar to those described for the $\dot{V}O_2$ max test.

The % $\dot{V}O_2$ max corresponding to a blood lactate concentration of 4 mmol·L$^{-1}$ was estimated by plotting each subject’s blood lactate concentration against % $\dot{V}O_2$ max and connecting the data points by linear extrapolation as previously described (Sjodin and Jacobs, 1981).
3.5.5.3. Variables measured
During the speed-lactate test the variables recorded were the same as those for the speed-$\dot{V} O_2$ test (described in section 3.5.3.3).

3.5.6. TRAINING PROTOCOL

3.5.6.1. A brief review of terminology and training principles
The major aim of a training programme, if it is well planned and executed, is to increase athlete's physical capabilities and optimise performance as the body adapts to the training stimulus (Matveyev, 1981; Letzelter, 1988; Harre, 1991; Zatsiorsky, 1995; Bompa, 1999). In biology, adaptation denotes a characteristic that favours survival in specific environments (Vander, 2001).

Exercise is an ideal stimulus for adaptation (Matveyev, 1981; Letzelter, 1988; Harre, 1991; Zatsiorsky, 1995; Bompa, 1999). From the practical point of view four features of the adaptation process are of primary importance for the design of a sport training programme: overload, accommodation, specificity and individualisation (Letzelter, 1988; Harre, 1991; Zatsiorsky 1995; Bompa, 1999).

There are two ways to vary the overload: to increase the training load (quantitative change in intensity and/or volume), or to change the type of exercise (change in quality (Letzelter, 1988; Harre, 1991; Zatsiorsky 1995; Bompa, 1999). For the present training study a change in training intensity and volume was used.

In biological terms accommodation is the decrease in response of a biological object to a continuous stimulus (Guyton and Hall, 1996, page 65). In sport, if the same training regimen is followed over long period accommodation may occur (Letzelter, 1988; Harre, 1991; Zatsiorsky, 1995; Bompa, 1999). Thus the training intensity was increased at intervals throughout the training studies presented in this thesis to ensure overload and prevent accommodation.

Finally, the training stimulus was individualised carefully for the training studies reported within thesis with each subject running for 30 min at a speed calculated to
elicit a specific percentage of $\dot{V} O_2 \text{ max}$ that was precisely determined from the regression equation between oxygen uptake and speed.

### 3.5.6.2. Rationale

The training protocol was designed, not to compromise the performance of the 30 s sprint so that recovery from a set amount of work could be examined before and after training. The training programme was also designed to improve both the endurance capacity (as indicated by the % $\dot{V} O_2 \text{ max} @ 4 \text{ mmol} \cdot \text{L}^{-1}$ %) of the participants and maximum oxygen uptake of the subjects.

A brief overview of the relevant literature used to assist in the design of the training programme is outlined below. Cheetham (1987) concluded that 10 weeks of endurance training (6-8 h per wk for 10 wk on the top of their recreational running programme) at 70 % of their $\dot{V} O_2 \text{ max}$ resulted in an increase in the time taken to reach peak speed during a 30-s running sprint post-training in the experimental group while the reduction on fatigue index for speed was blunted. These findings were corroborated 10 years later by Harrison (1997) where a reduction of MPO during the first 10 s of sprint 1 and an elevated fatigue index for speed was observed following 6 weeks continuous training on the cycle ergometer at 70 % of their $\dot{V} O_2 \text{ max}$. In contrast, Treweek (1994) deploying an identical programme did not find any detrimental effects on sprinting performance. It seems that the advice of Treweek (1994) to his subjects to continue their regular activity during the experimental period compensated any negative effects associated with endurance training. Similarly, Hardman et al. 1986 revealed that endurance cycle exercise for 30 min at 85 % $\dot{V} O_2 \text{ max}$ improves $\dot{V} O_2 \text{ max}$ by 18 % and sub-maximum endurance performance by 200 % and did not alter the performance profile during a 30-s maximal sprint. Judging from these investigations it can be assumed that continuous endurance exercise of about 30 min and at approximately 85 % $\dot{V} O_2 \text{ max}$ not only improves aerobic power and endurance capacity, but also has no detrimental effects upon sprinting performance. Finally, it is worth mentioning that this intensity is also considered appropriate for endurance training by training experts when continuous running is the method of the choice (Zintle, 1993).
Based on this intensity (85 % $\dot{V} O_2 \text{max}$) the duration of training required to elicit improvements in endurance fitness should be identified. Six weeks of continuous endurance training can improve both aerobic power and endurance capacity as indicated by improvements in $\dot{V} O_2 \text{max}$ and % $\dot{V} O_2 \text{max}$ @ 4 mmol·L$^{-1}$, respectively (Gaesser and Rich, 1984; Hardman et al., 1986; Williams and Nute, 1986).

The cellular changes inherent induced as a result of a training programme are restricted to the muscle fibres recruited to perform the exercise task (Fitts and Widrick, 1996). Thus an important factor for the design of this training programme was whether the intensity and duration used could recruit all the fibres and especially the fast twitch fibres that seem to play the major role in recovery of such activity (Casey et al., 1996b). Evidence in the literature supports the view that an intensity of 85 % $\dot{V} O_2 \text{max}$ is sufficient to recruit all fibre types (Andersen and Sjøgaard, 1975; Andersen and Henriksson, 1977b) during 30 min exercise.

Consistent with the above criteria, the frequency (repetitions per week) of the training regimen of 3 days per week can be considered adequate (Gaesser and Rich, 1984; Hardman et al., 1986; Williams and Nute, 1986).

Previous reports adopting a similar training regimen have revealed no training-related injuries (Williams and Nute, 1986). However, in order to reduce the possibility of injury training sessions were conducted 48 h apart whenever possible. Where training sessions are placed in a week period, does not influence training effects (Moffatt et al., 1977).

Thus, in summary the training protocol was as follows:

- **Length** of the training period: 6 wk
- **Intensity** of the exercise: 85 % $\dot{V} O_2 \text{max}$
- **Duration** of the session: 30 min
- **Frequency** of the exercise every wk: 3· wk$^{-1}$
3.5.6.3. Procedures

Each subject reported to the lab 3 times a week for six weeks in order to comply with the training requirements. Subjects were advised to have a rest day between training sessions to recover. In addition, they were instructed not to interrupt or change their personal training schedule.

The treadmill speed was set at 85% of their \( \dot{V}O_2 \text{ max} \).

Before the start of the 30 min runs volunteers performed a warm-up consisting of 3-5 min jogging at about 50-70% of their \( \dot{V}O_2 \text{ max} \) and 3-8 min stretching. After this period they started the 30 min session. The first minute was run at a speed required to elicit 50%, the second minute 60%, and the third to fifth minute 70% of each subject’s \( \dot{V}O_2 \text{ max} \), so that there was progressive increase to the target speed of 85% \( \dot{V}O_2 \text{ max} \) (figure 3.6). Volunteers were strongly encouraged to complete all the 30 min session. Water was provided \textit{ad libitum}. To break the monotony of the regimen music (all studies) and TV (for the third main study) was provided. Moreover, fans and sponges were available for the participants. Every session was supervised by one or two experimenters.

During the first week of the training period expired air samples were collected at the 12\textsuperscript{th} min [since oxygen consumption reaches stabilisation after approximately 3 to 10 min of the training session as suggested by Farrell et al. (1979), Hagan et al. (1980) and Conley and Kranhenbuhl, (1980)] in order to verify whether the speed run did indeed elicited 85% \( \dot{V}O_2 \text{ max} \). The standard conditions adopted for the speed lactate (time of the day, diet, previous exercise) test were also followed here since the speeds for the training were based on that test.

To vary the training load and avoid accommodation the training intensity was increased progressively every two weeks or when the subjects and/or the experimenters felt it would be possible to run for 30 min at a new and faster speed.
### 3.5.6.4 Monitoring of training

As briefly mentioned in the previous section, training intensity was adjusted on the basis of the subjective judgement of the experimenter and on the basis of laboratory measures. The investigator (and author of this thesis) is qualified to make such subjective judgements as he is a training specialist in Athletics from the Aristotle University of Thessaloniki as part fulfilment of the requirements for his First Degree in Sports Science and Physical Education [Appendix L1]. Furthermore, he is certified from the National Strength and Conditioning Association from the United States of America as Strength and Conditioning Specialist [Appendix L2]. His practical experience comes from both his participation as an athlete in national level in Athletics and from his part-time work as a sprint coach (Head Coach) at

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**Figure 3.6. The schematic representation of the 30 min training protocol.**

<table>
<thead>
<tr>
<th>INTENSITY (%) $\dot{V}O_{2\text{,max}}$</th>
<th>MINUTES</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>90</td>
<td>2</td>
</tr>
<tr>
<td>85</td>
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<td>20</td>
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<td>10</td>
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</tbody>
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ANTONIS TSAMPOUKOS Ph.D. THESIS
Loughborough University. Most of the subjects were experienced athletes and therefore their subjective judgements were of great value.

Using laboratory measures the intensity of the training was determined by measuring oxygen consumption and examining whether the speed employed elicited the predetermined percentage of $\dot{V}O_2$ max. This was achieved by collecting an expired air sample during the 12th min of the run. It was not convenient to measure oxygen consumption in every training session and measurements are of limited value due to the rapid change in $\dot{V}O_2$ max in the first few weeks of training (e.g. Gaesser and Rich, 1984; Williams and Nute, 1986) and therefore measuring oxygen consumption after the first two weeks the information received might be incorrect (as in % of maximum oxygen uptake). Heart rate monitoring via short-range telemetry was the alternative approach. It has also the advantage of the immediate feedback for both the participant and the experimenter. Heart rate was monitored for every session during the whole period of the training regimen. The experimenters recorded the heart rate every 5 min after the subject had reached the target intensity [Appendix M]. Although heart rate can provide a precise measure of training intensity (Gilman, 1996), it can also be influenced by a number of factors such as cardiac drift due to the duration of the exercise and environmental heat stress (Gilman, 1996). In addition, despite the strong relationship between heart rate and exercise intensity, a given percentage of maximum heart rate does not necessarily reflect a similar percentage of $\dot{V}O_2$ max (Swain et al., 1998).

The blood lactate response to the training session also determined, but only was used once during the training due to the invasive nature of the fingerprick blood samplings. In one of the training sessions blood samples were taken at the 12th min of the training session, as was the case for the expired air samples. The process took place after the experimenter had verified the target intensity by means of $\dot{V}O_2$ measurement. The rationale for measuring lactate was to check, through this metabolic index, the intensity of the 30 min run. The treadmill speed for training was established according to the $\dot{V}O_2$ measurement, but the blood lactate concentration could give an additional indication of how difficult the exercise was for the subject (Weltman, 1995).
Finally, participants' perceptions about the training regimen was monitored every 5 min [Appendix N] by means of the Borg scale for rating of perceived exertion (Borg, 1982). This was an additional technique for evaluating the intensity of the exercise (Robertson et al., 1990; Dunbar et al., 1992). The advantage of RPE is that is non invasive and offers immediate feedback. Furthermore, RPE good correlates well with the blood lactate responses to exercise and is unaffected by gender (Demello et al., 1987), training state (Seip et al., 1991), specificity of training (Boutcher et al., 1989), or intensity of training with special reference to 30 min run (Steed et al., 1994).

3.6. ANTHROPOMETRIC MEASUREMENTS

3.6.1. STATURE
The stature was taken after the subjects stood straight, against an upright wall with a stadiometer (Holtain Ltd., Crymych, U.K.) touching the wall with back, buttocks and both heels. The head was oriented in the Frankfurt plane (Duquet and Carter, 1996). All the volunteers were instructed to stretch upward and take and hold a full breath. The height was then taken placing the ruler until it touched the vertex firmly, without exerting extreme pressure.

3.6.2. BODY MASS
Prior to each main trial participants were weighed in minimal clothing, standing in the centre of a high accuracy scale platform (Model 3306 ABV Avery Industrial Ltd., Leicester, UK). Body mass was recorded to the nearest kilogram (0.1).

3.7. CLEARPLAN HOME OVULATION TEST
One of the most difficult requirements of the present work (for main study 1) was to test the female subjects just prior to ovulation when the highest concentrations of estradiol exist (Paurestein et al., 1978; World Health Organisation, 1980) whereas progesterone circulatory levels remain relatively low (< 2 ng·ml\(^{-1}\)). To achieve this goal, the prediction of the preovulatory period on the same day of testing was required. Testing on the same day is essential condition, because high concentrations of estradiol remain only for about 1 day and coincide with the LH surge (Paurestein
et al., 1978; World Health Organisation, 1980; Hoff et al., 1983). In addition, the LH surge, as detected either in plasma or in urine, is generally accepted as the standard method to predict impending ovulation (World Health Organisation, 1980; Corsan et al., 1990).

A number of clinical and traditional methods for predicting ovulation were not appropriate for this study. The radioimmunoassay of LH is the valid and standard reference method for prediction of ovulation. However, it is time consuming which makes it practically impossible, to test on the same day that the LH surge is observed (Corsan et al., 1990). Another very reliable clinical technique, ultrasonic scanning, verifies rather than predicts ovulation making its use inappropriate. Basal body temperature is also inappropriate, since it identifies ovulation after the event rather than predicts it (Moghissi, 1980). Finally, new evidence discourages the use of saliva to predict ovulation (Braat et al., 1998).

However, recent advances in Monoclonal Antibodies (Monoclonal Antibodies, Inc. 1984) have made it possible, for the first time, to predict the LH surge in in-home test kits that require only a few minutes to perform (Corsan et al., 1990). The Clearplan Home Ovulation Test employed in the present work has been shown to have high levels of accuracy (99 %; Data on file, Unipath limited) and has been experimentally verified to correlate excellently with ultrasonography (Bourne et al., 1996; Miller and Soules, 1996) and serum LH (Testart and Frydman, 1982).

3.8. HEART RATE MONITORING

Heart rate was monitoring throughout the speed-$\dot{V}O_2$, $\dot{V}O_2$ max and speed-lactate tests as well as during the training sessions by means of short-range telemetry (Sport Tester™, PE3000, Polar Electro Oy, Kempele, Finland; sampling frequency 15 s). The highest value obtained was recorded as the maximum heart rate of the subject.
3.9. PERCEIVED EXERTION

During the last minute of each stage (middle point) for speed- \( \dot{V}O_2 \), \( \dot{V}O_2 \text{ max} \) and speed-lactate tests, and in training sessions at predetermined time periods, individuals were asked to point to an appropriate single number on the 15 point (6-20) Borg scale (Borg, 1982), held within easy reach by an investigator. Subjects had been familiarised with the use of this scale previously according to the instructions suggested by Hetzler et al. (1991) [Appendix O].

3.10. ENVIRONMENTAL CONDITIONS

3.10.1. TEMPERATURE/HUMIDITY

Prior to, or immediately after, all the main trials ambient dry and wet bulb temperatures were measured by means of a whirling hydrometer (Brannan Thermometers Ltd., Cumberland, U.K.). The readings were then typed in a excel spreadsheet which had been formatted [Appendix P] using a formula as suggested by Parsons (1993).

3.10.2. BAROMETRIC PRESSURE

Readings for Barometric pressure were taken using a Fortin barometer (F.D. and Company, Watford, U.K.).

3.10.3. TIME OF TESTING

Both sprint and sub-maximal performance (\( \dot{V}O_2 \text{ max} \), speed-\( \dot{V}O_2 \) and speed-lactate tests) tests appear to be influenced due time of the test (McConnell et al., 1988; Hill and Smith, 1991; Bernard et al., 1998; Giacomoni et al., 1999; Giacomoni and Falgairette, 1999). Thus, where repeated trials occurred in this thesis the same time of the day (or as close as possible) was chosen.
3.11. DIET CONTROL

Dietary records were kept on special sheets designed for this purpose [Appendix I]. For the requirements of the main study 1, individuals were asked to record and weigh their habitual diets for three representative days (two week days and one weekend day) by means of digital scales (EKS electronic, model 50013 CR). The dietary records were also used to ensure that subjects were free from eating disorders. Dietary analysis was performed by a dietician using a computerised version (Comp-Eat S.O., Nutrition Systems, London, UK) of UK food composition tables (Holland et al., 1991).

3.12. COLLECTION AND TREATMENT OF GAS

Inhaled ambient air was measured by indirect calorimetry and more particularly with the open circuit spirometry method. Expired air samples were collected using the Douglas Bag method in predetermined time points. Subjects breathed through a low resistance two way respiratory valve (Jakeman and Davies, 1979) connected to a 30 mm wide bore low resistance falconia tube (Falconia Flexible ducting, Baxter, Woodhouse and Taylor Ltd., Macclesfield, U.K.) into plastic Douglas bags (Plysu Protection Systems, Milton Keynes, U.K.). The Douglas bags had a 150 Litres capacity and always evacuated few minutes preceding the experiments. In order to prohibit breathing from the nose, a nose clip was used when the expired air sample was collected.

An aliquot of this air sample from the Douglas bags was used to determine the percentage of oxygen and carbon dioxide using a paramagnetic oxygen analyser (Servomex O2/CO2 Analyser, Model 1440) and an infrared carbon dioxide analyser (Servomex O2/CO2 Analyser, Model 1440). The volume of the expired air was assessed with the aid of Harvard Dry Gas meter (Parkinson-Cowan Ltd.) and the temperature of the expired was determined by an electronic thermistor and logger (Edale Instruments Ltd., type 2984, Model C) which had been fitted in the Dry Gas meter. This procedure was done immediately after the tests.
CHAPTER 3

GENERAL METHODS

Calibration before each experiment was a routine process and was achieved by using certified reference (nitrogen/oxygen-carbon dioxide) gases of known concentration (CryoService Ltd., Worcester, U.K.) The laboratory technician was also checked the oxygen-carbon dioxide gas against a “gold standard” reference gas to ensure the accuracy of the gas concentrations.

Inspired gas volumes were calculated by using the Haldane transformation with formulas provided by either Consolazio et al. (1963) or McArdle et al. (1996).

3.13. COLLECTION, TREATMENT, STORAGE AND ANALYSIS OF BLOOD SAMPLES

3.13.1. COLLECTION OF THE BLOOD SAMPLES

Venous blood samples were drawn at predetermined time points via an indwelling cannula (18 gauge/45 mm cannula, Venflon 2, BOC Ohmeda AB, Helsingborg, Sweden), inserted into an antecubital or forearm vein under local anaesthetic (Lignocaine hydrochloride 1% w/v. Antigen pharmaceuticals Ltd., Roscrea, Ireland). The cannula was connected to a three-way stopcock (Connecta, BOC Ohmeda AB, Helsingborg, Sweden). The venous blood was then drawn into appropriate sized syringes (Becton-Dichinson, Oxford, U.K.). Patency was ensured by flushing solution with 0.9% sodium chloride (B.Braun Medical Ltd., Buckinghamshire, U.K.). The resting sample was always taken after volunteers had rested a in semi-reclined position on the experimental couch for at least 20 min (Hagan et al., 1978; Lundvall and Bjerkhoel, 1994) in order to standardise initial plasma volume and thus minimise any confounding effect of posture (Shirreffs and Maughan, 1994). For the same reasons, great care was also taken to keep the sampling arm in the same position (Eisenberg, 1963). Posture was also standardised for post-exercise samples.

Capillary blood from a pre-warmed thumb (warmed in water at 40-42 °C) was collected in duplicate into 20 μL disposable non-heparinized glass capillary tubes (Accupette Pipettes, Baxter Healthcare Corporation, U.S.A.) by means of an autoclix automatic lancet (Boehringer Manheim U.K., Ltd., Lewes, U.K.), which was used to pierce the skin.
3.13.2. TREATMENT AND ANALYSIS OF BLOOD pH

After collecting the blood into the appropriate size syringes one aliquot (5mL) was dispensed into lithium-heparin coated tube (Sarstedt Ltd, Leicester, UK) to prevent coagulation. Then, blood pH was immediately determined by manually placing the lithium-heparin coated tube on the electrode of a blood pH automatic analyser (ABL™ Blood Gas System, Copenhagen, Denmark).

Calibration of the automatic analyser was performed prior to each experimental day by determining aliquots of standards with known concentrations (S2030, S2040, S2050 and S2060). Table 3.3. shows the coefficient of variation (cv) for intraassay variation for pH.

3.13.3. TREATMENT, STORAGE AND ANALYSIS OF BLOOD METABOLITES

The lithium-heparin coated tubes were mixed gently and then duplicate 20 μL blood samples were dispensed into pre-treated microcentrifuge tubes (Sarstedt Ltd., Leicester, U.K.) by means of disposable non-heparinized glass capillary tubes (Accupette Pipettes, Baxter Healthcare Corporation, U.S.A.). The pre-treatment of the microcentrifuge tube was achieved with 200 μL of 0.4 mol·L⁻¹ perchloric acid to ensure deproteinisation (Maughan, 1982). The deproteinised samples were then mixed gently and centrifuged in eppendorf centrifuge (Eppendorf-Anderman Centriuge 5414, Germany) for 3 min at 12000 g and then placed initially in a box containing ice (McCaughan et al., 1999) and then in a freeza at −20 °C for subsequent analysis [Appendix Q1]. Lactate was assayed by using an enzymatic fluorometric method as previously described by Maughan (1982) by means of fluorimeter (Locarte Model LF 8-9, Locarte, London, U.K.). Capillary blood samples for lactate were treated in exactly the same way.

When the Yellow Spring Instruments (YSI) automatic analyser (YSI Model 2300 STAT PLUS glucose and Lactate Analyser) was used for the determination of whole blood and plasma assessment the procedures were slightly different. For whole blood, the lithium-heparin coated tube was placed into the analyser outlet from where an
CHAPTER 3

GENERAL METHODS

An aliquot (25 µL) of blood was aspirated. For plasma lactate the lithium-heparin coated tubes were first centrifuged (Koolspin, Burkard Scientific Ltd., Uxbridge, U.K.) for 15 min at 6000 g at 3 °C and then the same procedure as for the whole blood was followed. An aliquot of this plasma was also dispensed to a microcentrifuge tube and then stored at −20 °C for later analysis of plasma lactate using the Cobas Mira Plus analyser which is described below. The calibration routine was performed prior to each experimental day in compliance with the manufacturer’s instructions. Internal (5 mmol·L⁻¹) and external (30 mmol·L⁻¹) standards, as well as serum-based quality control (Control Serum N, Boehringer Mannheim, GmBH; Assigned value for lactate: 1.27 mmol·L⁻¹), were used to check linearity and optimal operation of the analyser.

Table 3.3. Intraassay coefficient of variations (CV) for blood sample assays. Where *: from Morris (1999).

<table>
<thead>
<tr>
<th>ASSAY</th>
<th>n</th>
<th>Mean (mmol·L⁻¹)</th>
<th>± SD</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate</td>
<td>10</td>
<td>5.2</td>
<td>0.11</td>
<td>2.1</td>
</tr>
<tr>
<td>Ammonia*</td>
<td>10</td>
<td>85 µmol·L⁻¹</td>
<td>7</td>
<td>8.2</td>
</tr>
<tr>
<td>pH</td>
<td>10</td>
<td>7.33</td>
<td>0.01</td>
<td>0.07</td>
</tr>
<tr>
<td>Hct</td>
<td>10</td>
<td>39.4 %</td>
<td>0.2</td>
<td>0.5</td>
</tr>
<tr>
<td>Hb</td>
<td>10</td>
<td>12.175 g·dL⁻¹</td>
<td>0.245</td>
<td>2.016</td>
</tr>
<tr>
<td>Estradiol</td>
<td>9</td>
<td>98 pg·mL⁻¹</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Progesterone</td>
<td>8</td>
<td>2.9 ng·mL⁻¹</td>
<td>0.1</td>
<td>4.6</td>
</tr>
</tbody>
</table>

For the purposes of the methodological study 2 plasma lactate was also analysed by the aid of Cobas Mira Plus analyser (Cobas Mira Plus, Roche Diagnostic Systems, Basel, Switzerland) using a commercially available kit (Boehringer Mannheim, GmBH) and employing an enzymatic method according to manufacturer instructions. Calibration of the analyser was achieved by means of serum quality controls such as Precinorm U and Precipath U (Boehringer Mannheim, UK Ltd, Lewes, UK), Control Serum N [Control Serum N (Human), Roche Diagnostics; Range for lactate: 1.14-5.04 mmol·L⁻¹] and CFAS (calibrator for automatic systems).

Another aliquot of blood (1.5 mL) was dispensed immediately after the collection from the syringe in a pre-treated microcentrifuge tube with 20 U·mL⁻¹ calcium-
heparin. The samples were then centrifuged for 3 min at 12000 g and the supernatant was drawn off by the aid of Pasteur pipette (Sarstedt Ltd., Leicester, U.K.) into a screw top microcentrifuge tube (Screw top microcentrifuge tube, Sarstedt L.T.D., Leicester, U.K.) and stored at -70°C for later determination of ammonia. Plasma ammonia assay was determined spectrometrically (Digital Gratting Spectrophotometer Series 2, Model CE393, Cecil Instruments Ltd., Cambridge, U.K.) using a commercially available kit (MPR 1Ammonia, Boehringer Manheim U.K., Ltd., Lewes, U.K.). The ammonia assay was performed [Appendix Q2] within 48 h after the collection of the blood (Tsingas and Wilson, unpublished observations).

Finally, figure 3.7 demonstrates a flow diagram for the order of the sampling and dispensing process. Coefficient of Variation (%) \([(100 \cdot \text{standard deviation}) / \text{sample mean})\] (Cohen and Holiday, 1982)] for all blood sample assays are shown in table 3.3.

### 3.13.4. TREATMENT AND ANALYSIS OF HAEMATOCRIT AND HAEMOGLOBIN

A small aliquot of venous blood was filled in a Na-Hep microhaematocrit tube (Scientific Laboratory Supplies Ltd, Nottingham, U.K.) from the lithium-heparin coated tube for the determination of Haematocrit (Hct). Hct was assessed in triplicate. After gentle mixing, the bore tubes were placed in the rotor of micro-haematocrit centrifuge (micro-haematocrit centrifuge, Hawksley and Sons Ltd, Lancing, U.K.). Packed cell volume was then determined [Appendix Q4] by means of a micro-haematocrit reader (Hawksley and Sons Ltd, Lancing, U.K.).

Haemoglobin concentration was assessed as recommended by the International Congress of Haematology cyanmethaemoglobin method (Maughan et al., 2001) with the aid of spectrophotometer (Digital Gratting Spectrophotometer Series 2, Model CE393, Cecil Instruments Ltd., Cambridge, U.K.). For the purpose of the assay [Appendix Q3] blood was collected in duplicate 20 μL disposable non-heparinized glass capillary tubes (Accupette Pipettes, Baxter Healthcare Corporation, U.S.A.) and dispensed in mini scintillation vials (Scientific Laboratory Supplies Ltd, Nottingham, U.K.).
3.13.5. TREATMENT, STORAGE AND ANALYSIS OF SERUM HORMONES

An aliquot of 5 mL was dispensed from the blood collection syringe to a 5 mL serum tube with clot activator (Sarstedt Ltd, Leicester, UK) and was left to clot for 30 to 60 min. After this period the tubes were centrifuged (Koolspin, Burkard Scientific Ltd., Uxbridge, U.K.) for 15 min at 4000 rpm at ≈ 3 °C and the supernatant was removed with the aid of a Pasteur pipette and distributed to 3 mini microcentrifuge tubes and stored at −70 °C for later analysis of estradiol and progesterone.

The estradiol assay was performed with the Coat-A-Count Estradiol method [Appendix R1], which is a no-extraction, solid-phase ¹²⁵I radioimmunoassay designed for the quantitative measurement of estradiol in serum (Coat-A-Count Estradiol, Diagnostics Products Corporation, Los Angeles, U.S.A.). The estradiol assay was performed with the aid of an automated gamma counter (Cobra II, Packard Instrument Company Inc., U.S.A.).

The progesterone assay [Appendix R2] was performing using the same method (Coat-A-Count Progesterone, Diagnostics Products Corporation, Los Angeles, U.S.A.) and the same automated gamma counter (Cobra II, Packard Instrument Company Inc., U.S.A.).

3.14. COLLECTION, TREATMENT, STORAGE AND ANALYSIS OF MUSCLE SAMPLES

3.14.1. MUSCLE BIOPSY PROCEDURE

Muscle biopsies taken from the vastus lateralis muscle using the needle biopsy technique as reintroduced by Bergstrom (1962) with suction being applied (plate 3.3). Although there is some controversy in the literature concerning the muscle biopsy site [i.e. Costill (1988) claims that greater quantities of glycogen were present in the plantar flexion muscles rather than the quadriceps] evidence from both kinesiology (Hamill and Knutzen, 1995) and metabolic responses to sprint running (Cheetham et al., 1986; Nevill et al., 1989) support the notion of considerable involvement of quadriceps in running. The muscle biopsies were performed by Mr Leslie Boobis...
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After reporting to the laboratory subjects rested for 30 min on the experimental couch prior to the resting muscle sample being taken (plate 3.3). During this 30 min period the surgeon prepared the experimental leg for both the pre- and post-exercise biopsies.

Plate 3.3. Muscle biopsy procedure during the very exact moment when the needle is getting inside the subject's vastus lateralis by the surgeon Mr Leslie Boobis.
11.5 mL VENOUS LACTATE

- Dispense 1.5 mL into pre-treated microcentrifuge tube with Ca-Hep (1)
  - Measure pH ASAP (5)
  - Dispense 5 mL into serum tubes (white), mix gently and leave them to coagulate for 30 min (3)
  - Centrifuge in cool spin centrifuge for 15 min at 4000 rev-min⁻¹ (13)

- Dispense 5 mL into Li-Hep tubes (orange) and mix gently (2)
  - Dispense the plasma in screw top microcentrifuges (6)
  - Dispense 2 x 20 µL aliquots and dispense them into microcentrifuge tubes containing 2 x 200 mL of 2.5% PCA (8)
  - Mix well and centrifuge for 1 min at 13000 rev-min⁻¹ (9)
  - Dispose in liquid nitrogen ASAP (7)
  - Ammonia assay within 48 h (Appendix S2)
  - Dispense the supernatant in small microcentrifuge tubes (marked with blue colour) (14)
  - Store them at -70 °C till later analysis of estradiol (Appendix T1) (15)
  - Dispense the supernatant in small microcentrifuge tubes (marked with green colour) (14)
  - Store them at -70 °C till later analysis of progesterone (Appendix T2) (15)

- Dispense 2 x 20 µL aliquots and dispense them into 5 mL "drapkins" solution for subsequent determination of Hb (Appendix S3) (11)

- Fill 75% of 3 Na­Hep microhaematocrit tubes, mix them gently and seal one end with plasticine for later determination of Hct (Appendix S4) (12)

Figure 3.7. Flow diagram of blood collection and treatment. Numbers in the boxes indicate order of each process.
Two different local anaesthetics (plate 3.4) were used in order to better prepare the subjects for the biopsy: one local (0.9% sodium chloride, B.Braun Medical Ltd., Buckinghamshire, U.K.) and one deeper (2% Lignocaine Injection, B.Braun Medical Ltd., Buckinghamshire, U.K.) in the muscle area. Afterwards, three small "cuts" (as the number of biopsies) were made on the subject’s skin in order to facilitate the biopsy process. The time delay from the cessation of the sprint to the freezing of the sample in liquid nitrogen was on average 14 s. This time delay was recorded by the same experimenter in all biopsy samples.

Plate 3.4. Injection of the anaesthetic prior to the biopsy by the surgeon.

Muscle samples were removed from the needle under liquid nitrogen (approximately 30-100 mg wet weight), and stored in plastic screw-top microcentrifuge tubes immersed in liquid nitrogen until freeze-dried. The screw top microcentrifuge tubes had been previously pierced so that liquid nitrogen to easily circulate in and out of the tube.
3.14.2. INITIAL TREATMENT AND STORAGE OF MUSCLE SAMPLES

Muscle samples were divided into two pieces: main and back-up samples and were stored in two different plastic screw-top microcentrifuge tubes. Then main samples were freeze-dried (Moduyo 4K Freeze Dryer, Edwards High Vacuum International, U.K.) and then placed in a freezer at -70 °C within plastic bags surrounded by desiccant (silica gel) for later analysis. The back-up samples were kept in the liquid nitrogen.

The freeze dried muscle samples were washed twice with 1 mL petroleum ether to remove the fat and any blood present in the sample (Bogdanis, 1994). One mL Petroleum ether was added into each screw top microcentrifuge tube and mixed twice using a whirlimixer (Scientific Industries INC, Bohemia, N.Y. 11716 USA) before removing the ether. At the conclusion of the second wash the microcentrifuge tube was left open in a fume cupboard to facilitate complete drying of the sample.

Connective tissue and blood were dissected from the muscle and the sample was powdered with the aid of an agate pestle and mortar. The powdered samples were weighed using a high precision (accurate to 0.01 mg) electrical balance (Metler Toledo AG 245 balance, Greifence, Switzerland) and then stored back in -70 °C plastic bags surrounded by desiccant (silica gel) for later analysis.

3.14.3. ANALYTICAL METHODS

3.14.3.1. Extraction procedure

The extraction procedure was undertaken using a slight modified of the method described by Harris et al. (1974):

- Pre-weighed powdered muscle samples were removed from the -70 °C freezer and allowed to reach room temperature in silica gel (20 min) and out of the desiccant for about 30 min. The samples were then stored on ice.
- One batch of ten samples was then spun in an eppendorf centrifuge (Eppendorf-Anderman Centrifuge 5414, Germany) at high speed for 1 min to collect the muscle in the bottom of the tube. Then the experimenter gently tapped the tube to free the muscle pellet taking care not to disperse the muscle too much.
A known volume of perchloric acid was added into the muscle powder (volume = muscle weight (mg)/12.5. Note: if the extraction factor is 80 e.g. 8.75 mg of dry muscle dissolved in 700 μL of Perchloric acid). Then they were vortexed for 10 min keeping tube on ice in between.

The extract was then centrifuged at 14000 rev-min⁻¹ for 1-3 min (at 0-4 °C) and a known volume of supernatant was removed to a new screw-top microcentrifuge tube.

To neutralised the extract a volume of 2.2 M KHCO₃ equal to one quarter of the volume supernatant within the screw-top microcentrifuge tube was added. Then the solution was vortexed and the cap was removed immediately. Finally, to allow CO₂ to escape from the tube the tubes were left with loose caps on ice for about 5 min.

The last step involved centrifugion at 14000 rev-min⁻¹ for 1-3 min and removal of the clear supernatant to a new screw-top microcentrifuge tube. The treated muscle sample was then stored at -70 °C freezer.

3.14.3.2. Mixed muscle metabolites assays
All the muscle assays presented here were carried out on freeze-dried tissue (Harris et al., 1974) with the use of fluorometric techniques (Lowry and Passonneau, 1972). Adenosine 5' Triphosphate (ATP), Phosphocreatine (PCr), glucose 6-phosphate (G6P), Lactate and Creatine (Cr) were assayed enzymatically by a fluorometric analysis (Locarte, model 8-9). The coefficient of variation (% CV) for all muscle assays is shown at table 3.4. Total glycogen assay variability has been shown previously (Harris et al., 1974); however, no coefficient of variation (% CV) values are available.

The assays procedures are reported in detail in the appendices section [Appendix S]. Buffers, co-factors (grade-I) and enzymes used in this analysis were available commercially (Roche and Sigma). All reagents were treated with double-distilled water the day before the analysis and kept at 4 °C. All the standards were prepared on the day of the analysis using grade I chemicals. Linear regression was always achieved (r ≥ 0.998). Metabolite concentrations (mmol·kg dry muscle⁻¹) were calculated by means of the regression equation, taking into consideration double-
distilled water blanks, standards and dilution factors. One millilitre of extract was equivalent to 8 mg of muscle powder.

Table 3.4. Intraassay coefficient of variations (CV) for muscle sample assays. Data from Bogdanis (1994). Values are mmol· kg⁻¹ dry· muscle⁻¹.

<table>
<thead>
<tr>
<th>ASSAY</th>
<th>n</th>
<th>Mean</th>
<th>± SD</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>10</td>
<td>27.56</td>
<td>0.70</td>
<td>2.5</td>
</tr>
<tr>
<td>PCr</td>
<td>10</td>
<td>78.25</td>
<td>2.4</td>
<td>2.4</td>
</tr>
<tr>
<td>Cr</td>
<td>10</td>
<td>36.30</td>
<td>36.30</td>
<td>2.8</td>
</tr>
<tr>
<td>Lactate</td>
<td>10</td>
<td>30.10</td>
<td>0.45</td>
<td>1.5</td>
</tr>
<tr>
<td>G6P</td>
<td>10</td>
<td>14.62</td>
<td>0.48</td>
<td>3.3</td>
</tr>
</tbody>
</table>

All muscle metabolites concentrations with the exception of lactate and glycogen are corrected to an individual’s mean total creatine content (sum of PCr + Cr) as suggested by Harris et al. (1974). Lactate was not corrected because this compound exists both in the muscle and in the interstitial space (Katz et al., 1986), whilst glycogen was not corrected since the muscle sample analysed was different from that of total creatine. Normalisation to wet weight was not performed due to the water shifts into the muscle especially in post exercise samples (Harris et al., 1977).

3.15. METABOLIC CALCULATIONS

3.15.1. METABOLIC CALCULATIONS FOR GAS SAMPLES

Ventilation of expired air ($\dot{V}_E$) was measured initially at ATPS (Ambient Temperature, Ambient Pressure, and Saturated with water vapour). In order to express the gas volume in STPD (Standard conditions of Temperature, Pressure and Dry) the following equation was resolved:

$$\dot{V}_E_{STPD} = \dot{V}_E_{ATPS} \cdot \left[273 \cdot (273 - \text{T} ^\circ C)^{-1}\right] \left[(P_B - P_{H_2O}) \cdot (760)^{-1}\right]$$

Where $P_B$ : the barometric pressure, $P_{H_2O}$ : vapour pressure which can be predicted from the following equation: $P_{H_2O} = (1.1001 \cdot T) - 4.19$, where $T$ the temperature. $T ^\circ C$: is also the temperature.
Oxygen uptake was calculated by the equation known as Haldane transformation (Consolazio et al., 1963):

\[
\dot{V}O_2 = \dot{V} I \left[ \%O_2 I - (\%N_2 I/\%N_2 E) \cdot (\%O_2 E) \right]
\]

Where: \( \dot{V} O_2 \): oxygen uptake, \( \dot{V} I \): volume of inspired air, \( \%O_2 I \): percent of oxygen in inspired air, \( \%N_2 I \): percent of nitrogen in inspired air, \( \%N_2 E \): percent of nitrogen in expired air, and \( \%O_2 E \): percent of oxygen in expired air.

Similarly, carbon dioxide production per minute was calculated as follows:

\[
\dot{V}CO_2 = \dot{V} E \cdot (\%CO_2 E - 0.03\%)
\]

Oxygen uptake during recovery from the sprints (chapter 6) was calculated as the area under curve between the recovery \( \dot{V} O_2 \) curve and the baseline (Hermansen, 1969).

All these equations were used to format an excel spreadsheet [Appendix K].

**3.15.2. METABOLIC CALCULATIONS FOR BLOOD SAMPLES**

Plasma volume changes were estimated by using the equation developed by Dill and Costill (1974). In brief:

\[
\Delta \%PV_{after}, \% = 100 \cdot (PV_{after} - PV_{before}) / PV_{before}
\]

The appropriate formation based on Dill and Costill’s publication was created in excel spreadsheet for ease and speed of the calculation.

Blood buffering capacity was estimated with the following equation (Bishop et al., 2003b) based on Sahlin and Henriksson (1984):

\[
\text{Blood buffering capacity (}\beta\text{)} = \frac{\text{blood lactate (peak lactate post-sprint)} - \text{blood lactate (rest)} \div \text{blood pH (rest)} - \text{blood pH (immediately post-sprint)}}
\]

Again, the above equations were used to format an excel spreadsheet [Appendix T].
3.15.2. METABOLIC CALCULATIONS FOR MUSCLE SAMPLES

Anaerobic ATP utilisation was calculated from the values of ATP, PCr and Lactate using the formula suggested by Katz et al. (1986):

$$\text{ATP turnover (utilisation)} = 2(-\Delta\text{ATP}) - \Delta\text{ADP} - \Delta\text{PCr} + 1.5 \Delta L + 1.5 \Delta \text{Pyr}$$

Production of ATP from glycolysis was estimated from the accumulation of lactate and pyruvate (Cheetham et al., 1986):

$$\text{ATP from Glycolysis} = 1.5 \Delta L + 1.5 \Delta \text{Pyr}$$

Pyruvate (Pyr) and ADP (Adenosine 5' diphosphatase) were not measured since their omission is not expected to make a significant difference when sprinting is considered (Bogdanis, 1994).

Glycogenolytic rate (mmol glucosyl·kg dry muscle·s⁻¹) units were calculated from accumulation of glycolytic metabolites as reported by Bogdanis et al. (1996a):

$$\text{Glycogenolytic} = (\Delta G1P + \Delta G6P + \Delta F6P) + 0.5(\Delta L + \Delta \text{Pyr})$$

As previously mentioned Pyr, glucose 1-phosphate (G1P), Fructose 6-phosphate were not assayed because their contribution in the above equation is minimal and with not significant effect (Bogdanis, 1994).

Glycolytic rate was also based on Bogdanis et al. (1996a) and expressed in mmol glucosyl·kg dry muscle·s⁻¹:

$$\text{Glycolytic rate:} 0.5(\Delta L + \Delta \text{Pyr})$$

For the same reasons described above pyruvate omitted.

Muscle pH estimated using the following two equations (Sahlin et al., 1976):
\[ \text{pH} \approx 7.22 - [0.00521 \cdot (\text{lactate} + \text{pyruvate})], \text{for resting samples} \]
\[ \text{pH} \approx 7.06 - [0.00413 \cdot (\text{lactate} + \text{pyruvate})], \text{for post exercise samples} \]

Finally, it should be hypothesised that some lactate diffusion into the circulation during the sprints is possible. However, in short term maximal exercise lactate underestimation is considered minimal \((\text{Spriet, 1995})\).

### 3.16. INFORMATION TECHNOLOGY

In order to find the information necessary for the series of studies presented in this thesis a variety of approaches were employed. Routine database searching such as \text{SPORT DISCUS}, \text{EMBASE}, \text{MEDLINE}, \text{WEB OF SCIENCE}, \text{SMART}, \text{PUBMED} were appropriately scrutinised by typing in key words.

Traditional methods such as examining the reference lists of certain review and research papers was also used, especially when the articles of interest were prior to 1980 (large search engines do not include old articles).

A number of courses provided by Loughborough University were attended to improve information tracking skills [APPENDIX U].

### 3.17. STATISTICAL ANALYSES

Statistical analyses were undertaken using either Microsoft excel spreadsheets (descriptive statistics, 95% limits of agreement, ratio limits of agreement, [Appendix Y]) or using the Statistical Package for the Social Sciences \((\text{Kinnear and Gray, 2000; Bryman and Cramer, 2001; Coakes and Steed, 2001; Ntoumanis, 2001})\).

Analysis of Variance (ANOVA) was employed to evaluate differences within and between conditions and in the event of significant interaction (within) the Bonferroni method was used to perform multiple comparisons \((\text{Atkinson, 2002})\). Differences
between two variables were compared by the use of the pertinent t-test (*Thomas and Nelson*, 1990).

The strength of association between two variables was examined using the correlation coefficient (r). The functional relationship between variables was examined by means of a regression equation. Coefficient of determination ($r^2$) and/or percentage of variance were calculated when the portion/percentage of common association of the factors that influence the two variables was to be determined (*Thomas and Nelson*, 1990).

Where reproducibility or agreement between variables was considered, the 95 % limits of agreement were employed (*Bland and Altman*, 1986). However, when a positive relation between the absolute differences (trial 1 minus trial 2) and their mean revealed (that is, presence of heteroscedasticity) the ratio limits of agreement was the method of the choice (*Nevill and Atkinson*, 1997). In presence of heteroscedasticity coefficient of variation (CV) expressed in percentage (% CV) as suggested by *Nevill and Atkinson* (1997) was also performed [Appendix V].

Coefficient of Variation (CV) was employed to assess the variability of multiple measurements of the same variable (*Cohen and Holliday*, 1982) [Appendix V].

In all statistical analysis statistical significance was accepted at the 5% level. Results are presented as mean ± standard deviation (SD) unless otherwise stated.
3.18. METHODOLOGICAL STUDY 1: TEST-RETEST RELIABILITY OF THE PERFORMANCE VARIABLES AND BLOOD LACTATE RESPONSES TO A 30 s TREADMILL SPRINT PROTOCOL

3.18.1. INTRODUCTION

Minimal measurement error, that is reliability, is one of the most important aims in research (Thomas and Nelson, 1990). Better reliability implies greater accuracy and maximises the chances of tracking changes in the dependent variable as a result of the intervention with the independent variable. The need for high reproducibility also applies in sports medicine research where high precision measurements are required so that even the usual small changes in performance variables are identifiable (Atkinson and Nevil, 1998; Hopkins, 2000). In terms of maximal intensity exercise where maximal effort, motivation and sport-specific equipment are essential, achievement of high precision presents an even greater challenge to the investigators.

As far as maximal intensity exercise of 30-s duration is concerned (that is, the Wingate Anaerobic Test) examination or reports of the repeatability of certain variables have involved mainly sprint cycling (Bar-Or et al., 1977; Evans and Quinney, 1981; Kaczowski et al., 1982; Dotan and Bar-Or, 1983; Patton et al., 1985; Hebestreit et al., 1993; Weinstein et al., 1998) with fewer reports on treadmill sprinting (Cheetham, 1987; Sutton et al., 2000). Nevertheless, recently there has been an upsurge in interest in using nonmotorised treadmills in a sports science research setting (Winter and MacLaren, 2001) as indicated by the latest investigations in the field (Falk et al., 1996; Jaskolski et al., 1996; Jaskolska et al., 1999; Sutton et al., 2000; Tong et al., 2001). In these research groups the optimal resistance for the external peak power output was examined (Jaskolski et al., 1996), force-velocity relationships in treadmill running and cycling were compared (Jaskolska et al., 1999), the vertical component of power output was measured (Falk et al., 1996), the appropriateness of the non-motorised treadmill as an in-site experimental tool to assess children’s sprinting performance was evaluated (Sutton et al., 2000), and, finally the reliability of measuring performance in a homogeneous athletic group was
also considered (Tong et al., 2001). In spite of this renewed attention, and despite the general consensus that substantial periods of habituation should be considered before valid data can be obtained (Gamble et al., 1988; Falk et al., 1996; Greenhaff and Nevill, 1999; Sutton et al., 2000; Tong et al., 2001), reliability data are sparse and not well documented.

The few investigations reporting reproducibility to date are compromised by the duration of the protocol employed (< 30 s, Falk et al., 1996; Jaskolski et al., 1996; Jaskolska et al., 1999; Tong et al., 2001) and/or the statistical methods that used to examine repeatability (Cheetham, 1987; Falk et al., 1996; Jaskolski et al., 1996; Jaskolska et al., 1999). Until recently, the Pearson’s product moment correlation coefficient (r) was the method of the choice with coefficients for peak power output ranging from 0.76 to 0.94 for treadmill sprinting (Cheetham, 1987; Falk et al., 1996; Jaskolski et al., 1996; Jaskolska et al., 1999). Nonetheless, the correlation coefficient, r, is no longer regarded as an appropriate statistical method for examining agreement between variables (Bland and Altman, 1986; Nevill, 1996). According to Bland and Altman (1986), r measures the strength of the relationship between two variables and not agreement and is affected by the range of measured values in the sample (Bates et al., 1996). In addition, Nevill and Atkinson (1997) have recommended the use of the ratio limits of agreement when heteroscedasticity (when a positive relation between the absolute differences, trial 1 minus trial 2, and their mean exists between two variables) is present. The only study to date that has reported limits of agreement (using a 30 s protocol) is the paper from Sutton et al. (2000) where coefficients of repeatability for peak and mean power output were 28.4 W and 14.1 W, respectively. However, the authors do not state whether or not they checked the data for heteroscedasticity, variables such as speed were not mentioned, and their findings, which are based on children, are not validated with the criterion performance (sprint performance in the field tests).

There is currently a considerable formal debate in the literature with respect to the preferred statistical method to assess reliability (Bland and Altman, 1986; Nevill, 1996; Nevill and Atkinson, 1997; Atkinson and Nevill 1998; Mullineaux et al., 1999; Hopkins, 2000; Hopkins et al., 2001). Since current International Statistical Association opinion encourages the deployment of 95% limits of agreement and/or
ratio limits of agreement (ISO, 1994), the statistical evaluation in the reliability section of this thesis will rely on these techniques.

Even though the Wingate Anaerobic Test (WAnT) was initially invented for the non-invasive measurement of the "anaerobic" power (Bar-Or, 1987; Inbar et al., 1996), has also used as a tool for examining metabolic responses to both sprint cycling (i.e. Bogdanis et al., 1995; Bogdanis et al., 1996a) and treadmill sprinting (e.g. Nevill et al., 1989; Nevill et al., 1996a). However, surprisingly, only the study of Weinstein et al. (1998) using friction loaded cycle ergometer evaluated the reliability of metabolic measurements. Moreover, the latter investigation applied statistical techniques such as intraclass correlation coefficient (Weinstein et al., 1998), which has been criticised with regard to its appropriateness to determine repeatability (Atkinson and Nevill 1998).

Thus, it is the purpose of the present study to examine the reliability of the performance variables of a 30-s sprint by using the nonmotorised treadmill as the experimental model and employing 95 % limits of agreement (Bland and Altman, 1986) and/or where appropriate ratio limits of agreement (Nevill and Atkinson, 1997) as a statistical method. The reproducibility of the metabolic responses to a 30 s sprint as reflected by blood lactate concentrations is also evaluated.

3.18.2. METHODS

3.18.2.1. Subjects

Eight Loughborough University students (moderately trained) gave their informed consent to participate in the study after having informed about the potential risks, benefits and that they could withdraw from the study without giving explanation. Their age, stature and body mass were (mean ± SD), 20.6 ± 1.8 y, 1.7 ± 0.0 m and 65.9 ± 6.2 kg, respectively. All the volunteers but one (recreational runner) were games players who participated in their University teams. This study was approved by the Loughborough University Ethical Committee. The rest of the procedures described in section 3.2 were also followed here.
3.18.2.2. Ergometer

A comprehensive report of the ergometer has been presented in section 3.4.1.2. However, it is important here to present two modifications which arose from the experience of the main experimenter during the familiarisations and a pilot study (Tsampoukos and Nevill, unpublished observations, data not shown). The first of these modifications (previously mentioned in sections 3.4.1.2) concern the electric motor coupled permanently with the sprint treadmill to "warm-up" the sprint treadmill just before the experiment. The second modification concerns the harness passed around the subjects' waist securing the volunteers to the crossbar without restricting the movements of the limbs. When the original harness belt (Lakomy, 1987) was used, it tended to slip down during the sprint. Thus, another harness belt which was lightweight, cushioned and fully adjustable was used to prevent the belt from slipping down during the test.

3.18.2.3. Familiarisation

It was important to fully familiarise the subjects. Thus, volunteers were undertaken thorough the practise sessions as described in section 3.3.1.

3.18.2.4. Protocol and experimental design

The protocol as detailed in section 3.5.1 was employed here. In brief, a standardised warm-up consisting of 3 min jogging at 2 m·s\(^{-1}\) followed by 5 min stretching and two 30-s sub-maximal runs at 3.0 m·s\(^{-1}\) and 3.5 m·s\(^{-1}\), respectively, interspersed with a 30-s rest preceded each sprint. Five minutes after the completion of the standardised warm-up a 30-s "flat out" sprint from a rolling start (\(\approx 2\) m·s\(^{-1}\)) was performed on the "GO" command. Subjects were instructed to sprint maximally from start of the test and received strong verbal encouragement through the sprint. At the end of the sprint individuals were asked to lie down in a semi-reclined position on the experimental couch, so that blood samples could be drawn. Blood samples were taken at rest, post warm-up and at 1, 5 and 10 min post sprint. Twenty µL of capillary blood were drawn in duplicate from a pre-warmed thumb into disposable micropipettes. The blood was then deproteinised immediately with 2.5% of perchloric acid and stored at -20 °C for later enzymatic analysis (Maughan, 1982). The time plan of the familiarisations and
the main trials is shown in Figure 3.8. The protocol for the main trials has been previously described.

<table>
<thead>
<tr>
<th>WEEK</th>
<th>MON</th>
<th>TUE</th>
<th>WED</th>
<th>THU</th>
<th>FRI</th>
<th>SAT</th>
<th>SUN</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F1</td>
<td>F2</td>
<td>F3</td>
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<td></td>
<td>F5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>TRIAL 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>TRIAL 2</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Figure 3.8. Time plan for the present experimental design. Where F indicates familiarisations as described in paragraph 3.3.1.

Posture, time of the day, similar diet and tiredness were standardised for the second trial as mentioned in section 3.5.1.2. Volunteers reported to the laboratory after refraining for at least 4 h from food and 12 h and 24 h from caffeine and alcohol, respectively.

3.18.2.5. Statistical analysis.
Agreement between variables was initially assessed with the use of the 95% limits of agreement (Bland and Altman, 1986). However, when heteroscedasticity was present the ratio limits of agreement were employed (Nevill and Atkinson, 1997). The presence of heteroscedasticity necessitates the logarithmic transformation of the data (Nevill and Atkinson, 1997) so that a normal distribution is achieved and the data are described in a more meaningful manner (Bland and Altman, 1996; Nevill, 1997; Nevill and Atkinson, 1997). Nevertheless, since the sample size is very small a normal distribution can be assumed (Thomas and Nelson, 1990) even though a normal distribution was not observed after transformation. Normality was checked visually with a blom plot using SPSS. The presence of learning effects was evaluated by means of a paired t-test (Atkinson and Nevill, 1998; Hopkins, 2000). The sample sizes required to detect a 5-10 % difference in performance profile between trials were estimated with the aid of the nomogram introduced by Atkinson et al. (1999). Finally,
to facilitate comparison with other published studies the Pearson moment product correlation coefficient was also calculated.

For the blood lactate concentration each time point was compared separately in order to facilitate the use of the limits of agreement method.

Nonetheless, when heteroscedasticity was observed in the data the coefficient of variation (CV) was used as previously recommended (Atkinson and Nevill, 1998) and calculated according to Atkinson (2003) [APPENDIX Y]. Results are presented as mean ± SD unless otherwise stated. Significance was set at 5% level.

3.18.3. RESULTS

3.18.3.1. Performance variables

Mean ± SD for PPO, MPO, peak speed, mean speed, fatigue index for power and fatigue index for speed are presented in table 3.5. At a first glance the values seem very close and with very similar means. All but one of the variables fatigue index for power appeared to be heteroscedastic as indicated from their positive (not necessarily significant) correlation coefficient between the absolute differences and the mean (table 3.5). After log transforming the remaining variables and calculating the ratio limits of agreement (table 3.5), the data seem to be still highly reproducible as indicated by the narrow ratios and the estimated sample size estimation (table 3.5) of the need of less than 7 subjects for detecting a 10 % in means across trials (statistical power: 0.9).
Table 3.5. Performance data for a single 30-s treadmill sprint (mean ± SD, n = 8). Statistical analyses refer to paired t-test, 95 % limits of agreement (absolute limits), correlation coefficient between trial 1 and trial 2 (r), correlation coefficient between the absolute differences and the mean (R1), Ratio limits of agreement (ratio limits), coefficient of variation (CV), correlation coefficient between the absolute differences and the mean (R2) after the log transformation, and, sample size estimation for a future study involving test-treatment-test design. (10 % change, statistical power: 0.90). Where FIPO = fatigue index for power; FIsp = fatigue index for speed.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>t-test</th>
<th>Absolute limits</th>
<th>R1</th>
<th>r</th>
<th>Ratio limits</th>
<th>CV (%)</th>
<th>R2</th>
<th>Sample size estimation</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPO (W)</td>
<td>±427 ± 35</td>
<td>±422 ± 39</td>
<td>t = 0.511</td>
<td>5</td>
<td>-0.20</td>
<td>0.84</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>MPO (W)</td>
<td>±276 ± 29</td>
<td>±273 ± 27</td>
<td>t = 0.299</td>
<td>3</td>
<td>0.30</td>
<td>0.96</td>
<td>1.01%±1.06</td>
<td>2.2</td>
<td>0.25</td>
<td>3</td>
</tr>
<tr>
<td>FIPO (%)</td>
<td>±54 ± 7</td>
<td>±58 ± 8</td>
<td>t = 0.524</td>
<td>-3</td>
<td>-0.10</td>
<td>-0.50</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Peak speed (m·s⁻¹)</td>
<td>±5.46 ± 0.32</td>
<td>±5.42 ± 0.29</td>
<td>t = 0.284</td>
<td>0.04</td>
<td>0.21</td>
<td>0.94</td>
<td>1.01%±1.04</td>
<td>1.4</td>
<td>0.21</td>
<td>3</td>
</tr>
<tr>
<td>Mean speed (m·s⁻¹)</td>
<td>±4.61 ± 0.35</td>
<td>±4.61 ± 0.34</td>
<td>t = 0.771</td>
<td>0.01</td>
<td>0.13</td>
<td>0.99</td>
<td>1.01%±1.02</td>
<td>0.8</td>
<td>0.13</td>
<td>3</td>
</tr>
<tr>
<td>Flsp (%)</td>
<td>±29 ± 5</td>
<td>±29 ± 4</td>
<td>t = 0.868</td>
<td>0.4</td>
<td>0.03</td>
<td>0.08</td>
<td>1.01%±1.63</td>
<td>16.7</td>
<td>0.01</td>
<td>80</td>
</tr>
</tbody>
</table>

Figure 3.9. A Bland and Altman plot for PPO (n = 8).
In contrast, for fatigue index for speed even though the means were very similar for the two main trials (29 ± 5 % vs 29 ± 4 %) there was wide range of ratio limits, and the prerequisite for about 80 participants to detect a 5 % change in the mean. In line with this trend, fatigue index for power was also highly variable with absolute limits of agreement of −3.2 ± 13.40 %. Thus, if a subject had obtained a fatigue index for power of 54 % in the first trial, fatigue index for power might be (worst case scenario) as “low” as 29 % or as “high” as 81 % in the second trial. Obviously, these fluctuations in a performance variable cannot be considered acceptable in sports science research.

![Graph](image.png)

Figure 3.10. The histogram of the ratios for the MPO.

Finally, none of the variables were influenced by statistical bias as reflected by the paired t-test results (all values $P > 0.05$, see table 3.5). An example of a Bland and Altman plot (for PPO) and histogram of the ratios (MPO) are illustrated in figures 3.9 and 3.10, respectively.

**3.18.3.2. Blood lactate**

Table 3.6 summarises the results for blood lactate responses. These values are relatively more inconsistent in comparison with the mechanical data. Again the means and standard deviations were similar for the two main trials and although a more variable profile was revealed as the wider ratio limits of agreement implicated
(heteroscedasticity present in all but one occasions, see table 3.6 for details) all the individual ratios (trial 1+trial 2) were contained within the range specified from the ratio limits of agreement denoting good reliability.

Blood lactate recovery [% return to pre-exercise (resting) values from 5' post-sprint (average highest value)] was slightly less variable (ratio limits of agreement of 1.05*/±1.16, table 3.6) and the estimated sample size to detect 10 % difference between trials was less than 10 subjects. Lastly, no statistical bias appeared in the metabolic data, which is in concordance with the performance profile.

Finally, statistical analysis did not reveal any significant relationship between blood lactate responses and the mechanical data. The only significant positive correlation was between the blood lactate recovery in the second trial and mean speed ($r = 0.72$, $P < 0.05$).

Table 3.6. Blood lactate concentrations at rest, post warm-up and during 10 min recovery from a single 30-s treadmill sprint (mean ± SD, $n = 8$). Statistical analyses refer to paired t-test, 95 % limits of agreement (absolute limits), correlation coefficient between trial 1 and trial 2 ($r$), correlation coefficient between the absolute differences and the mean (R1), Ratio limits of agreement (ratio limits), coefficient of variation (CV), correlation coefficient between the absolute differences and the mean (R2) after the log transformation, and, sample size estimation for a future study involving test-treatment-test design. (10 % change, statistical power: 0.90).

<table>
<thead>
<tr>
<th>Variables</th>
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<th>Mean ± SD</th>
<th>t-test</th>
<th>Absolute limits</th>
<th>R1</th>
<th>r</th>
<th>Ratio limits</th>
<th>CV</th>
<th>R2</th>
<th>Sample size estimation</th>
</tr>
</thead>
<tbody>
<tr>
<td>REST</td>
<td>Trial 1</td>
<td>0.7 ± 0.8</td>
<td>0.829</td>
<td>0.02 ± 0.49</td>
<td>0.4</td>
<td>0.85</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>POST WARM-UP</td>
<td>Trial 2</td>
<td>0.2 ± 0.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>POST SPRINT 1</td>
<td>3.1 ± 3.1</td>
<td>1.2 ± 1.5</td>
<td>t = 0.941</td>
<td>-0.03 ± 0.54</td>
<td>0.94</td>
<td>0.76</td>
<td>0.92*±1.94</td>
<td>39.6</td>
<td>0.17</td>
<td>200</td>
</tr>
<tr>
<td>POST SPRINT 5</td>
<td>10.8 ± 10.3</td>
<td>1.7 ± 1.8</td>
<td>t = 0.325</td>
<td>0.4 ± 0.75</td>
<td>0.75</td>
<td>0.75</td>
<td>1.03*±1.24</td>
<td>11.8</td>
<td>-0.31</td>
<td>15</td>
</tr>
<tr>
<td>POST SPRINT 10</td>
<td>11.4 ± 11.1</td>
<td>2.0 ± 2.1</td>
<td>t = 0.531</td>
<td>0.3 ± 0.69</td>
<td>0.74</td>
<td>0.74</td>
<td>1.02*±1.29</td>
<td>12.5</td>
<td>0.05</td>
<td>16</td>
</tr>
<tr>
<td>POST SPRINT</td>
<td>10.9 ± 10.2</td>
<td>2.4 ± 2.2</td>
<td>t = 0.201</td>
<td>0.7 ± 0.42</td>
<td>0.61</td>
<td>0.61</td>
<td>1.06*±1.30</td>
<td>14.2</td>
<td>0.07</td>
<td>17</td>
</tr>
</tbody>
</table>
3.18.4. DISCUSSION

The main aim of the present study was to evaluate the reliability of the performance data during a single 30 s protocol using a nonmotorised treadmill as an experimental model. In addition, the reproducibility of blood lactate responses after the sprint and during the recovery was also appraised. Statistical analysis revealed that the majority of the variables introduced minimal measurement error and thus suggest that the experimental model adopted is a reliable tool for assessing performance and metabolic responses of maximal intensity exercise.

Overall, the present results are in agreement with published investigations in the field (Nevill et al., 1996a). PPO of 425 W (average of the two trials), MPO of 275 W and fatigue index for power of 56 % in the present study are consistent with the performance results of Nevill et al. (1996a), who reported values of 419-619 W, 277-381 W and 55-59 % for PPO, MPO and fatigue index for power, respectively. Blood lactate responses were also in line with Nevill et al. (1996a) as reflected by the blood lactate concentrations of ≈ 11 mmol·L⁻¹ in the present study and the range of values from 11 to 16 mmol·L⁻¹ in the study of Nevill et al. (1996a).

The purpose of the present study was to examine reliability. The results suggest that PPO and MPO are very stable performance indices with the ratios (MPO) between the mean of the two trials from all the participants falling within the range indicated by the ratio limits of agreement (1.08*±0.95 for MPO, figure 3.10). For PPO there was only a 5 W difference (427 W vs 422 W). Similar observations have been reported by Cheetham (1987) with only a 6 W difference between trial 1 and 2 (648 W vs 642 W) and a Pearson correlation coefficient of 0.91. The slightly higher correlation coefficient in the earlier study (as compared with 0.84 in this study) can be attributed to the wider range of the values in the earlier study (Bates et al., 1996). Comparable correlation coefficients were also given by other research groups ranging from 0.76 to 0.94 (Falk et al., 1996; Jaskolski et al., 1996; Jaskolska et al., 1999). The only other study to have used limits of agreement (Tong et al., 2001) has reported absolute limits of agreement of 105 ± 588 which are wider than those for the present experiment (5.2 ± 21). However, termination of practice sessions in Tong et al. (2001) study was permitted after the volunteers had reached a predetermined speed of 6.5 m·s⁻¹. In
contrast, in the present investigation subjects had to undertake at least 5 familiarisation sessions with the PPO in the last session (full sprint) to be similar to the highest achieved in the first 3 visits. Thus, slightly better habituation may have contributed to better agreement between trials in the present study. A more qualitative approach to the examination of the reproducibility of PPO revealed different observations, though. Considering the worst case scenario, as suggested by Nevill and Atkinson (1997), if in the first trial the PPO is 425 W (average PPO from the two tests) it is possible that the same participant could obtain PPO as low as 388 W (425 minus 37, table 3.5) or as high as 472 W (425 plus 47, table 3.5) on the second trial. Although this spread in values is much smaller in magnitude than that reported by Tong et al. (2001), these findings imply the need for caution when PPO is assessed.

On the other hand MPO appeared to be quite stable as indicated by the ratio limits of agreement (1.01*/±1.06) and the very similar mean of the two trials (276 W vs 273 W). Close values for MPO have been mentioned before with regard to a nonmotorised ergometer from Sutton et al. (2000) and Cheetham (1987) (142 W vs 144 W and 435 W vs 437 W, respectively). The correlation coefficient between MPO in trial 1 and trial 2 was very high (0.96) and compares favourably with that of Cheetham (0.97).

Studies reporting ratio limits for MPO using a 30 s protocol could not be traced in the literature. Nevertheless, one study, reported within a fuller publication by Nevill and Atkinson (1997) examined. “Wingate Mean Power” reliability and the ratio limits were 1.00*/±1.17. This range (0.85 to 1.17) is much larger than the that achieved in the present study (0.95 to 1.08) implying better reproducibility of MPO in the present study. Verification of the consistency of MPO is reflected from the figure 3.11 where all the participants’ ratios (ranging from 0.95 to 1.05) appeared to be contained between the calculated ratio limits (0.95-1.05) of agreement (Nevill and Atkinson, 1997).

The reliability of the speed data was even better than that reported for power output. Peak speed values were very similar in trial one and two whilst mean speed values appeared identical (table 3.5). Mean ratios (for peak speed) between the two trials of each individual were within the range determined from the ratio limits (1.01*/±1.04) and agree well (1.00*/±1.03) with previous findings (Tong et al., 2001). There are no previous studies in the literature examining the reproducibility of mean speed.
Nonetheless, judging from the results presented in table 3.5 (ratio limits: 1.00*/±1.02, identical means) mean speed is very reliable.

The phenomenon of the generally observed lower repeatability of PPO and peak speed relative to the corresponding MPO and mean speed in various studies is in line with the metanalytic review of Hopkins et al. (2001) and may be explained by: a) with the increase in the number of repetitions of limb movement the independent random errors in each repetition tend to be cancelled out and thus minimises the error, b) the software and/or hardware interfacing the ergometer is the computer may be less reliable in registering peaks than averaging power over a period of time. Clear-cut explanations are yet to be established.

A less consistent profile was revealed for the fatigue index for power and speed. For the latter, ratio limits of agreement were quite wide (1.01*/±1.63) and only two of the eight individuals had mean ratios that were between the calculated ratio limits (data not shown) indicating the lack of consistency of this index. On the other hand subjects' fatigue index for power did not seem to be heteroscedastic (table 3.5) since there was a negative relationship between the absolute differences (trial 1 minus trial 2) and their mean (Nevill and Atkinson, 1997). This is probably due to the small sample size. Fatigue index for power variability was less pronounced. Similar observations relative to fatigue index have been pointed out in the preliminary work of Cheetham (1987) who reported on only fatigue index for speed. Nevertheless, it should be noted that this variability was present even though the means between the two sprints were very similar for both of the indexes (table 3.5). One reason for this variability in fatigue indexes from sprint one to sprint two is the tendency for exhausted subjects to stop as soon as possible, sometimes in the 29th s of the sprint, but variable from one trial to another. This anticipation for premature termination of exercise has been previously reported with regards to brief maximal exercise (Caprioti et al., 1999). Although unfortunate, this finding is useful for later studies. Improvement could be achieved by either making the individuals run for one more second or by better emphasizing the need to run for the full 30 s.

Following familiarisations, learning effects did not influence any of the performance variables in this study as reflected by the paired t-tests results (Atkinson and Nevill,
1998; Hopkins, 2000). Thus, it can be concluded with confidence that the familiarisation schedule performed here was adequate even though time consuming (≥5). This substantial period of habituation has not been used in other studies, where 2 sessions are usually considered enough as far as maximal intensity exercise is concerned (Caprioti et al., 1999; Martin et al., 2000). However, these used a cycle ergometer as experimental model which is considered less dependent on technique than for nonmotorised treadmill (Lakomy, 1987). The longer duration of the current protocol (30 s) as comparing with the 7 s (Caprioti et al., 1999) and 3.5 s (Martin et al., 2000) may also change the number of familiarisations required. Further research is required to identify the exact number of familiarisation sessions needed before the typical error settles into its lowest values.

Another way to help interpret the above findings is through the employment of analytical goals (Atkinson and Nevill, 1998). By estimating the sample size required to detect a pre-set amount of difference for a certain dependent variable, one can judge quantitatively whether the ratio limits of agreement are narrow enough to be accepted. Use of a nomogram introduced by Atkinson et al. (1999), which is based on ratio limits of agreement suggests that for a hypothetical 10 % change in the dependent variable (statistical power = 0.9) a sample size of about 3 subjects or less appears adequate for most of the variables (MPO, peak speed, mean speed). In contrast, the estimated sample size for fatigue index for power and fatigue index for speed is more than 80 subjects. Judging from these figures MPO, peak speed and mean speed reliability can be accepted with confidence for the present thesis.

It has been claimed that sport specific ergometers are of little use to detect the small changes (1 %) that are associated with changes in performance for elite athletes (Hopkins et al., 1999; Hopkins, 2000; Tong et al., 2001). This view seems unfair and premature since these conclusions relied on experimental procedures with poor control of environmental conditions, tapering and the importance of the race and were based on statistical analysis (CV calculated differently with that of the present study or Tong’s et al that differs from the present study. Also there is a lack of experimental evidence of the reliability of the criterion performance (that is, field tests) either with elite or sub-elite performers. Even the original investigator that validated the current
ergometer (Lakomy, 1987) did not examine the reliability of sprinting in the fields. It is suggested that future experiments should address this issue by examining the reliability of the criterion performance and by comparing it with the in-site repeatability in both elite and sub-elite athletes. Moreover, analytical goals such as sample size estimation should also be included in these comparisons to finalise the decision making whether the particular ergometer can be considered of practical use with reference to sports science support.

In general, the metabolic profile as reflected by the blood lactate responses follows a more variable pattern than the performance data. Table 3.6 summarises the results of these responses. Ratio limits of agreement of 1.06*/±1.29 for 5 min post-sprint are wider than those for MPO, peak speed and mean speed (see tables 3.5 and 3.6). On the other hand recovery of blood lactate towards the resting values displayed a more consistent form with ratio limits of agreement reduced to 1.05*/±1.16. This disparity maybe due to the involvement of more data in the calculation of this index and thus some smoothing of data in the same way described for performance variables mean values. The present observations agree well with the study of Weinstein et al. (1998) where blood lactate data were also more variable than performance variables after 30 s Wingate protocol on a cycle ergometer. Their Pearson correlation coefficient of 0.86 for peak blood lactate concentrations matches favourably with the one here (0.74) and any difference could be attributed to the inherent limitations of such a coefficient as has already been discussed. It can be speculated that the less consistent profile for blood lactate concentration observed here compared with the performance figures is a consequence of the “accumulated” experimental error resulting from the extra steps to obtain the final value. Sampling technique, correct timing, standardised posture, dispensing and the use of manual assays are procedures that can definitely add some error to the final value. Nevertheless, sample size estimation for detecting a 10 % change (table 3.6) reveals that a sample of less than 20 (peak lactate values) or 8 (lactate recovery) participants are adequate implying that despite this variability lactate measurements are of practical use.

Thus, the present data contradict the opinion that males are more reliable than the females (Hopkins et al., 2001) relative to power output. The repeatability indexes here
are higher than previously reported in males (Tong et al., 2001) and despite the small sample size (n = 8) correlation coefficient of 0.84 and 0.96 (for PPO and MPO, respectively) is in line with previous reports using similar protocol (Cheetham, 1987; Falk et al., 1996; Jaskolski et al., 1996; Jaskolska et al., 1999). Therefore, the trend in the literature that women are less reliable than their males' counterparts for both performance (Hopkins et al., 2001) and metabolic data (Weinstein et al., 1998) are probably misleading and the result of the inherent limitations of the statistical techniques employed. Statistical methods such as limits of agreement that are not influenced by the range and magnitude of the values may be of great importance to elucidate these issues in future research.

In summary, the present study verified, with more appropriate statistical methods than those previously published, that the majority of the performance data is reproducible for a 30 s protocol and sprint treadmill as the experimental model. Further research is required to elucidate the underlying mechanisms of the general lower repeatability of peak as opposed to mean values and to establish the reproducibility of the criterion performance (e.g. sprinting in the field).
3.19. METHODOLOGICAL STUDY 2: EFFECTS OF DIFFERENT SAMPLING SITES AND ANALYSES ON BLOOD LACTATE CONCENTRATION

3.19.1. INTRODUCTION

Lactate occupies a key position in the pathways of carbohydrate metabolism (Brooks et al., 2000) and provides direct (muscle lactate) or indirect (blood lactate) information concerning the anaerobic energy supply of ATP via glycolysis. Blood lactate concentration has also been suggested to be a reliable metabolic response for monitoring and optimisation of training in both athletes (Jacobs, 1986; Weltman, 1995) and in patients with coronary heart disease (Coyle et al., 1983). Moreover, blood lactate can be used to assess tissue hypoxia in critically ill children (Murdoch et al., 1994). In terms of maximal intensity exercise, blood lactate is the most common metabolite measured (Cheetham et al., 1986; Nevill et al., 1989; Bogdanis et al., 1996a) since it is an important indicator of anaerobic metabolism (Spriet, 1995) and can be also used for evaluating sprinting performance (Ohkawa et al., 1984; Jacobs, 1986).

In spite of this tremendous interest in the measurement of blood lactate concentration, blood lactate analysis was, until the 1980s, cumbersome, time consuming, technically demanding and required a large amount of blood. Thus, technical limitations excluded blood lactate measurements during field testing and multiple measurements during in-site investigations. However, a methodological breakthrough was made in the early eighties with the publication of an enzymatic method that requires a volume of only 20 μL of blood obtained by means of fingertip samples (Maughan, 1982).

Nonetheless, even with the enzymatic methods the inherent drawbacks of the pre-treatment of the samples, the technical skills and the length of a manual assay were still present. The introduction of the automatic analysers such as Yellow Spring Instruments (YSI) eliminated the above disadvantages and provided an attractive alternative. YSI have been widely used in conjunction with both haemolysed (Foxdal et al., 1990; Bishop et al., 1992b; Thin et al., 1999; McCaughan et al., 1999) and not
haemolysed whole blood \cite{Weil1986, Wandrup1989, Bishop1992}. These methodological advances would be of no practical use if the sampling site required a catheter (for arterial blood) or cannula (for venous blood) to be placed for blood sampling. Both of these procedures demand well-trained personnel and there are associated risks for the subject \cite{ExerciseRisksAndDiscomforts}. However, Maughan \cite{Maughan1982}, as mentioned before, suggested a method whereby 20 µL of blood can be collected in disposable glass capillary tubes directly from the finger of a pre-warmed hand. Thus, it is possible to employ a less invasive technique which does not necessitate great experience \cite{Jacobs1986}. Nevertheless, investigations in the literature that examine blood lactate responses to maximal exercise have collected blood from different sampling sites such as finger capillary \cite{Ohkuwa1984}, arteries \cite{Oyono-Enguelle1989} and veins \cite{Nevill1996a}. Furthermore, lactate has been determined in different portions of blood such as plasma \cite{Ahmaidi1996} and whole blood \cite{Nevill1996a}.

Given the variety of sampling sites, analytical methods and different portions of blood analysed in previous studies examining recovery from maximal intensity exercise, and, the fact that lactate concentration can change according to the method used \cite{Foxdal1990}, it is surprising that no direct comparisons of methods with special reference to maximal intensity exercise has been undertaken. It is important to examine the different methods during maximal intensity exercise as Foxdal et al. \cite{Foxdal1990} and El-Sayed et al. \cite{El-Sayed1993} stress that their findings concerning lactate concentration in plasma, whole blood, capillary finger blood and erythrocytes (in sub-maximal exercise) may not apply in different experimental protocols. In addition, agreement between methods examining the effects of various sampling sites or analytical procedures \cite{Weil1986, Wandrup1989, Foxdal1990} have not been validated using appropriate statistical techniques such as the so-called 95% limits of agreement \cite{Bland1986} or ratio limits of agreement \cite{Nevill1997}. 

ANTONIS TSAMPOUKOS

Ph.D THESIS
Thus, it is the purpose of the present study, with special reference to maximal intensity exercise, to examine the following issues:

- Whether different sampling sites can influence lactate concentration (on this occasion, capillary finger blood versus forearm venous blood).
- If there is agreement between different analytical methods such as YSI and manual enzymatic assay (for venous whole blood lactate) and YSI and Cobas Mira Plus (for venous plasma lactate).

3.19.2. METHODS

3.19.2.1. Subjects

Eight female physical education students gave consent to participate in this investigation. Their age, stature, and body mass were $23 \pm 3$ y, $1.66 \pm 0.1$ m and $62 \pm 9$ kg, respectively. Participants were recruited as described in preliminary study 1. All participants were sportswomen participating predominantly in team sports who trained regularly with their team. The study was approved by the Loughborough University Ethical Committee. Participants were provided with details of the study both verbally and in writing, including their right to terminate participation at any point. Participants then gave consent to take part. The rest of the procedures were identical to those described in section 3.2.

3.19.2.2. Ergometer

A thorough familiarisation process as detailed in section 3.4.1 was performed here.

3.19.2.3. Familiarisation

The ergometer, a sprint treadmill was as described in general methods (section 3.3.1).

3.19.2.4. Protocol and experimental design

Figure 3.11 depicts the time plan of these experimental procedures. Subjects arrived in the laboratory after at least 4 h in the post absorptive state. Subsequently, a venous cannula was inserted in an antecubital vein and subjects relaxed in a recumbent position for 20 min whilst this instrumentation took place. After the cannula had been inserted in the vein the thumb of the same hand where the cannula was placed was
pre-warmed with hot water. Then the first blood sample was drawn from both sites (thumb and antecubital vein) simultaneously by two experimenters. A third experimenter was responsible for the dispensing of the blood and did the immediate analysis required for this experimenter as illustrated in figure 3.12. Blood samples were also taken after warm-up (-5 min) before the sprint (upright posture) and at 1, 5 and 10 min after the sprint (figure 3.1).

<table>
<thead>
<tr>
<th>WEEK</th>
<th>MON</th>
<th>TUE</th>
<th>WED</th>
<th>THU</th>
<th>FRI</th>
<th>SAT</th>
<th>SUN</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F1</td>
<td>F2</td>
<td></td>
<td>F3</td>
<td></td>
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<td></td>
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<tr>
<td>2</td>
<td>F4</td>
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<td></td>
<td>F5</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>TRIAL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 3.11. Time plan for the present experimental design. Where F indicates familiarisations as described in paragraph 3.3.1. Shaded areas denote days that do not require the presence of the participants in the lab.

3.19.2.5. Collection, treatment and storage of the blood samples.
A detailed description of the collection and treatment of the venous and capillary blood, the operation of the YSI (Model 2300 STAT PLUS) and Cobas Mira Plus automatic analysers (Roche Diagnostics System, Basel, Switzerland) was provided in section 3.13.3.

From the lithium-heparin coated tube an aliquot of blood was aspirated using the YSI automatic analyser and the remaining blood was centrifuged separate the plasma and
erythrocytes. A plasma sample was aspirated into the YSI for the determination of plasma lactate. The remaining plasma was kept in \(-20^\circ C\) for later plasma analysis with the Cobas Mira Plus automatic analyser.

The enzymatic assay for whole blood capillary and venous lactate was performed using a fluorimeter (Locarte Model LF 8-9, Locarte, London, U.K.) as described previously (Maughan, 1982). Samples for both sites were assayed in the same run and the order of analysis of venous and capillary whole blood was randomised. The YSI analyser was operated within 18 min of collection for each whole blood sample.

Figure 3.12. A flow diagram that portrays the treatment of the samples.
(Williams et al., 1992). The YSI analyser used in the present study measured the total (haemolysed, that is intracellular and extracellular using cell-lysing agent) concentration of lactate. Analysis of plasma lactate in Cobas Mira Plus automatic analyser was performed according to manufacturer’s guidelines using a commercially available kit (Boehringer Manheim, GmbH). Table 3.7 demonstrates an overview of the methods employed.

Table 3.7. The different analytical methods, sampling sites and blood portions utilised are tabulated below.

<table>
<thead>
<tr>
<th>ANALYTICAL METHODS UTILISED</th>
<th>WHOLE BLOOD LACTATE</th>
<th>PLASMA LACTATE</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAMPLING SITE</td>
<td>FINGER CAPILLARY</td>
<td>VENOUS WHOLE BLOOD</td>
</tr>
<tr>
<td>BLOOD LACTATE DISTRIBUTION</td>
<td>PLASMA</td>
<td>WHOLE BLOOD</td>
</tr>
</tbody>
</table>

For all the methods used quality controls and/or standards were used according to the guidelines of each method (for more details see paragraph 3.13.3). Finally, interassay variability of the methods examined is shown at table 3.8.

Table 3.8. Between assay means and coefficient of variations for the methods utilised (Asterisks indicate that these data are from Bruce et al. unpublished observations).

<table>
<thead>
<tr>
<th>METHOD</th>
<th>LACTATE CONCENTRATION (mmol L⁻¹)</th>
<th>CV (COEFFICIENT OF VARIATION)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MANUAL ENZYMATIC</td>
<td>2.4</td>
<td>3.1 %</td>
</tr>
<tr>
<td>YSI ANALYSER (WHOLE BLOOD)</td>
<td>1.3</td>
<td>2.2 %</td>
</tr>
<tr>
<td>YSI ANALYSER (PLASMA-SERUM BASED QC)*</td>
<td>1.3</td>
<td>2.2 %</td>
</tr>
<tr>
<td>COBAS MIRA PLUS (PLASMA LACTATE)*</td>
<td>4.6</td>
<td>2.0 %</td>
</tr>
</tbody>
</table>

3.19.2.5. Statistical analysis

Agreement between variables of each of the two analytical methods or sampling sites was assessed with the 95 % limits of agreement method (Bland and Altman, 1986).
However, when a positive relationship was found between the absolute differences (trial 1 minus trial 2) and their mean (that is, presence of heteroscedasticity) the ratio limits of agreement was the method of the choice (Nevill and Atkinson, 1997). In order to apply either of these two methods the differences over time during the protocol were not taken into consideration. In the presence of heteroscedasticity the coefficient of variation (CV) was calculated (Atkinson, 2003) [APPENDIX Y]. Paired t-tests were used to assess quantitative differences between certain sampling points (Field, 2000). Relationships between variables were determined by Pearson Product Moment Correlation coefficient. Significance was accepted at 5 % level. Results are presented as mean ± SD unless otherwise stated.

3.19.3. RESULTS

3.19.3.1. Performance variables

Participants’ performances were 452 ± 73 W, 300 ± 38 W and 56 ± 18 for PPO, MPO and fatigue index for power, respectively.

3.19.3.2. Venous lactate vs capillary lactate

Figure 3.13 portrays the time course of venous and capillary whole blood lactate and table 3.9 summarises the results. Capillary lactate was higher than venous lactate at all sampling points \( P < 0.05 \) apart from the resting values and 5 min post-sprint \( P = 0.086 \).

![Lactate Concentration Chart](chart.png)

**Figure 3.13.** Time course of venous and capillary whole blood lactate concentration at rest, post warm-up (PWP) and at 1, 5 and 10 min post-sprint (1'PS, 5'PS and 10'PS) following a single 30 s treadmill sprint (mean ± SD, \( n = 8 \)). Where \( ^a = \text{main effect: method, } P < 0.05; ^b = \text{main effect: time, } P < 0.01 \).
Table 3.9. Venous (VL) vs Capillary (CL) lactate (mean ± SD, n = 8). Statistical analyses refer to paired t-test, 95 % limits of agreement (absolute limits), correlation coefficient between trial 1 and trial 2 (r), correlation coefficient between the absolute differences and the mean (R1), Ratio limits of agreement (ratio limits), coefficient of variation (CV), correlation coefficient between the absolute differences and the mean (R2) after the log transformation, and, sample size estimation for a future study involving test-treatment-test design. (10 % change, statistical power: 0.90).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean ± SD</th>
<th>Mean ± SD</th>
<th>t-test</th>
<th>Absolute limits</th>
<th>R1</th>
<th>r</th>
<th>Ratio limits</th>
<th>CV</th>
<th>R2</th>
<th>Sample size estimation</th>
</tr>
</thead>
<tbody>
<tr>
<td>REST</td>
<td>0.7 ± 0.2</td>
<td>0.8 ± 0.2</td>
<td>P &gt; 0.05</td>
<td>-0.0 ± 0.3</td>
<td>-0.42</td>
<td>0.71</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>POST</td>
<td>3.3 ± 1.2</td>
<td>4.3 ± 1.5</td>
<td>P &lt; 0.05</td>
<td>-1.1 ± 0.9</td>
<td>-0.69</td>
<td>0.97</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>WARM-UP</td>
<td>10.2 ± 11.1</td>
<td>11.1 ± 1.5</td>
<td>P &lt; 0.05</td>
<td>-1.0 ± 2.1</td>
<td>-0.03</td>
<td>0.82</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>1st POST-SPRINT</td>
<td>1.7 ± 0.2</td>
<td>1.8 ± 0.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5th POST-SPRINT</td>
<td>12.0 ± 2.0</td>
<td>12.7 ± 2.1</td>
<td>P = 0.084</td>
<td>-0.7 ± 2.0</td>
<td>-0.15</td>
<td>0.88</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>10th POST-SPRINT</td>
<td>11.2 ± 2.4</td>
<td>12.2 ± 2.2</td>
<td>P &lt; 0.05</td>
<td>-0.9 ± 1.3</td>
<td>0.21</td>
<td>0.96</td>
<td>0.92* / 1.13</td>
<td>3.9</td>
<td>0.07</td>
<td>n = 3</td>
</tr>
</tbody>
</table>

Taking the worst case scenario for the peak values (5 min post-sprint) it can be speculated that if one participant's venous lactate was 12.4 mmol·L⁻¹ her capillary lactate could be as high as 13.77 mmol·L⁻¹. A diagrammatic example of the peak lactate (venous vs capillary) employing 95 % limits of agreement is depicted in figure 3.14.

Figure 3.14. A Bland and Altman plot for venous vs capillary lactate (n = 8).
3.19.3.3. Venous lactate vs YSI venous lactate

Blood lactate concentration in venous whole blood analysed enzymatically and with the YSI is shown in figure 3.15 and table 3.10. There was a consistent profile for lower lactate concentrations at all sampling points \( P < 0.05 \) with the manual enzymatic analysis, with the difference being greatest at 5 min post-sprint. Nevertheless, the ratios of the two methods employed (venous lactate divided by the YSI) were within the range (data not shown) dictated from the agreement ratio \( (\% / 1.13, \text{table 3.10}) \) indicating acceptable agreement between the methods. Employing the nomogram of Atkinson et al. (1999) it appears that only about 3 subjects are required to detect a difference of 10 % (table 3.10).

![Figure 3.15](image)

Figure 3.15. Time course of venous and capillary whole blood lactate concentration (mean ± SD, \( n = 7 \)) analysed enzymatically (VL) and with YSI (YSI-VL) at rest, post warm-up (PWP) and at 1, 5 and 10 min post-sprint (1’PS, 5’PS and 10’PS). Where "\( a \)" = main effect: method, \( P < 0.05 \); "\( b \)" = main effect: time, \( P < 0.01 \).
Table 3.10. Venous blood lactate analysed enzymatically (VL) vs YSI venous (YSI-L) blood lactate at rest, post warm-up and following a single 30-s treadmill sprint (mean ± SD, n = 7). Statistical analyses refer to paired t-test, 95 % limits of agreement (absolute limits), correlation coefficient between trial 1 and trial 2 (r), correlation coefficient between the absolute differences and the mean (R1), Ratio limits of agreement (ratio limits), coefficient of variation (CV), correlation coefficient between the absolute differences and the mean (R2) after the log transformation, and, sample size estimation for a future study involving test-treatment-test design. (10 % change, statistical power: 0.90).

<table>
<thead>
<tr>
<th>Variables</th>
<th>YSI-L</th>
<th>VL</th>
<th>t-test</th>
<th>Absolute limits</th>
<th>R1</th>
<th>r</th>
<th>Ratio limits</th>
<th>CV</th>
<th>R2</th>
<th>Sample size estimation</th>
</tr>
</thead>
<tbody>
<tr>
<td>REST</td>
<td>0.8 ± 0.2</td>
<td>0.7 ± 0.2</td>
<td>P &lt; 0.05</td>
<td>0.1 ± 0.2</td>
<td>0.73</td>
<td>0.93</td>
<td>1.14±1.25</td>
<td>9.2</td>
<td>0.08</td>
<td>≈3</td>
</tr>
<tr>
<td>POST WARM-UP</td>
<td>3.8 ± 1.4</td>
<td>3.3 ± 1.3</td>
<td>P &lt; 0.01</td>
<td>0.4 ± 0.2</td>
<td>0.64</td>
<td>1.00</td>
<td>1.13±1.09</td>
<td>2.3</td>
<td>-0.41</td>
<td>≈2</td>
</tr>
<tr>
<td>1' POST-SPRINT</td>
<td>10.7 ± 9.7</td>
<td>9.7 ± 1.2</td>
<td>P &lt; 0.01</td>
<td>1.2 ± 1.2</td>
<td>-0.44</td>
<td>0.88</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>5' POST-SPRINT</td>
<td>12.9 ± 11.5</td>
<td>11.5 ± 1.5</td>
<td>P &lt; 0.01</td>
<td>1.4 ± 1.3</td>
<td>0.08</td>
<td>0.90</td>
<td>1.13±1.13</td>
<td>3.9</td>
<td>0.54</td>
<td>≈3</td>
</tr>
<tr>
<td>10' POST-SPRINT</td>
<td>12.0 ± 10.7</td>
<td>10.7 ± 2.0</td>
<td>P &lt; 0.05</td>
<td>1.3 ± 2.0</td>
<td>-0.35</td>
<td>0.86</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

3.19.3.4.YSI plasma lactate vs Cobas Mira Plus plasma lactate.
Plasma lactate concentration determined by both the YSI and Cobas Mira Plus analysers is illustrated in figure 3.16. There is almost an identical profile between the analysers. Table 3.11 demonstrates the numerical values. It can be seen that the two analysers have very similar plasma lactate concentrations and no statistical bias (with paired t-test) observed.
Figure 3.16. Time course of plasma lactate with two automatic analysers, YSI (PL-YSI) and Cobas Mira Plus (PL-COBAS) at rest, post warm-up (PWP) and at 1, 5 and 10 min post-sprint (1'PS, 5'PS and 10'PS) (mean ± SD, n = 5) at rest following a single 30 s treadmill sprint. Where \( ^{b} \) = main effect; time, \( P < 0.01 \).

Table 3.11. YSI plasma (YSI-PL) vs Cobas Mira Plus plasma (COBAS-PL) lactate at rest, post warm-up and following a single 30-s treadmill sprint (mean ± SD, n = 5). Statistical analyses refer to paired t-test, 95 % limits of agreement (absolute limits), correlation coefficient between trial 1 and trial 2 (r), correlation coefficient between the absolute differences and the mean (R1), Ratio limits of agreement (ratio limits), coefficient of variation (CV), correlation coefficient between the absolute differences and the mean (R2) after the log transformation, and, sample size estimation for a future study involving test-treatment-test design. (10 % change, statistical power: 0.90).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean ± SD</th>
<th>Mean ± SD</th>
<th>t-test</th>
<th>Absolute limits</th>
<th>R1</th>
<th>r</th>
<th>Ratio limits</th>
<th>CV</th>
<th>R2</th>
<th>Sample size estimation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>YSI-PL</td>
<td>COBAS-PL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>REST</td>
<td>1.2 ± 0.2</td>
<td>1.2 ± 0.3</td>
<td>( P &gt; 0.05 )</td>
<td>0.0 ± 0.4</td>
<td>-0.45</td>
<td>0.74</td>
<td>N/A</td>
<td></td>
<td></td>
<td>0.08</td>
</tr>
<tr>
<td>POST WARM-UP</td>
<td>4.9 ± 2.0</td>
<td>5.1 ± 0.3</td>
<td>( P &gt; 0.05 )</td>
<td>-0.2 ± 0.4</td>
<td>-0.94</td>
<td>1.00</td>
<td>N/A</td>
<td></td>
<td></td>
<td>N/A</td>
</tr>
<tr>
<td>1' POST SPRINT</td>
<td>14.8 ± 1.1</td>
<td>14.1 ± 1.1</td>
<td>( P &gt; 0.01 )</td>
<td>0.8 ± 1.9</td>
<td>0.35</td>
<td>0.74</td>
<td>1.05*/+1.14</td>
<td>4.6</td>
<td>N/A</td>
<td>n=3</td>
</tr>
<tr>
<td>5' POST SPRINT</td>
<td>16.9 ± 1.3</td>
<td>16.4 ± 0.3</td>
<td>( P &gt; 0.05 )</td>
<td>0.4 ± 1.3</td>
<td>-0.41</td>
<td>0.96</td>
<td>N/A</td>
<td></td>
<td></td>
<td>0.54</td>
</tr>
<tr>
<td>10' POST SPRINT</td>
<td>15.4 ± 2.3</td>
<td>15.3 ± 2.8</td>
<td>( P &gt; 0.05 )</td>
<td>0.1 ± 1.4</td>
<td>-0.62</td>
<td>0.98</td>
<td>N/A</td>
<td></td>
<td></td>
<td>N/A</td>
</tr>
</tbody>
</table>

3.19.4. DISCUSSION

The key findings of the present study were that during recovery from maximal exercise capillary blood lactate concentrations were higher than venous blood lactate...
concentrations, that venous blood samples analysed enzymatically gave a lower blood lactate concentration than the same samples analysed using a YSI analyser, but the plasma lactate concentrations were similar using the Cobas Mira and YSI methods.

In general, the performance data for subjects in the present study are in accordance with those of methodological study 1 and published research (Nevill et al., 1996a). The PPO and MPO of 452 W and 300 W in this study are in agreement with those of 425 W (PPO) and 275 W (MPO) from preliminary study 1 or those of 419-619 W (PPO) and 277-381 W (MPO) from Nevill et al. (1996a). Similarly, lactate responses (capillary lactate) are in line with those in the preliminary study 1 of the present thesis (11.3 mmol·L\(^{-1}\) vs 12.7 mmol·L\(^{-1}\), previous and present study, respectively).

**Venous lactate versus capillary lactate.** Capillary blood lactate concentration was consistently higher than venous blood lactate concentration in the present study. For example capillary blood lactate at 5\(^{th}\) min into recovery (peak values) was 0.7 mmol·L\(^{-1}\) higher than the blood lactate concentration of venous blood and the 95 % limits of agreement for this value were \(-0.7 \pm 2.0\) with no heteroscedasticity present (table 3.9, figure 3.14). To help interpret these values it can be speculated (worst case scenario) that one participant’s peak lactate could be 12 mmol·L\(^{-1}\) if the sampling site was an arm vein and 13.3 mmol·L\(^{-1}\) if the sampling site was capillary blood from the thumb. Obviously, this range in values is too wide to be accepted by any researcher in the field of high-intensity exercise. In addition, the lactate differences between the two methods are greater than the arbitrary (not scientific evaluated) value of 0.5 mmol·L\(^{-1}\) (Dassonville et al., 1998; personal communication with colleagues at Loughborough University).

Overall, the present disagreement between the two sampling sites is in accordance with research in the area conducted either on a treadmill or on a cycle ergometer for sub-maximal exercise (Di Vico et al., 1989; Godsen et al., 1989; Robergs et al., 1990; Foxdal et al., 1990; Foxdal et al., 1991; Graeter et al., 1991; El-Sayed et al., 1993a; El-Sayed et al., 1993b; Dassonville et al., 1998). A report from Williams et al. (1992) is in conflict with this general trend, though, claiming that no significant differences existed between these two sampling sites. These discrepancies are more probably due
CHAPTER 3 GENERAL METHODS

to technical difficulties encountered as the authors highlight. Two other studies also report no alterations in lactate concentrations (Busse et al., 1984; Ferry et al., 1988) due to sampling site, but the lack of difference in these studies may be due to earlobe sampling (Feliu et al., 1999), or to the exercise protocol used (Busse et al., 1984) or due to inappropriate statistical techniques. The latter factor applies to all studies to date and the present study is the first to use the Bland and Altman method for examining whether or not there are differences in lactate concentration with sampling site and different analytical methods. Table 3.12 provides an overview of the studies that examined capillary in comparison venous whole blood lactate.

It is difficult to explain why capillary blood lactate concentration in the thumb is higher than venous blood lactate concentration in the arm. It has long been established that lactate concentrations are higher in the (venous) blood leaving the muscles than in the (arterial) blood arriving at the muscles (Jorfeldt et al., 1978). Assuming that capillary blood from the thumb is representative of arterial blood (Maughan et al., 2001), then, the lactate concentrations in capillary tissue give an average of whole body lactate concentration at a point in time (Oyonno-Enguelle et al., 1989). However, the results of the present study and others (Di Vico et al., 1989; Foxdal et al., 1990; El-Sayed et al., 1993a; El-Sayed et al., 1993b; Dassonville et al., 1998) clearly show that capillary lactate is higher than venous lactate. This gradient has been claimed to be a consequence of lactate uptake from the inactive arm muscle (Foxdal et al., 1990), a view that is suggested by experiments that directly compare arterial and venous lactate (De Coster et al., 1969; Yoshida et al., 1982a; Yeh et al., 1983; Oyonno-Enguelle et al., 1989) and demonstrated by Poortmans and colleagues (1978). This shift from lactate release to lactate uptake occurs when blood lactate reaches a certain level (Ahlborg et al., 1975). In terms of whole body exercise the agreement of the two sampling sites has only been examined to a limited extent (El-Sayed et al., 1993a; El-Sayed et al., 1993b Dassonville et al., 1998). However, exercising muscles in the arm will produce lower lactate in comparison with the average lactate of the whole body compartment due to smaller muscle mass (Righter et al., 1988; Van Hall et al., 2003). In addition, active muscle oxidises lactate to a greater extent than inactive muscle (Poortmans et al., 1978; Van Hall et al., 2003). It has also been suggested that during high intensity exercise, removal and metabolism
of lactate in the forearm muscle bed increases disproportionately to lactate delivery to the arm \((\text{El-Sayed et al., 1993a})\). This is

Table 3.12. Studies that examined finger (FC) or ear-lobe (ELC) capillary lactate vs venous (V). Where IT = incremental test.

<table>
<thead>
<tr>
<th>STUDY</th>
<th>n</th>
<th>TYPE OF EXERCISE</th>
<th>SAMPLING POINT</th>
<th>TYPE OF ANALYSIS</th>
<th>RESULTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>BUSSE et al. (1984)</td>
<td>15</td>
<td>Incremental discontinuous treadmill test to exhaustion (3 min stages, 30 s break)</td>
<td>ear lobe vs venous</td>
<td>enzymatic analysis</td>
<td>ELC = V</td>
</tr>
<tr>
<td>FERRY et al. (1988)</td>
<td>5</td>
<td>Continuous incremental (4 min stages) or discontinuous (1 min break) cycling</td>
<td>ear lobe vs venous</td>
<td>enzymatic analysis</td>
<td>ELC = V (discontinuous)</td>
</tr>
<tr>
<td>DI VICO et al. (1989)</td>
<td>10</td>
<td>Incremental cycling exercise (4 min stages)</td>
<td>fingertip vs venous</td>
<td>not reported</td>
<td>FC &gt; V</td>
</tr>
<tr>
<td>GODSEN et al. (1989)</td>
<td>31</td>
<td>maximal aerobic effort</td>
<td>fingertip vs venous</td>
<td>YSI</td>
<td>FC &gt; V</td>
</tr>
<tr>
<td>FOXDAL et al. (1990)</td>
<td>10</td>
<td>Incremental cycling exercise (5 min stages)</td>
<td>fingertip vs venous</td>
<td>YSI</td>
<td>FC &gt; V</td>
</tr>
<tr>
<td>ROBERGS et al. (1990)</td>
<td>7</td>
<td>Incremental cycling exercise to exhaustion</td>
<td>ear lobe vs venous</td>
<td>enzymatic analysis</td>
<td>ELC &gt; V</td>
</tr>
<tr>
<td>FOXDAL et al. (1991)</td>
<td>10</td>
<td>Incremental cycling exercise (5 min stages)</td>
<td>fingertip vs venous</td>
<td>YSI</td>
<td>FC &gt; V</td>
</tr>
<tr>
<td>GRAETZER et al. (1991)</td>
<td>13</td>
<td>maximal BRUCE treadmill test</td>
<td>fingertip vs venous</td>
<td>YSI</td>
<td>FC &gt; V</td>
</tr>
<tr>
<td>EL-SAYED et al. (1993a)</td>
<td>14</td>
<td>discontinuous graded treadmill exercise</td>
<td>fingertip vs venous</td>
<td>ANALOX GM7</td>
<td>FC &gt; V</td>
</tr>
<tr>
<td>EL-SAYED et al. (1993b)</td>
<td>8</td>
<td>30 min continuous test</td>
<td>fingertip vs venous</td>
<td>ANALOX GM7</td>
<td>FC &gt; V</td>
</tr>
<tr>
<td>DASSONVILLE et al. (1998)</td>
<td>312</td>
<td>6 min (bike and treadmill) and continuous (bike) or discontinuous IT to exhaustion (treadmill)</td>
<td>fingertip vs venous</td>
<td>KONTRON (LA640)</td>
<td>FC &gt; V in all occasions</td>
</tr>
</tbody>
</table>

in line with the view of \(\text{Freund et al., 1986}\) that there is an inverse relationship between lactate clearance during recovery and the intensity of prior exercise. Thus in summary, maximal intensity whole body exercise results in higher lactate concentrations arriving at the arm muscles. Some of this lactate is oxidised by the working arm muscles thereby reducing the lactate release into the neighbouring veins. As less lactate is being produced in the arms in comparison with the average whole body lactate, due to the smaller muscle mass in the arms, the net result is lower lactate concentration in the veins in comparison with the arterialised capillary.
The slightly higher resting (ns) lactate in capillary blood (0.1 mmol·L\(^{-1}\)) in comparison with venous blood agrees with other findings (Righter et al., 1988) and confirms the fact that lactate is being produced and utilised in fully aerobic conditions (Brooks, 2000). Higher resting arterial lactate is not universal finding, though. Poortmans et al. (1978), reported higher (significant) concentrations for venous lactate over arterial, even though these differences were very small. Careful inspection of the present data, and, more particularly the individual responses (data not shown) reveals that the trend of higher resting capillary than venous lactate was not apparent in all the volunteers. This disparity has been shown before (Yoshida et al., 1982a; Williams et al., 1992) and a possible explanation is that resting values are too low to be accurately measured.

One possible criticism of the present study is that the capillary samples were contaminated with sweat during the fingerprick procedure (Thoden, 1991). As sweat lactate concentrations are considerably higher than blood lactate concentrations this would increase the measured lactate concentration (Fellman et al., 1983; Lamont, 1987). In addition, venous samples can also be problematic in that contamination from the saline solution that is being used to maintain patency, may occur (Bishop and Martino, 1993). To avoid the artificial readings as a consequence of the above two sources of error the experimenters used the following two techniques: firstly, the thumb was wiped with an alcohol swab before the piercing and afterwards was cleaned with a tissue; secondly, for the venous sample, 2-3 mmol·L\(^{-1}\) of blood were discarded to avoid any contamination by the flushing solution utilised. This amount of blood has been suggested to be adequate in order to give a “clear” sample according to the guidelines of venous sampling technique (training that the experimenters had received, [Appendix A]).

It has also been claimed that the first drop of fingertip blood should be discarded and that “milking” the finger can dilute the sample by introducing extravascular fluid into the sample. However, evidence to date excludes this possibility (Godsen et al., 1991).

In summary, this sub-study suggests that whole-blood lactate from capillary and arm vein cannot be used interchangeably and researchers should be cautious when
comparing studies that involve whole body maximal exercise and the sampling site for drawing blood varies.

**YSI method versus enzymatic method for whole blood lactate determination.**

This sub-study attempted to assess the agreement between two methods that permit analysis of whole blood lactate, but utilise different approaches: a) lactate in deproteinized whole blood samples (that is, both extra- and intracellular lactate) with the aid of enzymatic analysis (*Maughan, 1982*), and, b) lactate in haemolysed whole blood samples using the YSI method as modified by *Foxdal et al. (1992)*, which includes a haemolysed agent in the YSI buffer (Triton X-100). Although the quantitative difference (paired t-test) was about 10.9% (*P* < 0.01), with the YSI method giving higher results than the standard enzymatic assay, qualitative analysis revealed that the two methods are in good agreement.

The statement that there is agreement between the automatic and manual methods, even though quantitative (paired t-test) differences were found, is made because all the individual ratios (method 1 divided by method 2, data not shown) at peak lactate concentrations are contained within the range (1.00-1.27) defined by the calculated agreement ratios (table 3.10). This range is tighter than that of 0.79-1.29 obtained for the reliability study (criterion measure, peak lactate concentrations, table 3.9). In addition, the sample size estimation in order to detect a 10% difference (statistical power of 0.90) due to an (simple) experimental intervention by applying this or similar protocol needs only ≈ 4 subjects. High significant positive correlations (*r* = 0.86-1.00, *P* < 0.05-0.01) between the two methods are in line with this notion. Similar findings were revealed for all the other sampling points (table 3.10). However, the differences exceeded the concentration of 0.5 mmol·L⁻¹ which is usually accepted for lactate assays (*Dassonville et al., 1998; personal communication with colleagues at Loughborough University*).

Thus, there are some limitations of the qualitative analysis undertaken here. Firstly, it should be emphasised that these findings are based on only 7 pairs of samples for both methods and all sampling points. *Bland and Altman (1999)* suggest that 40 subjects should be employed if the reliability between two variables is to be examined. In
addition, the YSI claims linearity until 14 mmol·L⁻¹ and some volunteers exceeded this lactate concentration 5 min post-sprint (peak values, data not shown). Moreover, different timings in the handling process between the two methods may influence somewhat the results. In figure 12 a flow diagram presents the steps for the blood collection and treatment. Close inspection of the figure reveals that some time elapsed between deproteinisation of the sample (step 3, enzymatic method) and aspiration using the YSI (step 5). The tubes containing the blood samples (Lithium-Heparin coated tubes) do not have any form of preservative (personal communication with technical personnel from Sarstedt Ltd, Leicester, UK) but only anticoagulant (Lithium-Heparin). Blood preserved with fluoride/oxalate is often recommended for lactate (Weiner, 1995) because it stops glycolysis (Bueding and Wortis, 1940). Bishop et al. (1992a) emphasises the importance of identical timing in deproteination (manual method) and reading lactates with YSI method (with lysed agent) while they utilised sodium fluoride and potassium oxalate as preservatives for lactate. Even though, they observed 1.5 % higher (ns) concentrations using the YSI in comparison with the manual enzymatic method.

Another source of error could derive from the equilibration of lactate across the red blood cell membrane which occurs rapidly at 37 °C (Johnson et al., 1945; Daniel et al., 1964; Juel et al., 1990). Equilibration is greatly slowed down while temperature approaches 0 °C with a time course as long as 100 hours (Johnson et al., 1945). Immediate cooling in melting ice was one of the common practices for this study and therefore methodological shortcomings were limited.

Nevertheless, in low to moderate physiological lactate concentrations good agreement has previously been shown between manual and YSI methods (Thin et al., 1999; Bruce, unpublished observations). This was true even when the more rigorous 95 % limits of agreement (Bland and Altman 1986) were employed.

In summary, this sub-study suggests that for whole blood, as long as the lactate concentrations are within the limits (up to 14 mmol·L⁻¹) that the manufacturer (YSI) claims and the timing in handling process is identical, manual and automatic (YSI) analyses can give lactate concentrations that reflect each other even though the
absolute values are higher for the YSI method. This study suggests that YSI is a reliable method for analysing whole blood lactate as long as the lactate concentrations are up to 14 mmol·L\(^{-1}\).

**YSI method versus Cobas Mira Plus method for plasma lactate determination.**
The purpose of this sub-study was to compare plasma lactate concentrations using the YSI and Cobas Mira Plus analysers methods. It was found that both analysers gave similar peak plasma lactate concentrations (at the 5\(^{th}\) min) and thus these two methods can be used interchangeably.

However, which ever method is used there are some important considerations when determining plasma lactate concentration (Lormes et al., 1998). Points of concern are that there should be fast cooling and centrifugation and that the blood should not be treated with any stabilising agent (Lormes et al., 1998). These suggested precautions do not appear to influence significantly plasma lactate readings though (Hoogeveen et al. in Lormes et al., 1998). Furthermore even though equilibration of lactate across the red blood cells membrane occurs quickly at 37 °C (Johnson et al., 1945; Daniel et al., 1964; Juel et al., 1990), equilibration is greatly slowed down when temperature approaches 0 °C with a time course as long as 100 hours (Johnson et al., 1945). Thus immediate cooling in melting ice should be employed.

In conclusion, the YSI and Cobas Mira Plus analyser can be used interchangeably for plasma lactate measurements, a notion which agrees with previous investigations for sub-maximal exercise (Foxdal et al., 1992; Bruce et al., unpublished observations).
3.20. METHODOLOGICAL STUDY 3: SEPARATE AND COMBINED INFLUENCE OF POSTURE AND SPRINTING UPON PLASMA VOLUME CHANGES

3.20.1. INTRODUCTION

Exercise has been associated with a redistribution of water between body fluid compartments commonly resulting in a reduction in plasma volume (Harrison, 1985; Kargotich et al., 1998). Moderate intensity exercise results in a decrease in plasma volume as water moves from the vascular compartment into both the interstitial and intracellular fluid compartments of contracting muscle (Costill and Fink, 1974; Harrison et al., 1975). Similar, but more pronounced plasma volume losses have been reported following short-term intense exercise (Brooks et al., 1988; Allsop et al., 1990; Nevill et al., 1996a).

Apart from exercise per se the magnitude of changes in plasma volume could be influenced by alterations in posture, heat stress, hydration and physical training (Harrison, 1985; Kargotich et al., 1998). In terms of brief (30 s) maximal intensity exercise and as long as environmental conditions are neutral, posture change seems to be the only remaining variable able to influence body fluids (Harrison, 1985; Pivarnik et al., 1986).

During moderate intensity exercise involving either cycling or swimming or running the confounding effects of posture have been studied in more detail. Pivarnik and colleagues (1986) examined whether or not posture and/or cycle exercise could influence plasma volume shifts concluding that each factor could contribute significantly, either independently or in combination. These findings were confirmed later by McNaughton (1989) using an exercise model involving swimming, cycling and running (sprint triathlon). He found significant plasma volume losses due to all types of exercise (swimming, cycling and running) which were independent of posture even though in the control trial significant haemoconcentration did occur. However, McNaughton's (1989) findings were masked by the effect of dehydration which was not mentioned, but implied from the statistically important ($P < 0.05$) body mass reduction as a consequence of the exercise trial. In addition, packed cell volume...
(haematocrit, Hct) was measured by Coulter Counter technique which is not considered a valid method for the determination of Hct when osmolality changes substantially (Maughan et al., 2001). Therefore, it remains unclear whether and to what extent running exercise per se can affect plasma volume.

In maximal intensity running exercise the usual reported plasma volume losses [estimated % relative plasma volume changes by Hct and haemoglobin (Hb) evaluation (Dill and Costill, 1974) pre- and post-exercise] in a thermoneutral environment are between 8.1 to 16.8 % for 30-s sprint running (table 3.13) with range of changes accounted for by different subject groups as endurance in comparison with sprint trained or males in comparison with female subjects (Cheetham et al., 1986; Brooks et al., 1988; Allsop et al., 1990; Brooks et al., 1990; Nevill et al., 1996a). Thus, it is not clear whether some of these reductions in plasma volume are due to sprinting per se or simply an artefact due to the confounding effects of posture. When moving from the supine position to standing the immediate reductions in plasma volume are about 10 % with a further increase to reach 15-17 % after 15 min standing (Hagan et al., 1978; Jacob et al., 1998). These values are very similar to the changes in plasma volume reported for sprinting (table 3.13). Thus, it may be that exercise in a standing position cannot alter further the fluid dynamics. This view is reinforced by the claim of a limit to the reduction in plasma volume which approaches a ceiling at about 20 % as indicated by centrifugation-induced loss in plasma volume in + Gz position (i.e. centrifugal acceleration acting longitudinally from head to foot) (Greenleaf et al., 1977). The view of limit for plasma volume losses is also consistent with the concept of the operation of local edema-preventing mechanisms (Aukland and Reed, 1993).

On the other hand there is also the notion that if exercise is sufficiently severe, net filtration might still exceed net absorption and lymphatic return (Harrison, 1985) and thus further (or independent from posture) reductions in plasma volume can occur.
Table 3.13. A summary of studies completed to date and reported plasma volume changes after treadmill sprinting.

<table>
<thead>
<tr>
<th>STUDY</th>
<th>SUBJECTS</th>
<th>TRAINING STATUS</th>
<th>PPO (W)</th>
<th>% PLASMA VOLUME LOSSES</th>
<th>SAMPLING TIME</th>
<th>POSTURE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sant'Ana Pereira</td>
<td>6 males</td>
<td>*</td>
<td>*</td>
<td>8.7</td>
<td>1 min post-sprint</td>
<td>no reported</td>
</tr>
<tr>
<td>Allsop et al. (1990)</td>
<td>10 males (7 active, 3 females)</td>
<td>692</td>
<td>15.4</td>
<td>immediately post sprint</td>
<td>(?)</td>
<td>no reported</td>
</tr>
<tr>
<td>Brooks et al. (1988)</td>
<td>10 males</td>
<td>physically active</td>
<td>653</td>
<td>11.5</td>
<td>3 min post sprint</td>
<td>recumbent</td>
</tr>
<tr>
<td>Brooks et al. (1990)</td>
<td>9 males</td>
<td>no reported</td>
<td>781</td>
<td>15.2</td>
<td>3 min post sprint</td>
<td>recumbent</td>
</tr>
<tr>
<td>Brooks et al. (1990)</td>
<td>9 females</td>
<td>no reported</td>
<td>583</td>
<td>13.2</td>
<td>3 min post sprint</td>
<td>recumbent</td>
</tr>
<tr>
<td>Cheetham et al. (1986)</td>
<td>8 females</td>
<td>highly trained</td>
<td>534</td>
<td>8.1</td>
<td>3 min post sprint</td>
<td>no reported</td>
</tr>
<tr>
<td>Hamilton et al. (1991)</td>
<td>6 males</td>
<td>endurance trained</td>
<td>777</td>
<td>13.1</td>
<td>1 min post sprint</td>
<td>no reported</td>
</tr>
<tr>
<td>Hamilton et al. (1991)</td>
<td>6 males</td>
<td>game players</td>
<td>839</td>
<td>12.2</td>
<td>1 min post sprint</td>
<td>no reported</td>
</tr>
<tr>
<td>Nevill et al. (1996a)</td>
<td>6 males</td>
<td>endurance trained</td>
<td>619</td>
<td>14.8</td>
<td>1 min post recumbent</td>
<td>sprint</td>
</tr>
<tr>
<td>Nevill et al. (1996a)</td>
<td>6 males</td>
<td>sprint trained</td>
<td>826</td>
<td>16.8</td>
<td>1 min post recumbent</td>
<td>sprint</td>
</tr>
<tr>
<td>Nevill et al. (1996a)</td>
<td>6 females</td>
<td>endurance trained</td>
<td>419</td>
<td>10.1</td>
<td>1 min post recumbent</td>
<td>sprint</td>
</tr>
<tr>
<td>Nevill et al. (1996a)</td>
<td>5 females</td>
<td>sprint trained</td>
<td>619</td>
<td>13.5</td>
<td>1 min post recumbent</td>
<td>sprint</td>
</tr>
</tbody>
</table>

Sprint cycling or running is certainly intense exercise with an estimated power output of about 300% $\dot{V}O_2$ max (Wootton, 1984). Enormous metabolic responses due to sprint running in both blood (Brooks et al., 1988; Nevill et al., 1996a) and muscle...
(Nevill et al., 1989; Greenhaff et al., 1994) reinforce the demanding nature of sprint exercise. It has been suggested (Harrison, 1985) and experimentally shown in animal research (Watson et al., 1993), that plasma volume shifts are influenced by osmotic gradients, phenomenon attributed to high concentrations of metabolites (e.g. lactate) which increases tissue osmolality and promotes movement of fluids into myocytes and interstitium. Additionally, increased arterial pressure and muscle contraction during exercise also appear to contribute in this view (Lundvall et al., 1972; Nose et al., 1991).

There currently exist two theories in the literature concerning the usefulness or not of correcting metabolic responses due to plasma volume shifts (Kargotich et al., 1998). The first theory is that if other factors such as exercise, posture and environmental conditions significantly influence body fluids, then, a solute within the plasma which seems to have been changed possibly reflects the changes in body fluids and not the true biological response. In contrast, the second theory that correction of plasma volume changes is not essential because the concentration of a metabolite at the time, irrespective of plasma volume shifts, is the important factor as it is this that determines the body’s response. It was not the aim of the current investigation to address this challenging issue.

The purpose of the present study is to evaluate the independent effects of posture and sprinting exercise upon the relative changes of plasma volume based on Hct and Hb determination (Dill and Costill, 1974) which is the most widely used technique in such exercise (Cheetham et al., 1986; Brooks et al., 1988; Nevill et al., 1989; Allsop et al., 1990; Brooks et al., 1990; Hamilton et al., 1991; Nevill et al. 1996a, see also table 3.13). Thus, we hypothesise that even though posture will have a significant effect on body fluid dynamics, sprint running per se will also have a statistically important impact on plasma volume changes.

3.20.2. METHODS

3.20.2.1. Subjects
Eight female physical education students gave consent to participate in this study. Their physical characteristics were (mean ± SD) 23.6 ± 1.8. y, 62.4 ± 5.9. kg and 1.67
± 0.06 m for age, body mass and stature, respectively. They were all involved in sports at Loughborough University and most of them were members of various athletic clubs. Subject recruitment started after the Loughborough University ethical committee had given formal permission.

3.20.2.2. Familiarisations
Habituation with the sprint treadmill was thorough and as previously described in this thesis (section 3.3.1).

3.20.2.3. Ergometer
The nonmotorised treadmill prior to modifications described in section 3.4.1 was used here.

3.20.2.4. Protocol and experimental design
Participants reported to the laboratory in the morning after an overnight fast or at midday after a light breakfast. Individuals were encouraged to consume identical meals on all occasions and to drink similar amount of water. No caffeine (12 h) or alcohol (24 h) was allowed prior to participation in the main trials. Experiments were conducted at the same time of the day 48 h apart (figure 3.18). Each subject completed two trials with the order randomised. Thus, in this investigation each subject served as her own control. On one of the visits volunteers performed a 30-s sprint with the normal laboratory procedures, in terms of posture changes. More particularly, participants were instructed to stay in a semi-supine position on an adjustable experimental couch for 20 min while at the same time a cannula was inserted into an antecubital vein. This position was identical in both trials and the 20 min time period was selected since posture-induced changes have been shown to be (literally) complete during this period (Hagan et al., 1978; Lundvall and Bjerkhoel, 1994). The first blood sample was taken immediately after this period (resting sample, semi-supine position). The subjects then completed a standardised warm-up identical to one described before (see methodological study 1/2). Then a second blood sample was taken (immediately post warm-up) in a standing position. Immediately, afterwards participants relaxed on the experimental couch for 5 min with additional 2 samples at min 1 (3rd sample) and 3 (4th sample) during this period. They then stood up again and performed (within 1 min) the 30-s sprint. Further samples were drawn
immediately post-sprint (5th sample) in an upright position and at 1 (6th sample), 5 (7th sample), 10 (8th sample), 20 (9th sample) and 30 min (10th sample) during recovery from the sprint while they were in the semi-supine position. On all occasions the dependent arm (the arm that blood was taken) remained in the same position (Eisenberg, 1963). On another occasion volunteers carried out the control trial where no exercise was undertaken and blood samples were taken at identical time points and postures. Figure 3.17 gives a schematic representation of the experimental protocol. Trial order was randomised.

![Diagram of experimental protocol](image)

**WHERE:**
- 20 min rest on the couch.
- SS, UP, semi-supine and upright posture, respectively.
- standardised warm-up.
- numbers below the arrows indicate minutes that include the whole experiment.
- ↑ indicates blood sample.
- ⏳ indicates resting period after the warm-up.

**Figure 3.17. The schematic representation of the protocol.**
Figure 3.18. Time plan for the present experimental design. Where F indicates familiarisations as described in paragraph 3.3.1.

3.20.2.5. Collection and treatment of the blood samples
A two-syringe technique was employed for blood sampling. The first sample was discarded and the second utilised for Hb/Hct determinations. A 5 mL syringe was used to collect venous blood from the antecubital vein which was immediately dispensed into a lithium-heparin coated tube where gently mixing was carried out. A small aliquot of this blood was then transferred to an NH4-heparinised microhaematocrit tube (Scientific Laboratory Supplies Ltd, Nottingham, U.K.) with the aim to cover 2/3 to 3/4 of its length. During this procedure great effort was made to avoid air bubbles in the bore tubes [the bore of the tubes obeyed the British Standard 4316 (1968) that stipulates that the capillary bore must be less ± 2% (Maughan et al., 2001)]. After gentle mixing, the microhaematocrit tubes were placed in the rotor of a micro-haematocrit centrifuge (micro-haematocrit centrifuge, Hawksley and Sons Ltd, Lancing, U.K.). Packed cell volume was then determined [Appendix Q4] by means of a micro-haematocrit reader (Hawksley and Sons Ltd, Lancing, U.K.). Hct was assessed in triplicate in order to reduce the experimental error and the mean value was used in subsequent calculations. No corrections were made for the sampling-induced decrease in intravascular erythrocyte (Lundvall and Lidgren, 1998). However, correction of 1 % for plasma trapping between the red cells was adopted as previously recommended (Dacie and Lewis, 1968).

Haemoglobin concentration was assessed by the International Congress of Haematology (Maughan et al., 2001) recommended cyanmethaemoglobin method (van Kampen and Ziiistra, 1961) with the aid of a spectrophotometer (Digital Gratting Spectrophotometer Series 2, Model CE393, Cecil Instruments Ltd., Cambridge, U.K.). For the purpose of the assay [Appendix Q3] blood was collected in duplicate.
20 μL disposable non-heparinized glass capillary tubes (Accupette Pipettes, Baxter Healthcare Corporation, U.S.A.) and dispensed in mini scintillation vials (Scientific Laboratory Supplies Ltd, Nottingham, U.K.).

The coefficient of variation (Cohen and Holliday, 1982) for intraassay variability was 0.7 % and 1.9 % for Hct and Hb determination, respectively.

Blood, cell and plasma volume (BV, CV and PV, respectively) relative changes were then estimated [section 3.15.2 and Appendix T1] as previously described (Dill and Costill, 1974).

Absolute changes in plasma volume were also estimated based on Hct, assuming an initial volume of 65 mL·kg−1 body mass for females subjects (Astrand and Rodahl, 1986) and the change in plasma volume was calculated [section 3.15.2 and Appendix T2].

Finally, all the assessments, for both Hb and Hct, were done by the same experimenter (the author of this thesis in this occasion) so as to minimise the experimental error.

3.20.2.6.Statistical analysis

A two-way analysis of variance for within subjects design was used to assess whether there were any differences in % estimate plasma volume changes between the sprint trial and the posture trial during the test (main effect: sprint by posture by time) and over time for each method separately (main effect: time). When a significant interaction was revealed adjusted paired t-tests, corrected by the Bonferroni method, were used. Correlations between variables were determined by Pearson Correlation coefficients. Results are expressed as mean ± SD, unless otherwise stated.
3.20.3. RESULTS

3.20.3.1. Body mass

Body mass was not changed between days (data not shown) and during the experiment (before and after the experimental procedures) a sample of 3 subjects had a negligible (150 g, data not shown) reduction which can be attributed to blood drawn during the experiment.

3.20.3.2. Environmental conditions

Ambient temperature was normal and similar \((P < 0.05)\) in both trials \([20.9 \, ^\circ\text{C} \text{ (range 19-25)} \text{ and } \text{21.2 } ^\circ\text{C} \text{ (range 19-25)}\) for sprint and control trial, respectively].

The calculated relative humidity [Appendix P] followed similar trends and it was 59.6 % (range 51-63) and 59.1 % (range 48-69) for the two trials with no significant differences observed.

3.20.3.3. Performance variables

Mechanical data for PPO, MPO, FIpo, peak speed, mean speed and FIsp were 461 ± 57 W, 330 ± 89 W, 50 ± 6 %, 5.60 ± 0.47 m·s\(^{-1}\), 4.85 ± 0.40 m·s\(^{-1}\) and 27 ± 4 %, respectively. No significant relationships were found between plasma volume changes and any of the above values.

3.20.3.4. Plasma volume changes

The changes in plasma volume for the control and sprint trial are depicted in figure 3.19 and summarised in table 3.15. Raw values for Hct and Hb are presented in table 3.14. In both conditions there was a significant change \((P < 0.05)\) in plasma volume over time and within the first 10 min of the experimental protocol (immediately after post warm-up vs standing) accounting for -12.5 % and -7.5 % for the sprint and control trial, respectively \((P < 0.05)\). This profile was constant in the rest of the sampling points for the exercise trial with the largest decreases immediately post-sprint in the upright position (-17.7 %, \(P < 0.05\)) whereas in the non exercise mode the highest value was observed after the first 10 min standing (-7.5 %, \(P < 0.05\)). As can be seen in figure 3.19 in the control trial the pattern was somewhat different with the plasma volume decreases not always as pronounced which ultimately ended in hemodilution in the last three samples. In contrast, 30 min after sprinting plasma
volume losses were still evident. Thus plasma volume changes were more pronounced in the sprint trial than during the trial with posture changes alone (figure 3.19). Even the two sub-maximal 30 s runs in the warm-up incurred larger fluid shifts than posture changes alone at the same sampling point (figure 3.19, 2nd blood sample, \( P < 0.05 \)).

In terms of absolute changes in plasma volume the results are presented in figure 3.20. Ten min of quite standing accounted for 313 mL·kg\(^{-1}\) losses in plasma volume (highest losses) whereas sprinting was responsible for twice as much (719 mL·kg\(^{-1}\)). The two strides resulted in 508 mL·kg\(^{-1}\) of plasma losses compared with the 313 mL·kg\(^{-1}\) of posture at the same time.

Table 3.14. Raw values for Hct and Hb at rest, post warm-up and during 10 min recovery from a single 30-s treadmill sprint between sprint and control trial (mean ± SD, n = 8). "PS" indicates post-sprint.
Figure 3.19. % Estimated relative changes in plasma volume (PV) at post warm-up and following a single 30-s treadmill sprint between the sprint and control trial (mean ± SD, n = 8). “a” indicates significant differences (P < 0.05) from the resting values, “ab” indicates significant differences (P < 0.05) between trials at the particular time point, PWP 1: 1st sample post warm-up, PWP 2: 2nd sample post warm-up, PWP 3: 3rd sample post warm-up, and PS1 to PS6 are the corresponding sampling after the sprint.

Table 3.15. % Estimated relative changes in plasma volume (PV) at rest, post warm-up and following a single 30-s treadmill sprint between the sprint and control trial (mean ± SD, n = 8). “a” indicates significant differences (P < 0.05) from the baseline value whereas “ab” indicates significant differences (P < 0.05) between sprint and control trial at the particular sampling point. “PS” indicates post-sprint.

<table>
<thead>
<tr>
<th>POSTURE</th>
<th>SAMPLING TIME</th>
<th>DURATION IN EACH POSTURE</th>
<th>SPRINT</th>
<th>CONTROL</th>
</tr>
</thead>
<tbody>
<tr>
<td>SEMI-SUPINE</td>
<td>0 min</td>
<td>20 min</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>UPRIGHT</td>
<td>9 min 30 s</td>
<td>10 min</td>
<td>-12.5 ± 3 a</td>
<td>-7.5 ± 5 a</td>
</tr>
<tr>
<td>SEMI-SUPINE</td>
<td>11 min</td>
<td>1 min</td>
<td>-9.6 ± 3 a b</td>
<td>-6.7 ± 4 a</td>
</tr>
<tr>
<td>SEMI-SUPINE</td>
<td>13 min</td>
<td>2 min 30 s</td>
<td>-5.2 ± 4 b</td>
<td>-3.8 ± 5</td>
</tr>
<tr>
<td>UPRIGHT</td>
<td>15 min</td>
<td>2 min 30 s (PS)</td>
<td>-17.7 ± 4 b</td>
<td>-6.0 ± 3 a</td>
</tr>
<tr>
<td>SEMI-SUPINE</td>
<td>16 min 30 s</td>
<td>1 min</td>
<td>-16.6 ± 3 b</td>
<td>-4.6 ± 6</td>
</tr>
<tr>
<td>SEMI-SUPINE</td>
<td>20 min 30 s</td>
<td>5 min</td>
<td>-14.4 ± 3 b</td>
<td>-1.7 ± 5</td>
</tr>
<tr>
<td>SEMI-SUPINE</td>
<td>25 min 30 s</td>
<td>10 min</td>
<td>-12 ± 3 b</td>
<td>1.1 ± 4</td>
</tr>
<tr>
<td>SEMI-SUPINE</td>
<td>30 min 30 s</td>
<td>20 min</td>
<td>-7.1 ± 4 b</td>
<td>3.1 ± 3 a</td>
</tr>
<tr>
<td>SEMI-SUPINE</td>
<td>35 min 30 s</td>
<td>30 min</td>
<td>-3.1 ± 3 b</td>
<td>0.8 ± 4</td>
</tr>
</tbody>
</table>
Figure 3.20. % Estimated absolute changes in plasma volume (PV) between sprint and control trial at rest, post warm-up and following a single 30-s treadmill sprint between the sprint and control trial (mean ± SD, n = 8). PWP 1: 1st sample post warm-up, PWP 2: 2nd sample post warm-up, PWP 3: 3rd sample post warm-up, and PS1 to PS6 are the corresponding sampling after the sprint.

3.20.3.5. Blood volume changes
A similar pattern, but smaller in magnitude, was observed when blood volume was the dependent variable. In the exercise trial the peak blood volume losses (-10.5 %, \(P < 0.05\)) happened immediately post-sprint while in the resting trial the greater change was after 10 min standing (-4.7 %, \(P < 0.05\)). The sprint trial caused larger blood volume losses than changes in posture alone at all sampling points \((P < 0.05\) in all cases apart the last sample). Table 3.16 gives an overview of these results which are also shown in figure 3.21.

3.20.3.6. Cell volume changes
In contrast to the reductions in plasma and blood volume there was an increase in relative changes in cell volume due to both trials \((P < 0.05)\). These results are tabulated in table 3.17 and portrayed in figure 3.22. However, there were not any statistically significant differences between the sprint and change in posture only trials (sprint-trial interaction, ns).
Table 3.16. Relative changes (%) in Blood Volume between sprint and control trial at rest, post warm-up and following a single 30-s treadmill sprint between the sprint and control trial (mean ± SD, n = 8). "a" indicates significant differences ($P < 0.05$) from the baseline value whereas "b" indicates significant differences ($P < 0.05$) between sprint and control trial at the particular sampling point. "PS" indicates post-sprint.

<table>
<thead>
<tr>
<th>POSTURE</th>
<th>SAMPLING TIME</th>
<th>DURATION IN EACH POSTURE</th>
<th>SPRINT</th>
<th>CONTROL</th>
</tr>
</thead>
<tbody>
<tr>
<td>SEMI-SUPINE</td>
<td>0 min</td>
<td>20 min</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>UPRIGHT</td>
<td>9 min 30 s</td>
<td>10 min</td>
<td>-7.6 ± 3 b</td>
<td>-4.7 ± 3 b</td>
</tr>
<tr>
<td>SEMI-SUPINE</td>
<td>11 min</td>
<td>1 min</td>
<td>-5.6 ± 3 a</td>
<td>-4.1 ± 3 a</td>
</tr>
<tr>
<td>SEMI-SUPINE</td>
<td>13 min</td>
<td>2 min 30 s</td>
<td>-4.5 ± 3 b</td>
<td>-2.1 ± 3</td>
</tr>
<tr>
<td>UPRIGHT</td>
<td>15 min</td>
<td>2 min 30 s (PS)</td>
<td>-10.5 ± 3 b</td>
<td>-3.7 ± 2 a</td>
</tr>
<tr>
<td>SEMI-SUPINE</td>
<td>16 min 30 s</td>
<td>1 min</td>
<td>-9.8 ± 2 a b</td>
<td>-2.8 ± 4</td>
</tr>
<tr>
<td>SEMI-SUPINE</td>
<td>20 min 30 s</td>
<td>5 min</td>
<td>-7.8 ± 2 a b</td>
<td>-1.0 ± 2</td>
</tr>
<tr>
<td>SEMI-SUPINE</td>
<td>25 min 30 s</td>
<td>10 min</td>
<td>-6.3 ± 2 a b</td>
<td>0.8 ± 2</td>
</tr>
<tr>
<td>SEMI-SUPINE</td>
<td>30 min 30 s</td>
<td>20 min</td>
<td>-3.4 ± 3 b</td>
<td>2 ± 2 a</td>
</tr>
<tr>
<td>SEMI-SUPINE</td>
<td>35 min 30 s</td>
<td>30 min</td>
<td>-0.8 ± 3</td>
<td>0.9 ± 2</td>
</tr>
</tbody>
</table>

Figure 3.21. % Estimated relative changes in blood volume (BV) between sprint and control trial at post warm-up and following a single 30-s treadmill sprint between the sprint and control trial (mean ± SD, n = 8). "a" indicates significant differences ($P < 0.05$) from the resting values, "b" indicates significant differences ($P < 0.05$) between trials at the particular time point, PWP 1: 1st sample post warm-up, PWP 2: 2nd sample post warm-up, PWP 3: 3rd sample post warm-up, and PS1 to PS6 are the corresponding sampling after the sprint.
Table 3.17. Relative changes (%) in cell volume between sprint and control trial at rest, post warm-up and following a single 30-s treadmill sprint between the sprint and control trial (mean ± SD, n = 8). " Share indicates significant differences (P < 0.05) from the baseline value. "PS" indicates post-sprint.

<table>
<thead>
<tr>
<th>POSTURE</th>
<th>SAMPLING TIME</th>
<th>DURATION IN EACH POSTURE</th>
<th>% CELL VOLUME CHANGES</th>
</tr>
</thead>
<tbody>
<tr>
<td>SEMI-SUPINE</td>
<td>0</td>
<td>20 min</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>UPRIGHT</td>
<td>9 min 30 s</td>
<td>10 min</td>
<td>0.5 ± 1</td>
</tr>
<tr>
<td>SEMI-SUPINE</td>
<td>11 min</td>
<td>1 min</td>
<td>0.9 ± 2</td>
</tr>
<tr>
<td>SEMI-SUPINE</td>
<td>13 min</td>
<td>2 min 30 s</td>
<td>1.6 ± 1*</td>
</tr>
<tr>
<td>UPRIGHT</td>
<td>15 min</td>
<td>2 min 30 s (PS)</td>
<td>1.3 ± 1*</td>
</tr>
<tr>
<td>SEMI-SUPINE</td>
<td>16 min 30 s</td>
<td>1 min</td>
<td>1.2 ± 2</td>
</tr>
<tr>
<td>SEMI-SUPINE</td>
<td>20 min 30 s</td>
<td>5 min</td>
<td>2.9 ± 3*</td>
</tr>
<tr>
<td>SEMI-SUPINE</td>
<td>25 min 30 s</td>
<td>10 min</td>
<td>2.9 ± 3*</td>
</tr>
<tr>
<td>SEMI-SUPINE</td>
<td>30 min 30 s</td>
<td>20 min</td>
<td>2.6 ± 3</td>
</tr>
<tr>
<td>SEMI-SUPINE</td>
<td>35 min 30 s</td>
<td>30 min</td>
<td>3.0 ± 2*</td>
</tr>
</tbody>
</table>

Figure 3.22. % Estimated relative changes in cell volume between the sprint and control trial at post warm-up and following a single 30-s treadmill sprint between the sprint and control trial (mean ± SD, n = 8). " Share indicates significant differences (P < 0.05) from the resting values, PWP 1: 1st sample post warm-up, PWP 2: 2nd sample post warm-up, PWP 3: 3rd sample post warm-up, and PSI to PS6 are the corresponding sampling after the sprint.

3.21.4. DISCUSSION

This investigation attempted to evaluate the independent effects of posture and sprinting on relative changes in plasma volume using the method introduced by Dill and Costill (1974). The principal finding was that whole body maximal exercise (sprint running) can alter significantly body fluids in addition to the changes caused as a result of changes in posture.
Performance during sprinting in the present study, in terms of power and fatigue, was similar to the performance of female subjects in earlier studies [e.g. present study PPO, MPO and fatigue index for power, of 461 W, 330 W, 50 %, respectively, Nevill et al. (1996a) 419-619 W, 277-381 W and 55-59%, respectively].

Estimated peak plasma volume reductions of 17.7 % relative to the resting levels appeared immediately after the sprint (in the upright position) and were the highest ever reported in such exercise (see table 3.15). The previously highest reported values in women after treadmill sprinting were 13.5 % by Nevill et al. (1996a) in sprint trained volunteers (n = 5). This discrepancy is probably due to methodological variations since in Nevill's study the blood sample was taken at 1 min post-sprint and in the recumbent position. It is well documented that shifts in plasma volume occur quickly (Hagan et al., 1978) and that the adoption of the supine/semi-supine posture induces hemodilution (Hagan et al., 1978; Shirreffs and Maughan, 1994) thereby minimising the plasma volume losses. In addition, Nevill et al. (1996a) did not mention the time that their subjects remained in the recumbent position for the baseline value, so this may have varied in comparison with the procedure in the present study. Such variations in body position, timing of blood sampling and subject differences in sex and training status may be responsible for the marked variations in changes in plasma volume across other reported studies (Harrison, 1985; Cheetham et al., 1986; Brooks et al., 1988; Nevill et al., 1989; Brooks et al., 1990; Allsop et al., 1990; Hamilton et al., 1991; Kargotich et al., 1998, see also table 3.13).

There was a 17.7 % loss in plasma volume immediately post-sprint; a value that was statistical significant \( (P < 0.05) \) from both the baseline value (a hypothetical 0 % change) and the corresponding 6.0 % reduction in plasma volume observed in the control trial at the same time point. This value (-17.7 %) was also significantly different \( (P < 0.05) \) from the highest value due to changes in posture observed after 10 min of standing (-7.5 %). The changes in plasma volume due to posture alone in the present study were somewhat different from those reported by Lundvall and Lindgren (1998) using the Hct/Hb method. In their study plasma volume reductions were 14.5 % after 8-10 min of standing in comparison with the 7.5 % in the present experiment. This controversy is probably due to the methodological differences as in
the present study the semi-supine position was used for baseline values as opposed to the supine position in the *Lundvall and Lindgren (1998)* investigation.

This is the first investigation that has examined plasma volume alterations due to maximal whole body exercise in an attempt to separate the confounding effects of posture. It was found that intense exercise of only 30 s duration can provoke enormous changes in body fluids as indicated by the estimated 11.7 % plasma volume loss due to sprinting per se (sprint trial – posture trial). In absolute terms this change represents 719 ml·kg\(^{-1}\) plasma loss from the intravascular compartment (figure 3.20). In order to calculate the absolute values it was assumed that there were not any changes in body mass before and after the experimental protocol. This assumption comes from the fact that no more that 0.2 kg differences appeared in a sample of the volunteers measured (n = 3, data not shown), the subjects did not urinate during the procedure and they were instructed to follow as similar diet as possible including fluids. The experiments were also conducted at the same time of the day for further compliance with these instructions. Thus, apart from the blood drawn from the antecubital vein no other fluid was directed out of the body. Plasma volume losses due to perspiration are considered negligible in this type of exercise. The fact that there were no changes in body mass and the two trials were performed in thermoneutral environment, suggests that short-term intense exercise induces transient plasma volume shifts. The view that running exercise can alter fluid dynamics has been supported by other experimental data (*McNaughton, 1989*), although there were methodological limitations to the study.

It has been previously postulated (*Harrison, 1985*) that running exercise per se, after moving from the supine position, will not result in further plasma volume losses due to four physiological mechanisms: a) during exercise the operation of muscle pump reduces the venous pressure in the dependent legs although recent data challenge this mechanism for the reduction of venous pressure (*Hamann et al., 2003*), b) during exercise there is an increased lymphatic return, and, c) the fact that the accumulation of fluid in active muscle is countered by rising the tissue pressure, plasma osmolality and the reciprocal changes in the colloid osmotic pressure of plasma and interstitial fluid. In the absence of evidence to the contrary this theory has prevailed to date, even
though it was not accompanied by direct experimental evidence. The present findings, which are the first on this topic, are in conflict with the theory of Harrison (1985), though.

The present data suggest that sprinting can cause a redistribution of water between body fluid compartments in addition to the effects of posture change. Although the commonly cited mechanism of plasma volume loss due to capillary fluid pressure increases (Kargotich et al., 1998), extensive fluid losses cannot be entirely explained by this mechanism (Bjornberg, 1990). Watson et al. (1993) showed that the major mechanism explaining changes in plasma volume was that high concentrations of metabolites and/or electrolytes enter the circulation (e.g. lactate and sodium, respectively) which in turn increase tissue osmolality and thereby facilitate the movement of an ultrafiltrate of plasma into muscle cells and interstitium. High levels of metabolites and electrolytes are typical characteristics of maximal exercise such as sprinting (Cheetham et al., 1986; Brooks et al., 1988; Nevill et al., 1989; Brooks et al., 1990; Allsop et al., 1990; Hamilton et al., 1991; Lindinger et al., 1992; Nevill et al. 1996a; Cherry et al., 1998). The proportional decrease in plasma volume with the increase in plasma electrolytes such as [Na\(^+\)] and [K\(^+\)], during treadmill sprinting (Sant' Ana Pereira, 1990) is in line with this view (Lindinger et al., 1992). Thus, during sprinting net filtration might still exceed net absorption and lymphatic return thereby leading to the excess body fluid loss in comparison with the corresponding plasma volume reduction due to posture change. Finally, the persistence of reductions in plasma volume during recovery from sprinting is probably related to water movements into previously exercised muscles (Sjøgaard and Saltin, 1982; Kowalchuk et al., 1988).

The physiological significance of these findings is difficult to interpret due to the lack of general consensus over the relevance of plasma volume interactions with plasma constituent levels (as described in the introduction), but it is now confirmed that sprinting per se as performed for the experimental work in this thesis does cause a shift in plasma volume.

It should be emphasised here that the results presented above have to be treated with caution since the changes in plasma volume are estimated relative values based on
Hct/Hb determination and not upon advanced technological methodology such as Evans blue dye dilution technique. The methodological error by Hct/Hb method can bring about an underestimation of 30 to 50 % (Johansen et al., 1998; Lundvall and Lindgren, 1998). However, the Evans blue dye dilution technique is not appropriate when repeated measurements are required since it takes some time before the dye is completely mixed (Johansen et al., 1998). In addition, when exercise is being considered, "it is the change relative to some steady-state control condition that is of primary interest" (Harrison, 1985).

Another interesting finding of the present study was the increase of cell volume of about 3 % due to sprint ($P < 0.05$, from the resting value) although these changes were not significantly higher as compared with the control trial. Increases in cell volume have been observed before when acidosis accompanied exercise (Costill and Fink, 1974; Beaumont et al., 1981). This erythrocyte swelling maybe due to the physiological mechanism of haemoglobin to buffer hydrogen ions while at the same time bicarbonate and chloride ions accumulate (Harrison, 1985). This is true in maximal exercise when intracellular acidosis does take place (Nevill et al., 1989). Opposingly, these data simply reflect methodological errors of the Hct and Hb concentration which have been shown to represent a range of $-3$ to $+3$ % (Harrison et al., 1982).

In conclusion, this is the first investigation to examine in detail the potential influence of maximal whole-body exercise on fluid dynamics employing the most typical technique of Hct/Hb. It was found that intense exercise of only 30 s can cause significant plasma volume shifts which are in addition to those changes induced by posture alone. With future methodological advances, which will enable direct measurements of fluid dynamics during exercise, it may be possible to further understanding of the mechanisms underlying such changes in plasma volume.
3.21. METHODOLOGICAL STUDY 4: BLOOD LACTATE STORAGE

3.21.1. INTRODUCTION

Many of the blood samples collected during the various studies in this thesis (particularly those collected during training studies) had to be stored for varying periods of time prior to analysis. Therefore it was important establish whether or not storage had any impact on the values recorded.

Buono (1986) has reported that freezing at \(-10^\circ C\) for 3 months provides a reliable storage process for 1 mL blood samples deproteinized with perchloric acid. It was the purpose of this experiment to extend these findings by determining if freezing free supernatant prolongs the stability of the lactate for more than 3 months where the supernatant was derived from 20 \(\mu L\) blood samples. More particularly this study was conducted in order to examine the effects of prolonged freezing and defrosting on three physiological concentrations of whole blood venous lactate: low (resting sample), moderate (post warm-up samples) and high (5 min post-sprint 2). The experimental design was identical to the repeated sprint protocol as described in section 3.5.2.

3.21.2. METHODS

The collection and treatment of the blood samples as well as the lactate assay were performed as was described in section 3.13.3. The whole procedure including the collection of the blood from the cannula, the dispensing of the samples and the final enzymatic analysis was carried out by the same experimenter (author of this thesis). Three pairs of samples from one subject were utilised for this study. The samples were assayed after 3, 9 and 13 months of storage, timed from the first day of storage at \(-20^\circ C\).

In order to examine whether defrosting influences the stability of lactate concentrations, the lactate samples from previous assays were defrosted and reassayed together with the non defrosted stored samples. Thus, the first pair of samples
for the three physiological concentrations was assayed 3 times (3 months pair), the second 2 times (9 months pair) and the third once (13 months pair). Table 3.18 summarises the time plan.

Table 3.18. Time schedule for the lactate assays. Where, RV: resting concentration; PWP: post warm-up; 5 PS2: 5 min post-sprint 2; 1st, 2nd and 3rd DEF: 1st, 2nd and 3rd that the samples defrosted (to be assayed).

<table>
<thead>
<tr>
<th>SAMPLES</th>
<th>RV</th>
<th>PWP</th>
<th>5 PS2</th>
<th>RV</th>
<th>PWP</th>
<th>5 PS2</th>
<th>RV</th>
<th>PWP</th>
<th>5 PS2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st pair</td>
<td>DEF</td>
<td>DEF</td>
<td>DEF</td>
<td>1st</td>
<td>DEF</td>
<td>DEF</td>
<td>2nd</td>
<td>DEF</td>
<td>DEF</td>
</tr>
<tr>
<td>2nd pair</td>
<td>1st</td>
<td>DEF</td>
<td>DEF</td>
<td>1st</td>
<td>DEF</td>
<td>DEF</td>
<td>2nd</td>
<td>DEF</td>
<td>DEF</td>
</tr>
<tr>
<td>3rd pair</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1st</td>
<td>DEF</td>
<td>DEF</td>
</tr>
</tbody>
</table>

Because only few samples were analysed no statistical analysis was performed. The participant was a healthy female basketball player (national calibre- participated in England women's team) with age, height and body mass of 18 y, 1.80 m and 63 kg.

3.21.3. RESULTS

Table 3.19 tabulates the results from the lactate assays. Resting, post warm-up and 5 min post-sprints concentrations were 1.1, 2.5 and 14.1 mmol·L⁻¹, respectively after 3 months freezing at -20 °C. Storage duration appeared to have no influence on the recorded blood lactate concentration. However, defrosting samples on one or two occasions seemed to result in higher blood lactate concentrations which were most noticeable at the highest concentration measured.
Table 3.19. Time schedule for the lactate assays. Concentrations are in mmol·L⁻¹. Where, RV: resting concentration; PWP: post warm-up; 5 PS2: 5 min post-sprint 2; 1ˢᵗ, 2ⁿᵈ and 3ʳᵈ DEF: 1ˢᵗ, 2ⁿᵈ and 3ʳᵈ that the samples defrosted (to be assayed).

<table>
<thead>
<tr>
<th></th>
<th>3 MONTHS</th>
<th></th>
<th></th>
<th>9 MONTHS</th>
<th></th>
<th></th>
<th>13 MONTHS</th>
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<tbody>
<tr>
<td></td>
<td>RV</td>
<td>PWP</td>
<td>5 PS2</td>
<td>RV</td>
<td>PWP</td>
<td>5 PS2</td>
<td>RV</td>
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<td></td>
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<tr>
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<td>2.5</td>
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<td>2ⁿᵈ</td>
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</tr>
<tr>
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<td>2.4</td>
<td>14.4</td>
<td>1.2</td>
<td>2.4</td>
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<td>1ⁿᵗ</td>
<td>1ⁿᵗ</td>
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<td>3ʳᵈ pair</td>
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<tr>
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<tr>
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</tr>
</tbody>
</table>

3.2.1.4. DISCUSSION

This methodological study examined the effects of prolonged freezing (−20 °C) and defrosting on low, moderate and high physiological concentrations of venous whole blood lactate. These findings suggest that prolonged freezing of deproteinized blood samples has no negative impact on lactate stability at least 13 months after the initial collection and storage of the blood. On the other hand repeated defrosting may result in higher lactate concentrations but only at higher physiological concentrations.

This investigation expands the findings of Buono (1986) where 3 months prolonged freezing (−10 °C) of deproteinized venous whole blood samples did not affect the lactate concentrations. The present findings also indicate that lactate is stable even after prolonged freezing and even using the very small volumes of blood (20 µL blood samples for this analysis).

However, repeated defrosting (more than once) appears to alter lactate concentration which may lead to erroneously elevated readings at high physiological lactate concentrations. At the 3ʳᵈ defrosting, lactate concentrations increased more than the arbitrary value of 0.5 mmol·L⁻¹ (table 3.19) which informally recognised as the upper
limit of experimental error for lactate (Dassonville et al., 1998; personal communication with colleagues at Loughborough University).

In his original paper, Maughan (1982) suggested that all stock solutions should be calibrated spectrometrically using scaled-up version with respects to the assay of interest. In addition, dilute working standard solutions should be prepared at frequent intervals. Neither of these procedures was followed here. To compensate for this methodological drawback quality controls for lactate (S 3005, Sigma Diagnostics, Poole Dorset, UK) were run together with each batch. The obtained concentrations were well inside the range set by the manufacturer (data not shown).

Finally, it should be acknowledged that the present findings are based on a restricted number of observations and therefore should be treated with caution. Further investigations with larger number of blood samples which are accompanied by appropriate statistical techniques should be performed to clarify these issues.

In summary, this study highlights the stability of lactate when deproteinized samples are stored at –20°. However, repeated defrosting may result in erroneously elevated concentrations at higher physiological lactate concentrations. Further studies employing larger numbers of samples are needed to fully elucidate these issues.
CHAPTER 4: THE EFFECTS OF MENSTRUAL CYCLE PHASE UPON SPRINT RUNNING PERFORMANCE

4.1. INTRODUCTION

From Kalipatira’s days (Kalipatira: was the mother of the famous ancient Greek family of Diagoridon from Rhodes whose members won many Olympic medals) - where women were not allowed either to participate in the Olympics Games or to attend them (Mouratidis, 1990), to Ekaterina Thanou’s days (silver medallist in the 100 m sprint in the Sydney Olympic Games), many socio-cultural advances with respect to women’s sporting participation have been achieved. As a consequence, women are now engaged in strenuous training programmes of a similar volume and intensity to those undertaken by men. This increase in participation in sport by women has led to increased interest in the physiological and metabolic responses of women to sport and exercise (e.g. Drinkwater, 2000).

It has also been important, therefore, to examine the potential effects of hormonal fluctuations, due to the female menstrual cycle, upon metabolism and performance. The two most biological active reproductive hormones of the adult female are 17-β-estradiol (estradiol) and progesterone (Norman and Litwack, 1997). Progesterone is produced by the adrenal gland in the follicular phase and the corpus luteum for the rest of the cycle (Jude et al., 1992). On the other hand the site of biosynthesis for estradiol is primarily the follicle and corpus luteum (Norman and Litwack, 1997).

Both estradiol and progesterone fluctuations during a normal menstrual cycle have been associated with alterations in athletic performance (Lebrun, 1993; Reilly, 2000) and metabolic responses during and after exercise (Bunt, 1990; Beidleman et al., 1999). More particularly, estradiol elevations during luteal phase have been suggested to contribute to muscle glycogen sparing during sub-maximal exercise at 70 % $\dot{V}O_2$ max (Hackney, 1999) possibly by affecting target cells either directly or indirectly via alterations in more classic metabolic hormones such as cortisol and growth hormone, even though these mechanisms are not clearly documented in the human muscle cell (Bunt, 1990; Ashley et al., 2000). Such views have been supported by some studies.
consistently (Nicklas et al., 1989; McCracken et al., 1994) but contradicted by others (De Souza et al., 1990). In terms of high intensity exercise it has been suggested that estradiol might contribute to an increase in muscle strength and significant increases (about 11%) in quadriceps and handgrip maximum voluntary isometric force have been reported during the mid-cycle phase (Philips et al., 1996; Sarwar et al., 1996). This muscle strengthening action possibly arises because estradiol can alter the negative effects of inorganic phosphate (Pi) upon cross-bridge kinetics (Pate and Cooke, 1989; Hellstrand, 1996). These speculations are also reinforced by the fact that women's muscles become weaker the following menopause and this can be prevented by hormonal replacement therapy (Rutherfold and Jones, 1992; Philips et al., 1993). If it is accepted that force increases can occur at ovulation and during the luteal phase when high levels of circulatory estradiol exist, it is possible that power output may be also affected (force x speed = power) raising the question of the significance of these changes for whole body maximal exercise such as sprinting.

Nevertheless, the effect of a given hormone is dependent not only on the plasma/serum concentration of the specific hormone in question, but also (among other factors) on the concentration of other hormones (Bunt, 1986). In the case of estradiol for example, its potential effects can be modified by corresponding levels of progesterone (Jensen et al., 1987; Bunt, 1990). However, the potential impact of this "antiestrogenic" effect of progesterone is unknown during maximal intensity exercise (Greeves et al., 1997).

Indeed, the effect of menstrual cycle phase on sprint performance is currently unknown due to the highly equivocal nature of the findings in the literature. Swimming performance expressed as the fastest time to complete 100 m free style was not altered in the premenstrual (days 13-28 from the beginning of the menses), menstrual (days 1-5 from the beginning of the menses) and postmenstrual phase (days 10-12 days from the beginning of the menses) in well-trained varsity level swimmers (Quadagno et al., 1991). Similarly, Miskic et al. (1997) using 15 x 15-s sprints interspersed with 2 min rest on a cycle ergometer found no difference in performance during menstruation (first 2 d of the cycle) and days 19-21 from the onset of the menses. Again during sprint cycling (about 36 s), De Bruyn-Prevost et al. (1984) found no difference in performance during menstruation (day 1-2 of the cycle),
ovulation (day 14 of the cycle) and at the end of the cycle (1-2 days before menstruation). Finally, Lynch and Nimmo (1998) found no variations in performance during repeated 20-s sprints with 100 s passive rest on an inclined treadmill during the mid-follicular (defined as day between 7-10 from the beginning of the menses) and the late luteal phase (defined at 12 after ovulation assuming a 28 days cycle). Opposingly, in the very first few days of the cycle (Brooks-Gunn et al., 1986), or, in the follicular phase (8th day from the onset of the menses) (Bale and Nelson, 1985) improved sprint swimming performance has been reported. In addition, improved performance during the follicular phase has been reported during repeated 30-s sprints on a cycle ergometer (Parish and Jakeman, 1987). To further complicate these findings improvement of performance in the luteal phase of the cycle has also been reported as indicated by higher power output during a 30-s cycle sprint (Masterson, 1999).

These discrepancies in findings may be due to the lack of hormonal documentation of the cycle phase in some studies (De Bruyn-Prevost et al., 1984; Bale and Nelson, 1985; Quadagno et al., 1991; Miskec et al. 1997; Masterson, 1999). Alternatively, variations in findings between studies could be due to perimenstrual problems (Giacomoni et al., 2000) towards the end of the cycle or during the first days of the bleeding episode (Bale and Nelson, 1985; Brooks-Gunn et al., 1986; Parish and Jakeman, 1987; Masterson, 1999). Finally, lack of circulatory evidence of both estradiol and progesterone (Bunt, 1986) may also account for some of the variation in findings between studies (Lynch and Nimmo, 1998).

Another general point that characterises all the studies to date is the lack of testing just prior to ovulation when there exist high concentrations of estradiol while progesterone values remain low, in order to isolate any effects of estradiol. However, it has been well recognised (Bunt, 1990) as well as experimentally shown (Horvath and Drinkwater, 1982) that it is very difficult to test subjects just prior to ovulation as exercise testing has to take place on the same day that a luteinising hormone (LH) surge is detected (which determines the pre-ovulation period).

Given the equivocal nature of findings in the literature, the lack of information on sprint running per se, the sparse of information on repeated sprints and the perceived
methodological limitations of previous studies, the purpose of the present study is to test the hypothesis that repeated sprint running performance and metabolic responses to such exercise will be influenced by menstrual cycle phase just prior to ovulation where peak estradiol concentrations occur. Therefore, in brief subjects undertook two maximal 30-s sprints with a 2 min passive recovery period, with performance and metabolic responses recorded, during three distinct and carefully controlled phases of the menstrual cycle (figure 4.1, see methods for detail). Furthermore, in order to avoid perimenstrual problems (Giacomoni et al., 2000) testing is not conducted on the first days or the last days of the cycle.

![Hormonal Concentration vs Day of the Cycle](image)

**Figure 4.1.** A hypothetical 28 d ovulatory cycle. Dark blue arrows indicate testing. In favour of clarity the scale is hypothetical.

### 4.2. METHODS

#### 4.2.1. Subjects

Eight female physical education students volunteered to participate in the present investigation. They were all highly active and members of the university team in their sports. All but one (distance runner) of the subjects were involved in multiple sprints or power events (hockey, soccer, athletics, basketball and rugby). Their mean ± SD age, body mass and stature were 20.4 ± 1.3 y (range 18-22), 1.67 ± 0.03 m and 62.8 ± 6.0 kg, respectively. Volunteers were informed of the purpose of the study, any
known risks, benefits and the right to terminate participation at will both orally and in writing. Then an informed consent form was signed. The experimental protocol had the approval of the Ethical Committee of Loughborough University.

All the subjects were eumenorrheic and had not used oral contraceptives for at least six months (except one volunteer who had ceased oral contraceptives use 4 months prior to experiment) before their participation in experimental procedures. Their cycle length was 24-40 days. Subjects were non-smokers and were not on any medications that could interfere with the experimental procedures. At the end of the experiment each subject was asked to complete an evaluation form [Appendix Z] regarding the use of the Clear Plan Home Ovulation Test and to give her own opinion as to whether or not performance changed during her menstrual cycle phase and whether or not her training programme had remained constant throughout the study.

4.2.2. Ergometer

The non-motorised treadmill with the modifications described in methodological study 1 was employed in this investigation.

4.2.3. Familiarisations

A thorough undertaken by the subjects as described in section 3.3.1.

4.2.4. Menstrual cycle phase information

Information for normal cycle length was initially obtained by a cycle-history questionnaire [Appendix G]. Evidence of ovulation was obtained by using the Clear Plan Home Ovulation Test (Unipath Limited, Bedford U.K.), which detected (within 24-36 h) the onset of the LH surge by using urine samples. The accuracy of these kits has been tested experimentally and shown to be satisfactory (Bourne et al., 1996; Miller and Soules, 1996 and discussion in section 3.7 in general methods chapter). The Clear Plan Home Ovulation Test was used so that subjects could undertake one of the repeated sprints test when estradiol levels are high while progesterone levels are still low (figure 4.1). Depending on each participant’s cycle length, urine testing started 9-17 days after the onset of the menses and continued until the LH surge was
detected. Verification of cycle phase was obtained later by hormonal analysis of estradiol and progesterone.

4.2.5. Protocol and Experimental design

Volunteers undertook two single 30-s sprints with a 2 min passive recovery period on 3 occasions at approximately the same time of the day (one sprint test in each menstrual cycle phase) in order to minimise time of the day effect (Bernard et al., 1998; Giacomoni and Falgarette, 1999). The tests were randomised to avoid any learning effects. Subjects were tested at day 6-8 of the beginning of the menses and always after the termination of the bleeding [follicular phase trial: both estradiol and progesterone have low concentrations], prior to ovulation as detected using the Clear Plan Home Ovulation Test kit [mid-cycle trial: estradiol levels high and progesterone levels still low], and, 9-11 days after the after the mid-cycle trial [luteal phase both estradiol and progesterone levels are high]. For the mid-cycle trial the subjects rang the laboratory on the morning of the positive Clear Plan Home Ovulation Test result and reported to the laboratory for testing later on that same day. Figure 4.1 gives a schematic representation of the testing in parallel with the hormonal fluctuations.

![Figure 4.2. The schematic representation of the protocol.](image-url)

This design meant that the present study was novel in that subjects were tested just prior to ovulation when only estradiol concentration was high (Pauerstein et al.,...
which practically was a rather short period of time (about a day). The Clear Plan Home Ovulation Test kit detects not the peak LH concentration, but rather the LH surge, which has been found to be a better predictor of ovulation than the peak LH concentration (Corsan et al., 1990). The mid-cycle surge in serum LH has been shown to precede ovulation by approximately 24-36 h (World Health Organisation, 1980) a period that is in line with the urine detection of LH (Bourne et al., 1996; Miller and Soules, 1996). Finally, peak estradiol levels are attained 24-36 h prior ovulation (Pauerstein et al., 1978; World Health Task Force Investigators, 1980). Thus theoretically, the method employed in the present study should have facilitated exercise testing at approximately the time of the estradiol peak.

The performance protocol consisted of two 30-s sprints interspersed with 2 min passive recovery on the non-motorised treadmill. Prior to these sprints a standardised warm-up (for more details see section 3.5.1.2 in general methods) was followed. The experimenters were aware of each subjects' menstrual cycle phase due to the great care taken to verify cycle phases. However, the extrinsic motivation given to the subjects (i.e. verbal encouragement) was constant across phases. Figure 4.2 illustrates the schematic representation of the protocol.

4.2.6. Clinical biochemistry

Blood sampling and analysis was performed as mentioned in detail in the general methods chapter (section 3.13).

4.2.7. Maximum oxygen uptake and speed-lactate test

These tests were undertaken as described in the general methods chapter (sections 3.5.4 and 3.5.5).

4.2.8. Dietary analysis

Each participant was instructed to record her habitual diet during three representative days (two week days and one weekend day) and to repeat this prior to each test as described in the general methods section (section 3.11). For the mid-cycle test...
sometimes the diet was recorded for more than 3 days due to uncertainty as to when the LH surge would occur.

4.2.9. Statistical analysis.

A two-way analysis of variance for within subjects design was used to assess whether there were any differences in performance variables among menstrual cycle phases (main effect: cycle phase) and between the first and second sprints in each phase (main effect: sprint). One-way analysis of variance for within subjects design was used to examine if any differences existed in recovery of mechanical data among menstrual cycle phases (main effect: cycle phase). Two-way analysis of variance for within subjects design was also used to ascertain any differences in metabolic responses among menstrual cycle phase (main effect: cycle phase) and the response to each subject with respect to time (main effect: time). When significant interaction (phase by sprint, or, phase by time) was revealed the Bonferroni method was used for multiple comparisons. Relationships between variables were evaluated by means of Pearson Product Moment Correlation coefficients. Results are expressed as mean ± SD, unless otherwise stated.

4.3. RESULTS

4.3.1. Hormonal documentation of menstrual cycle

Resting serum estradiol and progesterone concentrations (figures 4.3 and 4.4) confirmed the menstrual cycle phases as indicated with the 4.3- and 3-fold increase in estradiol \((P < 0.01\) in both cases) and 2.3- and 13.2-fold increase in progesterone at the midcycle and luteal phases, respectively. Table 4.1 summarises these values.
Table 4.1. Hormonal profile for resting 17β-estradiol and progesterone during the follicular, mid-cycle and luteal phase of the menstrual cycle. Where \(^a\) indicates \(P < 0.01\) from follicular concentrations, and, \(^b\) \(P < 0.01\) from the other two concentrations (mean ± SD, \(n = 8\)).

<table>
<thead>
<tr>
<th>Phase</th>
<th>Hormone</th>
<th>Follicular</th>
<th>Mid-Cycle</th>
<th>Luteal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>progesterone (ng·mL(^{-1}))</td>
<td>0.70 ± 0.29(^b)</td>
<td>1.61 ± 0.34(^{ab})</td>
<td>9.25 ± 3.09(^{ab})</td>
</tr>
<tr>
<td></td>
<td>17β-estradiol (pg·mL(^{-1}))</td>
<td>46 ± 16(^b)</td>
<td>199 ± 54(^{ab})</td>
<td>139 ± 42(^{ab})</td>
</tr>
</tbody>
</table>

Figure 4.3. Resting progesterone concentrations during follicular, mid-cycle and luteal phase of the menstrual cycle. Where \(^a\) indicates \(P < 0.01\) from follicular concentrations, and, \(^b\) \(P < 0.01\) from the other two concentrations (mean ± SD, \(n = 8\)).
4.3.2. Performance profile

Body mass was not altered significantly due to menstrual cycle phase (62.9 ± 2 kg, 63.1 ± 2 kg and 62.8 ± 2 kg for follicular, mid-cycle and luteal, respectively, $P > 0.05$), and, therefore data are not normalised for body mass (e.g. divided by body mass).

Performance data are presented in tables 4.2 to 4.7. None of these variables was altered due to menstrual cycle phase ($P > 0.05$). However, the performance profile in sprint 1 was always higher than in sprint two ($P < 0.01$), apart from the fatigue index for power and speed which were not different ($P > 0.05$). Percentage recovery of power and percentage recovery of speed were also unaffected by menstrual cycle phase (tables 4.3 and 4.5).
Table 4.2. Power output profile [PPO, MPO and fatigue index for power (FI_{po})] during two 30-s sprints interspersed with 2 min passive recovery during the follicular, mid-cycle and luteal phase of the menstrual cycle (mean ± SD, n = 8). Where "a" indicates main effect: sprint, P < 0.01.

<table>
<thead>
<tr>
<th>Phase</th>
<th>PPO (W)</th>
<th>MPO (W)</th>
<th>FI_{po} (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SPRINT 1</td>
<td>SPRINT 2</td>
<td>SPRINT 1</td>
</tr>
<tr>
<td>follicular</td>
<td>463 ± 51a</td>
<td>395 ± 48</td>
<td>302 ± 41a</td>
</tr>
<tr>
<td>midcycle</td>
<td>443 ± 43a</td>
<td>359 ± 44</td>
<td>298 ± 37a</td>
</tr>
<tr>
<td>luteal</td>
<td>449 ± 52a</td>
<td>397 ± 48</td>
<td>298 ± 39a</td>
</tr>
</tbody>
</table>

There was a significant correlation between recovery of MPO in the mid-cycle trial and estradiol (r = 0.75, P < 0.05). On the other hand relationships between estradiol and power output data were somewhat different during luteal phase as revealed by the negative correlation with MPO (-0.72, P < 0.05). Progesterone had a significant negative relationship with PPO during the luteal phase (r = -0.76, P < 0.05 and r = -0.84, P < 0.01 for sprint 1 and 2, respectively) and MPO during the luteal phase (r = -0.84, P < 0.01, sprint 1).

Table 4.3. Recovery of power output profile [PPO, MPO and fatigue index for power (FI_{po})] during two 30-s sprints interspersed with 2 min passive recovery during follicular, mid-cycle and luteal phase of the menstrual cycle (mean ± SD, n = 8).

<table>
<thead>
<tr>
<th>Phase</th>
<th>PPO RECOVERY (%)</th>
<th>MPO RECOVERY (%)</th>
<th>FI_{po} RECOVERY (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>follicular</td>
<td>86 ± 9</td>
<td>84 ± 5</td>
<td>105 ± 16</td>
</tr>
<tr>
<td>midcycle</td>
<td>81 ± 8</td>
<td>83 ± 5</td>
<td>91 ± 18</td>
</tr>
<tr>
<td>luteal</td>
<td>89 ± 11</td>
<td>85 ± 5</td>
<td>101 ± 12</td>
</tr>
</tbody>
</table>
Table 4.4. Speed profile [(peak speed, mean speed and fatigue index for speed $F_{1sp}$)] during two 30-s sprints interspersed with 2 min passive recovery during follicular, mid-cycle and luteal phase of the menstrual cycle (mean ± SD, n = 8). Where "a" indicates $P < 0.01$ from sprint 2.

<table>
<thead>
<tr>
<th></th>
<th>peak speed (m·s$^{-1}$)</th>
<th>mean speed (m·s$^{-1}$)</th>
<th>$F_{1sp}$ (m·s$^{-2}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SPRINT 1</td>
<td>SPRINT 2</td>
<td>SPRINT 1</td>
</tr>
<tr>
<td>follicular</td>
<td>5.63 ± 0.3$^a$</td>
<td>5.28 ± 0.3</td>
<td>4.87 ± 0.3$^a$</td>
</tr>
<tr>
<td>midcycle</td>
<td>5.58 ± 0.3$^a$</td>
<td>5.15 ± 0.3</td>
<td>4.88 ± 0.3$^a$</td>
</tr>
<tr>
<td>luteal</td>
<td>5.59 ± 0.2$^a$</td>
<td>5.16 ± 0.2</td>
<td>4.82 ± 0.3$^a$</td>
</tr>
</tbody>
</table>

Mean speed had a negative correlation with estradiol in the mid-cycle ($r = -0.74$, $P < 0.05$), with a similar profile of progesterone as indicated by the negative relationships with peak speed of the first sprint, peak speed of the second sprint and mean speed of the first sprint in the luteal phase ($r = -0.90$, $P < 0.01$, $r = -0.73$, $P < 0.01$, $r = -0.76$, $P < 0.01$, respectively).

Table 4.5. Recovery of speed profile [(peak speed, mean speed and fatigue index for speed $F_{1sp}$)] during two 30-s sprints interspersed with 2 min passive recovery during follicular, mid-cycle and luteal phases of the menstrual cycle (mean ± SD, n = 8).

<table>
<thead>
<tr>
<th></th>
<th>peak speed recovery (%)</th>
<th>mean speed recovery (%)</th>
<th>$F_{1sp}$ recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>follicular</td>
<td>94 ± 4</td>
<td>90 ± 3</td>
<td>111 ± 29</td>
</tr>
<tr>
<td>midcycle</td>
<td>92 ± 2</td>
<td>90 ± 3</td>
<td>104 ± 20</td>
</tr>
<tr>
<td>luteal</td>
<td>92 ± 3</td>
<td>90 ± 3</td>
<td>111 ± 16</td>
</tr>
</tbody>
</table>

Table 4.6. Force profile (peak force and mean force) during two 30-s sprints interspersed with 2 min passive recovery during follicular, mid-cycle and luteal phases of the menstrual cycle (mean ± SD, n = 8). Where "a" indicates $P < 0.01$ from sprint 2.

<table>
<thead>
<tr>
<th></th>
<th>peak force (N)</th>
<th>mean force (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SPRINT 1</td>
<td>SPRINT 2</td>
</tr>
<tr>
<td>follicular</td>
<td>89 ± 6$^a$</td>
<td>81 ± 6</td>
</tr>
<tr>
<td>midcycle</td>
<td>88 ± 5$^a$</td>
<td>74 ± 8</td>
</tr>
<tr>
<td>luteal</td>
<td>87 ± 8$^a$</td>
<td>83 ± 9</td>
</tr>
</tbody>
</table>
Statistical analysis revealed a significant positive relationship between the recovery of mean force and estradiol only during the mid-cycle phase ($r = 0.73, P < 0.05$), while progesterone had a negative relationship with all the force variables reaching significance for the peak force in sprint 2 during the luteal phase ($r = -0.77, P < 0.05$) and mean force in sprint 1 during the luteal phase ($r = -0.84, P < 0.01$).

Table 4.7. Recovery of force profile (peak force and mean force) during two 30 s sprints interspersed with 2 min passive recovery during follicular, mid-cycle and luteal phases of the menstrual cycle (mean ± SD, $n = 8$).

<table>
<thead>
<tr>
<th></th>
<th>peak force recovery (%)</th>
<th>mean force recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>follicular</td>
<td>92 ± 7</td>
<td>93 ± 3</td>
</tr>
<tr>
<td>midcycle</td>
<td>84 ± 7</td>
<td>92 ± 3</td>
</tr>
<tr>
<td>luteal</td>
<td>85 ± 10</td>
<td>93 ± 3</td>
</tr>
</tbody>
</table>

4.3.3. Blood metabolites

All the blood metabolic responses were increased over time ($P < 0.01$). However, no change due to menstrual cycle phase was observed. Figures 4.5 to 4.8 illustrate these data.

During the follicular phase trial blood lactate after sprint 1 was positively correlated with PPO and peak speed in the first sprint ($r = 0.86, P < 0.01$, $r = 0.76, P < 0.01$) but negatively with recovery of MPO ($r = -0.88, P < 0.01$) and recovery of mean speed ($r = -0.76, P < 0.05$). Post-sprint 1 and 5 min post-sprint 2 lactate concentrations were positively correlated with the corresponding ammonia values and negatively with pH values (data not shown). These relationships were similar in the other two trials.
Figure 4.5. Venous whole blood lactate concentrations at rest, post warm-up (PWP), post-sprint 1 (PS1), post-sprint 2 (PS2) and at 5 (5), 10 (10), 15 (15), 20 (20) and 30 min (30) of recovery after the second sprint at follicular, mid-cycle and luteal phases of the menstrual cycle (mean ± SD, n = 8). Where "a" indicates $P < 0.01$ from resting concentrations.

Figure 4.6. Venous plasma ammonia concentrations at rest, post warm-up (PWP), post-sprint 1 (PS1), post-sprint 2 (PS2) and at 5 (5), 10 (10), 15 (15), 20 (20) and 30 min (30) of recovery after the second sprint at follicular, mid-cycle and luteal phases of the menstrual cycle (mean ± SD, n = 8). Where "a" indicates $P < 0.01$ from resting concentrations.
Figure 4.7. Venous whole blood pH at rest, post warm-up (PWP), post-sprint 1 (PS1), post-sprint 2 (PS2) and at 5 (5), 10 (10), 15 (15), 20 (20) and 30 min (30) of recovery after the second sprint at follicular, mid-cycle and luteal phases of the menstrual cycle (mean ± SD, n = 8). Where “a” indicates $P < 0.01$ from resting concentrations.

Figure 4.8. % Estimated plasma volume changes at rest, post warm-up (PWP), post-sprint 1 (PS1), post-sprint 2 (PS2) and at 5 (5), 10 (10), 15 (15), 20 (20) and 30 min (30) of recovery after the second sprint at follicular, mid-cycle and luteal phases of the menstrual cycle (mean ± SD, n = 8). Where “a” indicates $P < 0.01$ from resting concentrations.
4.3.4. Maximum oxygen uptake and the percentage of maximum oxygen uptake at a blood lactate concentration of 4 mmol·L⁻¹ (% \( \dot{V}O_2\) max @ 4 mmol·L⁻¹).

Table 4.8. summarises the subjects' \( \dot{V}O_2\) max as well as % \( \dot{V}O_2\) max @ 4 mmol·L⁻¹ together with their training background. A significant positive correlation was found between % \( \dot{V}O_2\) max @ 4 mmol·L⁻¹ and MPO recovery during the follicular phase (\( r = 0.72, P < 0.05 \)) and during the mid-cycle phase (\( r = 0.89, P < 0.01 \)), while \( \dot{V}O_2\) max was significantly correlated with recovery of PPO during luteal phase (\( r = 0.82, P < 0.05 \)).

Table 4.8. \( \dot{V}O_2\) max, % \( \dot{V}O_2\) max @ 4 mmol·L⁻¹ and training background of the subjects.

<table>
<thead>
<tr>
<th>SUBJECTS</th>
<th>( \dot{V}O_2) max (mL·kg⁻¹·min⁻¹)</th>
<th>% ( \dot{V}O_2) max @ 4 mmol·L⁻¹ (%)</th>
<th>SPORT</th>
<th>LEVEL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>49.3</td>
<td>92</td>
<td>orienteering</td>
<td>recreational</td>
</tr>
<tr>
<td>2</td>
<td>47.2</td>
<td>91</td>
<td>football</td>
<td>varsity</td>
</tr>
<tr>
<td>3</td>
<td>52.2</td>
<td>83</td>
<td>hockey</td>
<td>varsity</td>
</tr>
<tr>
<td>4</td>
<td>62.5</td>
<td>84</td>
<td>400/800 m</td>
<td>national</td>
</tr>
<tr>
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<td>51.0</td>
<td>68</td>
<td>Basketball</td>
<td>varsity</td>
</tr>
<tr>
<td>6</td>
<td>41.2</td>
<td>69</td>
<td>hockey</td>
<td>recreational</td>
</tr>
<tr>
<td>7</td>
<td>47.7</td>
<td>73</td>
<td>400 m</td>
<td>varsity</td>
</tr>
<tr>
<td>8</td>
<td>49.9</td>
<td>85</td>
<td>rugby</td>
<td>national</td>
</tr>
<tr>
<td>MEAN±SD</td>
<td>50.1 ± 6.0</td>
<td>81 ± 6.0</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>RANGE</td>
<td>41-62</td>
<td>68-92</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>
4.3.5. Dietary analysis
The results from dietary analysis from habitual diet (2 week days and one weekend day) are depicted in figure 4.9.

![Figure 4.9. Percentage of fats, carbohydrates (CHO) and proteins (PRO) in habitual diet (n = 7).](image)

4.4. DISCUSSION

The principal finding of the present study was that the performance profile during two maximal 30-s sprints with 2 min passive recovery was not altered by the hormonal fluctuations of estradiol and progesterone. In addition, the blood metabolic responses to a repeated sprint were also unaffected by menstrual cycle phase.

In the present study the menstrual cycle phase was successfully verified using serum estradiol and progesterone. The resting levels of serum estradiol and progesterone (table 4.1) are within the reference range for eumenorrhic women in the respective phases (Johanson et al., 1971; Landgren et al., 1980, DPC, 1998). Moreover, for each individual, progesterone elevations $\geq 5$ ng·mL$^{-1}$ were accepted as indication of ovulation (Landgren et al., 1980; DPC, 1998). Determination of cycle phase using conventional methods such as basal body temperature is not considered a reliable method for ovulation detection (Bauman, 1981). In addition, basal body temperature identifies ovulation after the event, making its use impractical for experiments like the present investigation. Using basal body temperature is also possible to test when estradiol is high and progesterone low but the progesterone levels will be above 2
ng·mL⁻¹ and therefore will start adverse the positive effects as it was the case prior to ovulation (Simon et al., 1987). Studies have used the calendar method assuming a normal cycle length must be also treated with caution, since a cycle that is normal in length can be anovulatory (Johanson et al., 1971; Landgren et al., 1980). Moreover, considerable variation exists in the timing of ovulation, and thus the hormonal fluctuations, from cycle to cycle even in the same woman (Treolar et al., 1967; Vollman, 1977). Thus, a combination of indirect (Clear Plan Home Ovulation Test kit and cycle history questionnaire) and direct (hormonal documentation) methods is of crucial importance when the precise timing of experimental procedures in the context of menstrual cycle phase are to be performed. The need for a well-controlled methodology is even greater when the subjects are young (≈ 20 years old) as in the present study because of the high incidence of anovulatory cycles that occur about that age (Collett et al., 1954).

Performance results in the follicular phase are similar to those published previously for female subjects using the same ergometer and a similar experimental design. PPO in the present study (table 4.2) was 463 ± 18 W in comparison with 419 ± 25 W (endurance trained females) and 619 ± 30 W (sprint trained females) (Nevill and others, 1996a). The slightly different values probably reflect the training and performance specialisms of subjects in earlier studies (Nevill et al., 1996a) in comparison with the wider range of physiological characteristics in the present study [\( \dot{V}O_2 \text{max} \text{ (mL·kg}^{-1}·\text{min}^{-1}) : 41-62, \% \dot{V}O_2 \text{ max} \text{ @ 4 mmol·L}^{-1} \text{ (%) : 68-93, PPO (W) : 345-503}.\]

The lack of impact of menstrual cycle phase on sprint running performance is in agreement with several earlier studies using different exercise modes (De Bruyn-Prevost et al., 1984; Quadagno et al., 1991; Miskec et al., 1997; Lynch and Nimmo, 1998; Giacomoni et al., 2000) and when subjects did not present with premenstrual or menstrual syndrome (Giacomoni et al., 2000). However, the present findings of the lack of impact of menstrual cycle phase on sprint running performance contrast with the findings of Parish and Jakeman (1987), Masterson (1999), Bale and Nelson (1985) and Brooks-Gunn and co-workers (1986). However, these previously reported studies did not undertake hormonal verification of cycle phase. In addition, in one
CHAPTER 4

MENSTRUAL CYCLE AND SPRINT PERFORMANCE

project (Masterson, 1999) perimenstrual problems as consequence of testing in the first two days of the cycle were evident. Such perimenstrual problems are associated with sharp decreases of estradiol and its potential link with pain and stiffness in the last few days of the cycle and at the beginning of the menses (Halbreich et al., 1986). The impairment of physical performance in the late luteal phase due to premenstrual syndrome could also explain the previously reported improved swimming performance during menstruation over the premenstruation period (Brooks-Gunn et al., 1986). In their protocol menstruation was defined as day 8 and premenstruation as 4 d prior to onset of the menses. However, great importance has to be given to the definitions of the phases. The term “menstruation” has usually been reserved for the days where the bleeding occurs (e.g. Masterson, 1999; Giacomoni et al., 2000), but Brooks-Gunn and colleagues (1986) considered day 8 as menstruation which has contributed to a misleading interpretation of their results. The improved performance of the swimmers during day 8 in comparison to 4 days prior to the menses of the menstrual cycle may be due to perimenstrual pain 4 days prior to the menses, which was not reported by the authors. Finally, the studies of Parish and Jakeman (1987) and Bale and Nelson (1985) used exercise tests which are generally thought to be too daunting for subjects to perform maximally from the onset of exercise, thus questioning their findings with respect to sprinting performance.

It was hypothesised in the introduction that estradiol has muscle-strengthening effects and that force will be increased just prior to ovulation (mid-cycle trial in the present experiment). According to this hypothesis power output would also be increased since force $\times$ speed = Power. This scenario was based on the research of Philips et al., (1996) and Sarwar et al. (1996), who claimed that estradiol can alter the negative feedback of Pi upon cross-bridges kinetics [Pi increases the rate of cross-bridge detachment relative to attachment, decreases both cycle time and the proportion of bridge in the force-generating state (Pate and Cooke, 1989; Hellestrand, 1996)]. Inorganic phosphate (Pi) has also been postulated as a limiting factor in high-intensity whole body exercise (MacLaren et al., 1989; Maughan et al., 1997) even though its independent effects are difficult to study in in vivo experimental perturbations due to simultaneous depletion of PCr and Pi in such exercise (Bogdanis et al., 1995). Nevertheless, it has recently been demonstrated that recovery of power output is not associated with Pi elevations during repeated 30-s sprints (Bogdanis et al., 1996a). It
is also unclear whether the response of sedentary subjects, as of Philips et al. (1996) and Sarwar et al. (1996), differs from that of elite athletes or/and Philips et al. (1996) and Sarwar et al. (1996) had different ratio of estradiol and progesterone due to the lack of hormonal documentation of menstrual cycle phase in their studies. In the present study, progesterone did not seem to play any role in improvement of force generation since they were not changes in performance during the mid-cycle and significant negative correlations were revealed between progesterone and PPO in the first sprint (-0.76, \( P < 0.05 \)), second sprint (-0.84, \( P < 0.01 \)) during the luteal phase of the menstrual cycle. However, there was a significant positive correlation between resting estradiol concentrations during the mid-cycle phase and recovery of MPO (0.75, \( P < 0.05 \)) reflecting some association between ovarian hormones and recovery of performance.

It has been speculated that well-trained individuals (in comparison with recreationally active or untrained subjects) may respond differently in terms of performance during various phases of the cycle (Schoene et al., 1981). However, the protocol adopted for the present investigation could only be performed by trained volunteers (Loughborough Ethical Committee comments), so it is uncertain whether sprinting performance would have been affected if sedentary subjects had participated. It is well known that high-intensity endurance and resistance training may reduce resting circulatory estradiol (Consitt et al., 2002) and the physiological significance of reducing resting estradiol is unknown. Beidleman et al. (1999) postulated that one of the possible reasons for the differences between their results and those of Williams and Krahenbuhl (1997) concerning oxygen cost, were the different ratios of estradiol to progesterone. Thus, it is uncertain whether the longitudinal effects of the training routine that each individual undertook as part of their sports training programmes could influence the circulatory levels of these hormones and, if these changes could affect the results in this experiment.

The present study did not reveal any influence of menstrual cycle on recovery of performance (tables 4.2 - 4.7). There are postulations relative to the connection of ovarian hormones and PCr recovery rates as suggested by the slower rates of plantar flexion exercises in amenorrheic-endurance athletes in comparison with eumenorrheic (Harber et al., 1998). Nonetheless, neither in this study nor in the Giacomoni et al.,
(2000) study was the recovery of performance influenced by high levels of ovarian hormones suggesting that in whole body maximal exercise estradiol and progesterone are not related to the recovery of PPO. No relationship was found between PPO recovery and estradiol in the present study and significant negative relationships were found between progesterone and PPO in first sprint (-0.76, P < 0.05), PPO in the second sprint (-0.84, P < 0.01) and peak speed in the first and seconds sprints (-0.90, P < 0.01 and -.73, P < 0.05, respectively) during the luteal phase of the menstrual cycle. However, there was a significant positive correlation between resting estradiol concentrations at mid-cycle phase and recovery of MPO (0.75, P < 0.05) reflecting some association between ovarian hormones and recovery of performance.

Finally, blood metabolites were not altered as a result of cycle phase in the present study suggesting that possibly muscle metabolic responses did not change as well. This was an expected outcome since estradiol has been linked with glycogen sparing in favour of fat oxidation at intensities < 70 % \( \dot{V}O_2 \) max (Nicklas et al., 1989; Hackney, 1999) but the contribution of fat (Greenhaff et al., 1993) and exogenous glucose (Katz et al., 1986) is negligible during maximal intensity exercise. These results are in agreement with previous observations examining the effects of menstrual cycle phase on metabolic responses after short-intense exercise (De Bruyn-Prevost et al., 1984; Miskec et al., 1997; Lynch and Nimmo, 1998).

In conclusion, this study has shown that performance during two 30-s running sprints separated by 2 min passive recovery is unaffected by menstrual cycle phase. Furthermore, blood metabolites following such a repeated sprint were also unaffected by menstrual cycle phase, suggesting that in future studies with trained athletes it is not necessary to control the timing of testing due to the menstrual cycle phase.
CHAPTER 5: THE EFFECTS OF ENDURANCE TRAINING ON POWER OUTPUT RECOVERY AND BLOOD METABOLIC RESPONSES AFTER SPRINTING.

5.1. INTRODUCTION

The importance of recovery from maximal exercise was first stated by the Ancient Greek Philosopher and coach Flavius Philostratus in his book “Handbook for the Athletics Coach and Gymnasticus” (Philostratus, A.D. 170-240). This statement has been fully confirmed by contemporary sport scientists and it is now recognised that the processes occurring during recovery from exercise are as important as those occurring during exercise itself (Bowers and Fox, 1992) both in terms of the quality of any subsequent performance and in terms of furthering understanding of physiological mechanisms. The ability to recover quickly is of great significance especially when physical activities require multiple sprints interspersed with small recovery periods (Tomlin and Wenger, 2001).

In spite of the fact that recovery from sub-maximal exercise has been examined by physiologists since the early sixties (e.g. Astrand et al., 1960; Christensen et al., 1960), research examining recovery from maximal (sprint) intensity exercise did not commence until the eighties (e.g. Wootton and Williams, 1983; McCartney et al., 1983). This delay was not due to the lack of interest on this type of exercise but because of methodological limitations, namely inappropriate protocols (Cheetham et al., 1986) and the lack of a suitable ergometer for the study of maximal intensity exercise (Nevill et al., 1996b).

Since these methodological difficulties have been overcome authors have begun to examine the factors that govern recovery from sprint exercise and subsequently the aetiology of fatigue. For example, Bogdanis, Nevill, Boobis and Lakomy (1996a) found that power output recovery after a 30 s sprint was associated with endurance fitness as indicated by the significant relationship between % $\dot{V}O_2 max @ 4 mmol\cdot L^{-1}$ and % MPO (10 s) and % SP (10 s) ($r = 0.75-0.94; P < 0.05$ to $P < 0.01$). In addition, the same research group found similar correlations between muscle PCr recovery after sprinting and % $\dot{V}O_2 max @ 4 mmol\cdot L^{-1}$ %. These results have been confirmed by
other authors utilising various maximal intensity exercise protocols (Hakkinen et al., 1990; Hamilton et al., 1991; Dawson et al., 1993; Bogdanis et al., 1995; Bogdanis et al., 1996a; Hoffman et al., 1997; Hoffman et al., 1999; Tomlin and Wenger, 2002) and imply that endurance training could be beneficial for power athletes.

The importance of endurance training for athletes participating in “multiple-sprint” sports such as soccer, hockey, rugby, basketball is recognised by training experts (Thoden, 1991; Plisk, 1991; Hedrick, 1999). In addition, in individual sports endurance fitness can also be crucial in facilitating quality repetitions during training (Bogdanis, 1996).

Moreover, endurance training per se has been shown to increase various metabolic variables such as muscle blood flow (Ekblom and Hermansen, 1968), muscle capillarisation (Anderson and Henriksson, 1977a; Saltin and Rowell, 1980), mitochondrial number, size and surface area (Holloszy and Coyle, 1984) and oxygen extraction from the blood (Magel et al., 1978). It is believed that such metabolic changes provide the underlying mechanisms for the beneficial effects of aerobic training in enhancing power output recovery after sprinting (Tomlin and Wenger, 2001).

Although the literature appears compelling with respect to the positive benefits of endurance fitness on power output recovery, all information has been collected from studies which are cross-sectional in nature and which use sprint cycling as the exercise mode. In addition, very little data exist for female subjects and for sprint running per se. Thus, the purpose of the present study is to test the hypothesis that short-term (6 weeks) endurance training will enhance power output recovery from a maximal 30-s sprint.
5.2 METHODS

5.2.1. Subjects
Fifteen female physical education students volunteered to participate in the present investigation. All the participants were members of the university teams in their respective sports. They were predominantly games players, although a small number participated in individual sports and field events (sprints, long jump and hammer throw). Their mean ± SD age, stature and body mass were 24 ± 4, 1.67 ± 0.09 and 63.5 ± 9.6. Volunteers were informed of the purpose of the study, any known risks, both orally and in writing, and of their right to terminate participation at will. Then an informed consent form was signed. The experimental protocol had the approval of the Ethical Committee of Loughborough University.

5.2.2. Ergometers
The non-motorised treadmill (section 3.4.1) with the modifications presented in methodological study 1 was employed in this investigation. For the \( \dot{V}O_2 \) max, speed-\( \dot{V}O_2 \) and speed-lactate tests the motorised treadmill, as described in section 3.4.2, was used.

5.2.3. Familiarisations
A thorough habituation as described in section 3.3 was followed here.

5.2.4. \( \dot{V}O_2 \)max and speed-lactate test
A detailed description of these procedures is given in the general methods chapter and more particularly in sections 3.5.3-3.5.5.

5.2.5. Protocol and experimental design
Volunteers reported to the laboratory after an overnight fast and 48 h after completing the \( \dot{V}O_2 \) max and speed-lactate test. Anthropometry, environmental conditions, control of diet and, metabolic calculations were all undertaken as described in the general methods.

The performance protocol consisted of two 30-s sprints interspersed with a 2 min passive recovery on the nonmotorised treadmill. Prior to these sprints a standardised
warm-up (for more details see section 3.5.2.1 in general methods) was followed. Figure 5.1 illustrates this protocol. This test was performed before and after 6 weeks of training as described in the general methods (section 3.5.6). Finally, the time plan for this study is illustrated in figure 5.2.

Figure 5.1. The schematic representation of the protocol.
<table>
<thead>
<tr>
<th>WEEK</th>
<th>MON</th>
<th>TUE</th>
<th>WED</th>
<th>THU</th>
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<th>SAT</th>
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<tbody>
<tr>
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<tr>
<td>3</td>
<td>TRIAL 1</td>
<td></td>
<td>VO2max-1</td>
<td>SPLA-T 1</td>
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<td></td>
<td>VO2max-2</td>
<td>SPLA-T 2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 5.2. Time plan for the present experimental design. Where F indicates familiarisations as described in paragraph 3.3.1. VO2max-1: $\dot{V} O_2 \text{ max}$ test before training; VO2max-2: $\dot{V} O_2 \text{ max}$ test after training; SPLA-T 1: speed-lactate test before training; SPLA-T 2: speed-lactate test after training.

5.2.6. Menstrual cycle information
Menstrual cycle information was obtained initially from menstrual cycle phase questionnaires [Appendix G]. Verification of menstrual cycle phase was obtained later by hormonal analysis of estradiol and progesterone.

5.2.7. Clinical biochemistry
Collection, treatment, storage and analysis of blood samples were performed as mentioned in detail in General Methods Chapter (section 3.13).

5.3.8. Statistical analysis
A three-way analysis of variance for mixed subjects design -within (before and after training) and between subjects (between training and control group)- was applied to assess whether there were any differences in performance before and after training (main effect: training), between the first and second sprint (main effect: sprint) and between training and control group (main effect: group). Two-way analysis of variance for mixed subjects design –within (before and after training) and between subjects (between training and control group)- was used to examine if any differences
existed in recovery of mechanical data before and after training (main effect: training) and between groups (main effect: group). Three-way analysis of variance for mixed subjects design - within (before and after training) and between subjects (between training and control group) - was also employed to ascertain any variations in metabolic responses to endurance training (main effect: training), the response to each group with respect to time (main effect: time) and between training and control group (main effect: group). When significant interactions (training by sprint by group, or, training by group, or, training by time by group) were found adjusted unpaired t-tests, corrected by the Bonferroni method, were used to detect the differences. The relationships between variables were evaluated using the Pearson Product Moment Correlation coefficients. Results are expressed as mean ± SD, unless otherwise stated.

5.3. RESULTS

5.3.1. ENDURANCE TRAINING ADAPTATIONS

5.3.1.1. Training volume
Volunteers in the training group exercised for approximately 72 ± 15 min in the 1st week (3 sessions a wk) to 85 min ± 8 min in the last week of training. They ran 13 500 m (1st week) and 16 900 m in the last week. Figures 5.3 and 5.4 give a detailed graphical representation of these values.

![Graph](Figure 5.3. Treadmill running time (min) for each week of training for the training group (means ± SD, n = 8).)
5.3.1.2. $\dot{V} O_2\text{max}$

$\dot{V} O_2\text{max}$ (figure 5.5) was increased 3 % in the training group (from 48.7 ± 4.4 to 50.17 ± 5.1 mL·kg$^{-1}$·min$^{-1}$ post-trial) and decreased by 1.9 % in the control group (from 50.4 ± 1.3 to 49.4 ± 1.2 mL·kg$^{-1}$·min$^{-1}$ post-trial). These changes in $\dot{V} O_2\text{max}$ were significant differently different between the groups in both relative and absolute terms (training by group interaction, $P < 0.05$).
Maximal heart rate values were decreased 2 beats·min⁻¹ in the training group after training, while in the control group were increased 2 beats·min⁻¹ (from 187 vs 191 to 185 vs 194 beats·min⁻¹, in training group vs control group, before and after training respectively, training by group, $P = 0.061$).

Maximal ventilation increased 6.2 % after training in the training group while decreased 5.8 % in the control group (training by group interaction, $P < 0.01$).

Finally, the ventilatory equivalent for oxygen was 1.6 % higher in training group and reduced 4.8 % in the control group (training by group, $P = 0.095$). All these results can be seen in table 5.1.
Table 5.1. $\dot{V} O_2 max$ (mL· kg$^{-1}$· min$^{-1}$) test physiological profile in training group (TG,) and control group (CG), pre (PRE) and post (POST) training. Where “c”: training by group interaction, ($P < 0.05$). (mean ± SD, n = 8 for TG, n = 7 for CG).

<table>
<thead>
<tr>
<th></th>
<th>$\dot{V} O_2 max$</th>
<th>$\dot{V} E$</th>
<th>$\dot{V} E/\dot{V} O_2$</th>
<th>Heart rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TG</td>
<td>CG</td>
<td>TG</td>
<td>CG</td>
</tr>
<tr>
<td>PRE</td>
<td>48.7 ± 4.4</td>
<td>50.4 ± 1.3</td>
<td>90.5 ± 5.9</td>
<td>97.8 ± 5.6</td>
</tr>
<tr>
<td>POST</td>
<td>50.1 ± 5.1</td>
<td>49.4 ± 1.2</td>
<td>95.6 ± 8.5</td>
<td>92.2 ± 6.4</td>
</tr>
</tbody>
</table>

5.3.1.3. Speed-lactate test

The physiological responses to sub-maximal speed-lactate test are summarised in table 5.2. $\dot{V} O_2$ during the 16 min test was elevated during each stage (main effect: time, $P < 0.01$) but remained unaltered after the six weeks experimental period. Similarly, $\dot{V} E$ and $\dot{V} E/\dot{V} O_2$ were increased during exercise ($P < 0.01$) but were unaffected by training. Perceived exertion, R and heart rate were increased during the test ($P < 0.01$) but were unaffected by training. Blood lactate during the sub-maximal test tended to be lower after training with the differences being most evident at the two fastest running speeds (time by group by training interaction, $P = 0.074$, table 5.2). Figures 5.6 and 5.7 depict these trends.

The % $\dot{V} O_2 max @ 4$ mmol-L$^{-1}$ was 3 % higher in the training group (from 82 to 84 %) as compared with 1 % decrease in control group (from 81 to 80 %). These changes in % $\dot{V} O_2 max @ 4$ mmol-L$^{-1}$ due to endurance training showed a strong tendency towards statistical significance (training by group interaction, $P = 0.058$).
Table 5.2. Metabolic and psychological profile of the training group (n = 8) and control group (n = 7) before (PRE) and after training (POST). Where, 4, 8, 12 and 16 indicate collection min during the 16 min speed-lactate test. \( \dot{V} O_2 \), \( \dot{V} E \), \( \dot{V} CO_2 \), \( O_2/\dot{V} E \), is in mL·kg\(^{-1}\)·min\(^{-1}\), while lactate in mmol·L\(^{-1}\) (mean ± SD).

<table>
<thead>
<tr>
<th></th>
<th>TRAINING GROUP</th>
<th></th>
<th>CONTROL GROUP</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PRE</td>
<td>POST</td>
<td>PRE</td>
<td>POST</td>
</tr>
<tr>
<td>( \dot{V} O_2 )</td>
<td>26.4</td>
<td>31.4</td>
<td>36.5</td>
<td>40.8</td>
</tr>
<tr>
<td>± 9.5 ± 9.7 ± 10.4 ± 10.2 ± 9.3 ± 9.6 ± 10.2 ± 10.2 ± 5.7 ± 3.3 ± 2.6 ± 2.7 ± 4.0 ± 2.5 ± 1.9 ± 1.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \dot{V} CO_2 )</td>
<td>25.4</td>
<td>31.2</td>
<td>36.0</td>
<td>44.2</td>
</tr>
<tr>
<td>± 4.2 ± 4.1 ± 3.9 ± 4.9 ± 4.0 ± 3.8 ± 5.5 ± 5.7 ± 4.5 ± 2.1 ± 3.0 ± 2.2 ± 3.5 ± 2.4 ± 2.5 ± 2.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \dot{V} E )</td>
<td>40.6</td>
<td>50.0</td>
<td>59.1</td>
<td>73.1</td>
</tr>
<tr>
<td>± 6.6 ± 8.6 ± 11.8 ± 12.6 ± 5.5 ± 6.7 ± 9.6 ± 10.3 ± 8.4 ± 7.8 ± 10.8 ± 11.9 ± 6.7 ± 7.4 ± 10.3 ± 12.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \dot{V} O_2/\dot{V} E )</td>
<td>22.5</td>
<td>23.6</td>
<td>24.8</td>
<td>27.2</td>
</tr>
<tr>
<td>± 1.3 ± 2.0 ± 2.2 ± 2.5 ± 1.8 ± 1.7 ± 2.3 ± 2.4 ± 2.0 ± 1.4 ± 2.2 ± 2.0 ± 1.9 ± 1.9 ± 2.3 ± 2.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>0.89</td>
<td>0.92</td>
<td>0.93</td>
<td>1.01</td>
</tr>
<tr>
<td>± 0.03 ± 0.02 ± 0.07 ± 0.04 ± 0.03 ± 0.02 ± 0.04 ± 0.04 ± 0.02 ± 0.03 ± 0.03 ± 0.03 ± 0.03 ± 0.04 ± 0.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RPE</td>
<td>10</td>
<td>12</td>
<td>14</td>
<td>16</td>
</tr>
<tr>
<td>± 1 ± 1 ± 2 ± 1 ± 1 ± 1 ± 1 ± 1 ± 2 ± 2 ± 2 ± 2 ± 1 ± 2 ± 1 ± 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>heart rate</td>
<td>142</td>
<td>158</td>
<td>171</td>
<td>179</td>
</tr>
<tr>
<td>± 16 ± 13 ± 11 ± 9 ± 10 ± 11 ± 12 ± 10 ± 17 ± 10 ± 6 ± 4 ± 17 ± 13 ± 6 ± 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>lactate</td>
<td>1.9</td>
<td>2.0</td>
<td>3.1</td>
<td>5.8</td>
</tr>
<tr>
<td>± 0.7 ± 0.7 ± 1.4 ± 0.6 ± 0.6 ± 0.8 ± 1.7 ± 0.6 ± 0.5 ± 0.5 ± 0.6 ± 0.6 ± 0.6 ± 0.6 ± 0.6 ± 1.9</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
Figure 5.6. The speed-lactate test with an example of the speeds that correspond to 4 mmol·L⁻¹ before (PRE) and after (POST) training in the training group (means ± SD, n = 8).

Figure 5.7. The speed-lactate test with an example of the speeds that correspond to 4 mmol·L⁻¹ before (PRE) and after (POST) training in the control group (means ± SD, n = 8).
5.3.2. SPRINT PERFORMANCE RESPONSES

Body mass was unaltered after six weeks of training in both the training (64.2 ± 11.1 vs 63.8 ± 10.6 kg before and after training, respectively, ns) and control group (62.7 ± 8.3 vs 62.0 ± 7.5 kg before and after training, respectively, ns). Therefore performance parameters are presented in absolute terms (e.g. not divided by body mass).

The performance profile in the first sprint was unaltered during the experimental period (for all subjects, table 5.3).

There was no difference in PPO (first sprint) or PPO recovery as a result of training. However, recovery of MPO was higher after six weeks in the training group in comparison with the control group (main effect: training by group interaction, \( P < 0.05 \)). Also, fatigue index for power was unchanged as a result of training (main effect: training by group interaction, \( P > 0.05 \)). Table 5.3 summarises these data.

Similarly there were no differences in peak speed (sprint 1 or 2) or in peak speed recovery as a result of training (although there was a tendency for training by group interaction, for the recovery of peak speed \( P = 0.113 \)). There was no difference in mean speed (sprint 1 or 2) as a result of training, but there a strong tendency for an improvement in the recovery of mean speed (training by group interaction, \( P = 0.082 \)). No differences were found for fatigue index for speed. These results are shown at table 5.3.
Table 5.3. Sprint Performance parameters for the training group (TG, mean ± SD) and control group (CG) before and after training. Where "c": training by group interaction (P < 0.05); FI_po = fatigue index for power; FI_sp = fatigue index for speed. (mean ± SD, n = 8 for TG, n = 7 for CG).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>TG PRE</th>
<th>TG POST</th>
<th>CG PRE</th>
<th>CG POST</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPO (1st sprint) (W)</td>
<td>462 ± 85</td>
<td>461 ± 46</td>
<td>443 ± 66</td>
<td>445 ± 88</td>
</tr>
<tr>
<td>PPO (2nd sprint) (W)</td>
<td>417 ± 63</td>
<td>453 ± 63</td>
<td>355 ± 56</td>
<td>366 ± 51</td>
</tr>
<tr>
<td>RECOVERY OF PPO (%)</td>
<td>91 ± 11</td>
<td>98 ± 8</td>
<td>81 ± 12</td>
<td>83 ± 11</td>
</tr>
<tr>
<td>FI_po (1st sprint) (%)</td>
<td>48 ± 9</td>
<td>56 ± 5</td>
<td>53 ± 4</td>
<td>47 ± 8</td>
</tr>
<tr>
<td>FI_po (2nd sprint) (%)</td>
<td>55 ± 11</td>
<td>57 ± 7</td>
<td>52 ± 5</td>
<td>52 ± 7</td>
</tr>
<tr>
<td>MPO (1st sprint) (W)</td>
<td>321 ± 31</td>
<td>320 ± 44</td>
<td>302 ± 35</td>
<td>309 ± 42</td>
</tr>
<tr>
<td>MPO (2nd sprint) (W)</td>
<td>260 ± 28</td>
<td>280 ± 37</td>
<td>242 ± 16</td>
<td>252 ± 27</td>
</tr>
<tr>
<td>RECOVERY OF MPO (%)</td>
<td>81 ± 3</td>
<td>88 ± 6</td>
<td>81 ± 6</td>
<td>82 ± 4</td>
</tr>
<tr>
<td>Peak Speed (1st sprint) (m·s⁻¹)</td>
<td>5.70 ± 0.34</td>
<td>5.66 ± 0.23</td>
<td>5.55 ± 0.34</td>
<td>5.45 ± 0.38</td>
</tr>
<tr>
<td>Peak Speed (2nd sprint) (m·s⁻¹)</td>
<td>5.36 ± 0.24</td>
<td>5.54 ± 0.31</td>
<td>5.10 ± 0.24</td>
<td>5.07 ± 0.28</td>
</tr>
<tr>
<td>RECOVERY OF Peak Speed (%)</td>
<td>94.2 ± 2.3</td>
<td>97.6 ± 2.5</td>
<td>92.0 ± 5.3</td>
<td>93.0 ± 2.5</td>
</tr>
<tr>
<td>FI_sp (1st sprint) (%)</td>
<td>24 ± 6</td>
<td>26 ± 5</td>
<td>25 ± 5.63</td>
<td>22 ± 5.15</td>
</tr>
<tr>
<td>FI_sp (2nd sprint) (%)</td>
<td>33 ± 9</td>
<td>32 ± 5</td>
<td>28 ± 4.42</td>
<td>28 ± 5.61</td>
</tr>
<tr>
<td>Mean Speed (1st sprint) (m·s⁻¹)</td>
<td>5.04 ± 0.34</td>
<td>4.99 ± 0.28</td>
<td>4.92 ± 0.22</td>
<td>4.89 ± 0.32</td>
</tr>
<tr>
<td>Mean Speed (2nd sprint) (m·s⁻¹)</td>
<td>4.48 ± 0.33</td>
<td>4.61 ± 0.31</td>
<td>4.36 ± 0.09</td>
<td>4.37 ± 0.23</td>
</tr>
<tr>
<td>RECOVERY OF Mean Speed (%)</td>
<td>88.8 ± 4.0</td>
<td>92.4 ± 2.3</td>
<td>89.0 ± 3.5</td>
<td>89.0 ± 1.6</td>
</tr>
</tbody>
</table>

5.3.3. BLOOD METABOLIC RESPONSES

All the blood metabolic responses changed over time (P < 0.01), which reflects the influence of sprinting on the selective metabolic indexes measured in the present study.

Whole blood lactate responses are illustrated in figure 5.8. There were not any alterations in lactate time course due to training in either group (main effect: training, ns), or by comparing the two groups (training by group, ns). Mean peak whole blood lactate concentration was 15.2 ± 2.4 vs 14.8 ± 2.0 mmol·L⁻¹ in the training group and 16.7 ± 1.8 vs 16.6 ± 1.4 mmol·L⁻¹ in the control group, before and after the six weeks experimental period (main effect: group, P = 0.061).
CHAPTER 5  ENDURANCE TRAINING AND PO RECOVERY

Figure 5.8. Whole blood lactate responses after each sprint and recovery from the sprints, before (PRE) and after (POST) six weeks of experimental period in training (TG) and control (CG) group; “b” = main effect time, \( P < 0.01 \); PWP = post warm-up; PS1 = post-sprint 1; PS2 = post-sprint 2; 5, 10, 15, 20 and 30 = min during recovery from sprints in which blood sampling took place. (mean ± SD, \( n = 8 \) for TG, \( n = 7 \) for CG).

Blood pH values are depicted in figure 5.9. Blood pH had a tendency to be higher during recovery in the training group after training (figure 5.9, training by time by group, \( P = 0.082 \)). The lowest pH values were 7.04 vs 6.97 and 7.05 vs 7.01 before and after training in the training group in comparison with the control group, respectively (figure 5.9).

Blood buffering capacity was 4 % higher (ns) during the first sprint after training in the training group and did not change in the control group (26.8 ± 6.8 and 30.3 ± 8.2 vs 30.9 ± 5.1 and 30.8 ± 2.4 \( \mu \text{mol} \cdot \text{L}^{-1} \cdot \text{pH} \text{unit}^{-1} \) before and after training in training group vs control group, respectively). In the 2nd sprint Blood buffering capacity was rather similar in terms of changes between groups (42.0 ± 2.5 and 43.0 ± 4.6 vs 39.6 ± 5.3 and 41.6 ± 5.4 \( \mu \text{mol} \cdot \text{L}^{-1} \cdot \text{pH} \text{unit}^{-1} \) before and after training in training group vs control group, respectively, ns).
Fig. 5.9. Blood pH responses after each sprint and recovery from the sprints, before (PRE) and after (POST) six weeks of experimental period in training (TG) and control (CG) group; \( b \) = main effect time, \( P < 0.01 \); PWP = post warm-up; PS1 = post-sprint 1; PS2 = post-sprint 2; 5, 10, 15, 20 and 30 = min during recovery from sprints in which blood sampling took place. (mean ± SD, n = 8 for TG, n = 7 for CG).

Training had no effect on plasma ammonia (time by training by group interaction, ns) as compared training group vs control group. In general, the control group appeared to have slightly higher (ns) ammonia concentrations (135.2 ± 48.1 vs 175.5 ± 40.9, and 143.0 ± 42.6 vs 167.1 ± 37.7 μmol·L⁻¹, in training group vs control group, pre- and post-training, respectively). Figure 5.10 illustrates these results.
Estimated changes in plasma volume. There was a 17 to 20 % estimated percentage loss in plasma volume due to the first sprint with further 1-4 % losses accompanying the 2nd sprint (main effect: time, $P < 0.01$, figure 5.11). Finally, training did not produce changes in the estimated decrease in plasma volume as a result of sprinting in either group (ns).
Figure 5.11. Estimated percentage changes of plasma volume responses after each sprint and recovery from the sprints, before (PRE) and after (POST) six weeks of experimental period in training (TG) and control (CG) group; \( \text{PRE-TG} \); \( \text{POST-TG} \); \( \text{PRE-CG} \); \( \text{POST-CG} \). (mean ± SD, \( n = 8 \) for TG, \( n = 7 \) for CG).

5.3.4. MENSTRUAL CYCLE PHASE INFORMATION

Table 5.4 tabulates individual values from the radioimmunoassays of estradiol and progesterone for both groups. Subjects No 2, No 8 (training group) and No 2, No 7 (control group) have progesterone values higher than 5 ng·mL\(^{-1}\) post-training, suggesting that these volunteers were in luteal phase of their menstrual cycle. Subject 4 (control group) was probably at the mid-cycle phase before training and the luteal phase after training. Finally, in the rest of the trials volunteers had very low values of both hormones either because they were in the follicular phase or due to the use of oral contraceptives (subjects 1, 3, 4, 5, 7 from the training group and 1, 4, 5 from the control group, table 5.5). Perimenstrual pain during the last/first days of the menstrual cycle reported by subject No 6 from the control group and No 2, No 3, No 7 and No 9 from the training group (table 5.5). No subject was tested during the period of perimenstrual pain (questionnaire prior to each test, data not shown).
Table 5.4. Individual values for progesterone and estradiol before (PRE) and after (POST) training in training (n = 8) and control group (n = 7).

<table>
<thead>
<tr>
<th>SUBJECTS</th>
<th>TRAINING GROUP</th>
<th>CONTROL GROUP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estradiol (pg·mL⁻¹)</td>
<td>Progesterone (ng·mL⁻¹)</td>
</tr>
<tr>
<td>1</td>
<td>9 PRE 9 POST 0.5 PRE 0.5 POST</td>
<td>1 PRE 1 POST 0.4 PRE 0.4 POST</td>
</tr>
<tr>
<td>2</td>
<td>89 70 PRE 1.7 PRE 11.8 POST</td>
<td>31 PRE 41 POST 0.5 PRE 0.9 POST</td>
</tr>
<tr>
<td>3</td>
<td>16 11 PRE 0.5 PRE 0.5 POST</td>
<td>93 PRE 20 POST 0.5 PRE 0.9 POST</td>
</tr>
<tr>
<td>4</td>
<td>0 0 PRE 0.4 PRE 0.4 POST</td>
<td>212 PRE 70 POST 0.8 PRE 3 POST</td>
</tr>
<tr>
<td>5</td>
<td>4 2 PRE 0.5 PRE 0.4 POST</td>
<td>0 PRE 0 POST 0.8 PRE 0.9 POST</td>
</tr>
<tr>
<td>6</td>
<td>65 82 PRE 0.3 PRE 1.6 POST</td>
<td>28 PRE 57 POST 1 PRE 1 POST</td>
</tr>
<tr>
<td>7</td>
<td>6 5 PRE 0.7 PRE 0.8 POST</td>
<td>30 PRE 176 POST 0.5 PRE 9 POST</td>
</tr>
<tr>
<td>8</td>
<td>56 94 PRE 0.7 PRE 13.9 POST</td>
<td></td>
</tr>
</tbody>
</table>

Table 5.5. Oral contraceptive details for individual oral contraceptive users.

<table>
<thead>
<tr>
<th>Brand name</th>
<th>Number of subjects</th>
<th>Oestrogen (µg)</th>
<th>Progestin (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovranette</td>
<td>2</td>
<td>30 ethinyl estradiol</td>
<td>15 levonorgestrel</td>
</tr>
<tr>
<td>Cilest</td>
<td>1</td>
<td>35 ethinyl estradiol</td>
<td>250 levonorgestrel</td>
</tr>
<tr>
<td>Logynon</td>
<td>1</td>
<td>30 ethinyl estradiol</td>
<td>15 levonorgestrel</td>
</tr>
<tr>
<td>Microgynon</td>
<td>1</td>
<td>30 ethinyl estradiol</td>
<td>15 levonorgestrel</td>
</tr>
</tbody>
</table>

5.3.5. CORRELATIONS

Recovery of MPO was negatively correlated with lactate (r = -0.68, P < 0.01) after training in the pooled data (both groups together). Recovery of MPO was positively correlated with pH both prior to training (r = 0.52, P < 0.05) and in the training state (r = 0.70, P < 0.01). However, when the groups analysed separately the correlation between pH and recovery of MPO reached significance only in the control group (post-training only) when correlation was conducted for each group separately (r = 0.77, P < 0.05). Recovery of MPO was also positively correlated with % \( \dot{V} O_2\ max \) @ 4 mmol·L⁻¹ (r = 0.64, P < 0.05) after training in pooled data. When only the training
group was evaluated a higher (r = 0.67, ns), but non significant relationship was revealed (control group, r = 0.37, ns).

A positive association was found between % $\dot{V}O_2$ max @ 4 mmol·L⁻¹ and recovery of peak speed (r = 0.59, $P < 0.05$) as well as recovery of mean speed (r = 0.65, $P < 0.01$) after training in the pooled data. Before training this association was true only for the training group (r = 0.72, $P < 0.05$). Post-training a negative correlation appeared between the recovery of mean speed and lactate after the first sprint (r = -0.63, $P < 0.05$). Finally, recovery of peak speed was correlated with blood lactate (r = -0.52, $P < 0.05$) and blood pH (r = 0.65, $P < 0.05$) after the 1st sprint post-training.

Similarly, recovery of PPO was negatively correlated with blood lactate (r = -0.58, $P < 0.05$) and pH (r = 0.73, $P < 0.01$) after the 1st sprint post-training (pool data).

As expected blood lactate, pH and plasma ammonia after the 1st sprint were all related before and after training (data not shown).

5.4. DISCUSSION

To our knowledge this is the first longitudinal study that has examined the effects of endurance training upon the recovery of sprinting performance and selective blood metabolites. Training resulted in a small, but significant, change in $\dot{V}O_2$ max (3 % mL·kg⁻¹·min⁻¹) together with a tendency for changes in the sub-maximal blood lactate response to exercise (% $\dot{V}O_2$ max @ 4 mmol·L⁻¹ increased 3 %, $P = 0.058$) which resulted together in an enhanced recovery of MPO in the experimental in comparison with the control group. Endurance training had also a positive impact on mean speed recovery, despite the fact that this change was of marginal statistical significance (training by group by time interaction: $P = 0.082$). On the other hand, the training stimulus did not influence the blood metabolic responses to the sprint as reflected by blood lactate, plasma ammonia and estimated changes in plasma volume. However, there was a strong tendency for elevated pH during recovery after training in the training group in comparison with the control group (time by group by training interaction: $P = 0.082$) suggesting that endurance training did cause alterations in
Acid-base balance. Recovery of MPO was significantly correlated with pH post-sprint 1 after training ($r = 0.70, P < 0.05$) as well as with $\dot{V}O_2\max \, @ \, 4 \, mmol\cdot L^{-1}$ ($r = 0.64, P < 0.05$).

In general, the metabolic and mechanical responses to sprinting (1st sprint) match well with observations reported in the literature. PPO in the present study of approximately 450 W and MPO of 250 W (see also table 5.3) are similar to those reported by Nevill and colleagues, 1996: (treadmill sprinting PPO 400 to 600 W and MPO 270 to 380 W). Small differences between the studies may be due to subject selection and training status. Likewise, the metabolic responses in the present study are similar to those reported by Nevill et al. (1996) (see also figures 5.8 to 5.11).

In the present study the training challenge resulted in a moderate, but significant, increase in $\dot{V}O_2\max$ (3 % mL· kg$^{-1}$· min$^{-1}$) in the training group in comparison with the control group (3 % increase vs 1.9 % decrease, respectively, $P < 0.05$). This small increase in $\dot{V}O_2\max$ is in agreement with previous experimental observations in female game players (Williams and Nute, 1986) and in recreational college athletes (Callister et al., 1988) participating in similar training regimens. Nevertheless, these findings disagree with those of Gaesser and Rich (1984), Hardman et al. (1986) and Mayes et al. (1987) who reported larger improvements in $\dot{V}O_2\max$ (12, 18 and 24 %, respectively) and employed similar training routines. It is known though, that increases in $\dot{V}O_2\max$ are inversely related to the initial values (Pollock, 1973; Mayes et al., 1987) and perhaps the participants of the present study were close to their genetic potential with respect to aerobic power. For example, in the present investigation and in previous work of this research group (Williams and Nute, 1986) subjects had relatively high initial fitness levels [48.7 mL·kg$^{-1}$·min$^{-1}$ (3.1 L·min$^{-1}$) and 48.9 mL·kg$^{-1}$·min$^{-1}$, respectively] compared with 43.3 mL·kg$^{-1}$·min$^{-1}$ in Gaesser and Rich (1984) and 2.42 L·min$^{-1}$ in Mayes et al. (1987). Moreover, values of about 48.7 mL·kg$^{-1}$·min$^{-1}$ are considered above average for women of 24 years of age (American College of Sports Medicine, 2000). Variations in improvements in $\dot{V}O_2\max$ between training studies may also be attributed to differences in the length (Andersen and Henrikson, 1977a; Andersen and Henrikson, 1977b; Cunningham et al., 1979; Yoshida et al., 1982b), duration (Andersen and Henrikson, 1977a) and frequency.
(Andersen and Henrikson, 1977a; Andersen and Henrikson, 1977b) of the training programmes.

In parallel with the improvements in $\dot{V}O_{2\text{max}}$, maximal ventilation increased by 6.2 % in the training group and was reduced 5.8 % in the control group ($P < 0.01$). This is again in concordance with previous research (Williams and Nute, 1986; Yoshida et al., 1982b) where significant increases in $\dot{V}O_{2\text{max}}$ after short-term endurance training were found. The physiological significance of this increase in ventilation may be that an elevated $\dot{V}O_{2\text{max}}$ induces higher requirements for oxygen and the analogous need to eliminate carbon dioxide (McArdle et al., 2001).

The influence of training on the sub-maximal exercise responses was very moderate in magnitude (Table 5.2). $\dot{V}E$, $\dot{V}O_2$, $\dot{V}E/\dot{V}O_2$, RPE, R and heart rate at a given speed did not change significantly in the training group in comparison with the control group. Nevertheless, there was a trend for lower values after training in the training group for RPE, R and heart rate (Table 5.2).

On the other hand there was a strong tendency towards lower lactate concentrations at a given sub-maximal running speed in the training group following training in comparison with the control group (training by group by time interaction, $P = 0.074$, Table 5.2 and figures 5.6 and 5.7). Visual inspection of the figures 5.6 and 5.7 and Table 5.3 indicates that this tendency was probably due to the lower lactate concentrations at the last two speeds (corresponding to 80 and 90 % of the pre-training $\dot{V}O_{2\text{max}}$). This is an expected outcome of endurance training and agrees well with numerous reports in the literature deploying short-term endurance training (Yoshida et al., 1982b; Williams and Nute, 1986; Hardman et al., 1986; Mayes et al., 1987). In spite of the formal debate concerning the underlying mechanisms of such adaptation towards either faster lactate clearance from the blood (Donovan and Brooks, 1983), or, lower rate of lactate production in the training state (Constable et al., 1987), there is a general belief that reduced lactate production rather than lactate clearance is the key factor (Green et al., 1991b; Coggan and Williams, 1995). Acceptance of this thought also supports the idea that a reduction of glycolysis is accompanied by a shift in substrate utilisation, namely towards noncarbohydrate...
sources and in particular towards fat oxidation (Henriksson, 1977; Holloszy and Coyle, 1984; Hurley et al., 1986). This shift in substrate metabolism can occur without any alteration in oxygen uptake at the same absolute work load following training (Henriksson, 1977), as was the case in this experiment, and is a function of improved muscle oxidative capacity (Henriksson, 1977; Hurley et al., 1986). Although mitochondrial enzymes were not measured in the present investigation a study with a similar training regimen demonstrated significant elevations in cytochrome oxidase and succinate dehydrogenase (Henriksson and Reitman, 1977), and thus oxidative capacity, giving further support for a similar plausible adaptation in the present study.

Adopting the assertion that improved oxidative capacity did occur, some proof is required that the volunteers of the present investigation were able to use fats at a greater rate after training. As there was no change in R following training such evidence was absent. However, R values were quite high (close to and above 1.00 for the last two speeds) which make this substrate index susceptible to errors inherent with indirect calorimetry such as increases in carbon dioxide elimination due to hyperventilation, and buffering by sodium bicarbonate in the blood to maintain acid-base balance (Livesey and Elia, 1988). In addition, no information is available concerning plasma free fatty acids and muscle triglycerides in the present study. Thus although it is not possible to state with certainty that there was a change in substrate metabolism in the present study the strong general trend for lower sub-maximal blood lactate concentrations following training, in parallel with the numerous reports in the literature, suggests that these findings may indeed reflect a greater utilization of fat after training.

There was a 3 % increase in % $\dot{V} O_{2} max @ 4 mmol\cdot L^{-1}$ in the training group (from 82 to 84 %) and a corresponding 1 % decrease in the control group (from 81 to 80 %) post-training. These values had a strong trend towards statistical significance (group by training interaction, $P = 0.058$) which indicates that the training routine applied here had a positive impact on endurance capacity. The $P$ value would be at least 0.034 though, without the presence of an outlier in the data (data not shown). Interestingly, neither Williams and Nute (1986) nor Mayes et al. (1987) at % $\dot{V} O_{2} max @ 2$
mmol·L⁻¹ and % $\dot{V}O_2\ max @ 4\ mmol\cdot L^{-1}$, respectively, found enhancements of this variable following a comparable training design. The findings of Hurley and co-workers (1984) may explain this disparity, where a longer and more severe training programme was applied (6 times per week for 12 weeks) resulting in a moderate outcome of a 7 % increase in % $\dot{V}O_2\ max @ 4\ mmol\cdot L^{-1}$ post-training. The finding of a 26 % increase in $\dot{V}O_2\ max$ in comparison with a 7 % increase in % $\dot{V}O_2\ max @ 4\ mmol\cdot L^{-1}$ in their protocol suggests that % $\dot{V}O_2\ max @ 4\ mmol\cdot L^{-1}$ may be rather difficult to change with training. Hence, the finding of a moderate change (present study) or no changes in % $\dot{V}O_2\ max @ 4\ mmol\cdot L^{-1}$/% $\dot{V}O_2\ max @ 2\ mmol\cdot L^{-1}$ (Williams and Nute, 1986; Mayes et al. 1987) is not a surprise. The present data also support suggestion of Hurley and co-workers (1984) that $\dot{V}O_2\ max$ and % $\dot{V}O_2\ max @ 4\ mmol\cdot L^{-1}$ are determined by different underlying metabolic or physiological mechanisms. The lack of correlation between $\dot{V}O_2\ max$ and % $\dot{V}O_2\ max @ 4\ mmol\cdot L^{-1}$ in the present study ($r = 0.15$, ns pre- and post- training and $r = 0.19$, ns, when the percentage changes in each variable was correlated) is in line with the above view.

In the present study endurance fitness was improved while the performance characteristics and metabolic responses to the first sprint were unaltered in the training state. The lack of change in the first sprint after training was important to the study design as the percent recovery of PPO, MPO, peak speed and mean speed is strictly dependent upon the initial value. Hardman et al. (1986) using a similar design with sprint cycling as the exercise mode, found no change in sprint performance (1st sprint) or in the blood lactate response 5 min post-sprint (and probably peak values). Opposingly, Cheetham (1987) employing a lower intensity (60-70 % $\dot{V}O_2\ max$) and larger volume training regimen (6-8 h per wk for 10 wk on the top of their recreational running programme) found an increase in the time taken to reach peak speed and lower $F_{1sp}$ post-training in the experimental group. Thus, it seems that the volume of training rather than the intensity of exercise is the prevailing factor in causing changes in the performance of the first sprint. Studies in animals confirm this contention as Green and co-workers (1983) concluded that any transformation in fibre type is function of the total amount of activity rather than intensity of exercise.
Six weeks of endurance training at 85% $\dot{V}O_{2max}$ resulted in an improved recovery of MPO in the second sprint in the training group in comparison with the control group (81 to 88 and 81 to 82 %, in the training and control group, respectively, $P < 0.05$). Likewise, recovery of peak speed ($P = 0.113$) and mean speed ($P = 0.082$) showed a strong trend towards significant improvements with no changes in the other performance variables (table 5.3). In addition, a significant relationship between $\% \dot{V}O_{2max} @ 4 \text{ mmol} \cdot \text{L}^{-1}$ and recovery of MPO was revealed ($r = 0.64$, $P < 0.05$). These data corroborate and extend the conclusion of Bogdanis and co-workers (1996a) that individuals who posses higher endurance fitness may also have a faster recovery in performance during repeated sprints. It should be noted though, that this correlation between $\% \dot{V}O_{2max} @ 4 \text{ mmol} \cdot \text{L}^{-1}$ and recovery of MPO only reached significance when both groups were analysed together. When only the training group data was used the correlation was not statistically significant ($r = 0.67$, ns), probably as a result of the small range of the values and the small sample size (Bates et al., 1996) rather than lack of association (as suggested when the analysis involved the pooled data). Although there was a relationship between endurance fitness and the recovery of MPO in the present study there was no relationship between $\dot{V}O_{2max}$ and power output recovery. Similarly Bogdanis et al. (1996a) found no relationship between $\dot{V}O_{2max}$ and power output recovery for sprint cycling exercise. However, Tomlin and Wenger (2002) as well as Graham et al. (1995) did find such a relationship. An explanation for such variation in findings between studies might relate to the magnitude of the $\dot{V}O_{2max}$. For example Hoffman et al. (1999) found that recovery rate was related to $\dot{V}O_{2max}$ only in soldiers with a $\dot{V}O_{2max}$ below the population mean. Alternatively, the range in $\dot{V}O_{2max}$ may be important as McMahon and Wenger (1998) found a relationship between $\dot{V}O_{2max}$ and recovery with a range of values from 38 to 63 mL·kg$^{-1}$·min$^{-1}$. Specificity of the performance protocol may also play a role as Dawson et al. (1993) found a significant negative relation between power decrement and $\dot{V}O_{2max}$ (treadmill) during repeated track short sprinting, but not between $\dot{V}O_{2max}$ (cycling) and power decrement during repeated short-term cycle sprinting.
The finding that the reduction in blood pH was attenuated following sprint 1 after training leads to the conclusion that endurance training may improve acid-base balance. The significant positive relationship between recovery of MPO and blood pH before and after training (r = 0.52, \( P < 0.05 \) and r = 0.70, \( P < 0.01 \)) reinforces this thought. Reductions in pH have long been associated with inhibition of glycogen degradation by lowering the activity of glycogen phosphorylase (Chasiotis et al., 1983) and the glycolytic rate by reducing the activity of phosphofructokinase (Trivedi and Danforth, 1966). Inhibition of the glycogenolytic/glycolytic rate has been implicated as major cause of fatigue during sprinting and especially is thought to inhibit MPO (Spriet et al., 1989; Gaitanos et al., 1993; Maughan et al., 1997). However, there is limited evidence to support the influence of low pH on the glycolytic rate in in vivo models (Sahlin et al., 1975; Dobson et al., 1986). This lack of influence of acidic conditions upon glycogenolytic/glycolytic rate can be explained by the high accumulation of allosteric activators for phosphofructokinase and glycogen phosphorylase such as inorganic phosphate, AMP, IMP and ADP during sprinting. The price of maintaining high levels of glycolysis at a low pH is increased levels of AMP and ADP which, in turn, may impair the contractile process (Donaltson et al., 1978; Cooke et al., 1988).

A further possible reason for the enhanced recovery of power after endurance training in the present study is improved central and peripheral blood flow during recovery from the sprint 1 as a result of an elevated \( \dot{V}_{O_{2}}^{\text{max}} \) (Bassett and Howley, 2000). In the periphery this process will be a consequence of improved capillarisation of type I, IIA and IIB fibres (Andersen and Henriksson, 1977b), more efficient oxygen extraction from the muscle (Rowell, 1993), and, possibly better muscle vascular conductance, elevated oxygen diffusion, and venous compliance (Henriksson and Hickner, 1998). More centrally, an increase in oxygen-haemoglobin affinity will further add to the benefits of increased blood flow (Kjellberg et al., 1949).

Improved blood flow will result in faster transportation of \( H^+ \) from the sites of their production (muscle) to the sites of their removal (to the lungs or kidneys). The functional characteristic of blood buffer molecules (bicarbonate, mainly, phosphate and protein) which can be used again (Wilmore and Costill, 1999) in combination
with increased blood flow will further facilitate the buffering process. Increased buffering capacity during the sprint, in addition to during the recovery, will result in higher pH values post-sprint (Edge et al., 2002) as found in the present study. In the current experimental design pH values can be considered as mixed sprint-recovery values (Allsop et al., 1990; Nevill et al., 1996a) since blood sampling time averaged 30 s to 1 min and 30 s. The significant correlation between muscle buffering capacity and citrate synthase activity (Sahlin and Henriksson, 1984), which connects buffering capacity with oxidative potential of skeletal muscle thereby endurance fitness, corroborates the above observations at the cellular level.

An alternative attractive explanation of the faster recovery of MPO in the training state other than the effect of acidosis, is improved PCR restoration. Previously, Bogdanis and co-workers (1996a) reported a very similar pattern of recovery for PCR, power and speed after sprint cycling exercise. The high significant correlations between power and speed recovery and PCR resynthesis (r = 0.84-0.91) verified their contention. Moreover, a link between endurance fitness (% $\dot{V} O_2$ max @ 4 mmol-L$^{-1}$) and PCR recovery as well as power and speed recovery in the first 10 s of the second sprint was also shown (r = 0.75-0.94). These data fit well with previous observations of oxygen dependency of PCR resynthesis (Idstom et al., 1985; Quistorf et al., 1992). Thus, it is possible that greater blood flow during recovery from sprint 1 as a result of elevated $\dot{V} O_2$max will in turn accelerate PCR replenishment, thereby enhancing MPO recovery. The close association in the present study between recovery of MPO and % $\dot{V} O_2$ max @ 4 mmol-L$^{-1}$ reinforces this suggestion (r = 0.64, P < 0.05).

Plasma ammonia was only marginally elevated above pre-training values (fig. 5.10). It has been shown that ammonia decreases or remains stable at the same absolute or relative sub-maximal workload (< $\dot{V} O_2$max), respectively, as a result of adaptation to endurance training (Lo and Dudley et al., 1987; Denis et al., 1989). In the present study improved MPO in the second sprint would have resulted in a higher energy demand unless there was an improvement in efficiency after training. Increased energy demand in the second sprint may have resulted in greater ATP loss and subsequently enhanced stoichiometric increases in IMP and NH$_3$ in muscle (Maughan et al., 1997) and probably net ammonia efflux from the site of production to blood.
Thus, it seems possible that ammonia would increase after endurance training after sprint 2. Ammonia efflux from the muscle is influenced by the membrane potential which in turn is being determined from $K^+$ gradients (Sjøgaard et al., 1985; Knepper et al., 1989). During high intensity exercise $K^+$ loss from the muscle (corresponding to increases in the circulation) can bring about a drop in the membrane potential from a mean value of 89 mV at rest to at least 75 mV at the end of intense exercise (Sjøgaard et al., 1985). Subsequently, (more) ammonia should appear in the blood. Had endurance exercise attenuated extracellular $K^+$ the lowering of the membrane potential would also be blunted thereby reducing ammonium efflux from the muscle (Saltin et al., 1968; Kiens and Saltin, 1986). Subsequently, less ammonia will appear in the blood. However, when endurance training results in a higher work rate during intense ($>\dot{V}O_2max$) exercise, as it is the case here, serum arterial $K^+$ is accentuated (Saltin et al., 1968; Fosha-Dolezal and Fedde, 1988) and therefore ammonia increases in the blood could occur as found in the present study. Another mechanism that affects ammonium transport from the intracellular environment is the competition between ammonium and $K^+$ for the $K^+$ channels (Knepper et al., 1989). Again, hyperkalemia would also contribute to increased ammonium export from the cell (Graham et al., 1990). So, it seems that the slight, but in no means significant, increase in plasma ammonia matches well with the mechanisms at the cellular level.

Post-training post-sprint lactate concentrations were unchanged (fig. 5.8.) in both groups even though for the training group MPO in sprint 2 was higher. There is general consensus that blood lactate concentration is reduced in the endurance-trained state at the same absolute exercise intensity (Coggan and Williams, 1995) reflecting either attenuated production (Constable et al., 1987) or enhanced clearance (Donovan and Brooks, 1983). Possibly in the present study there was an enhanced lactate production as a result of faster recovery of muscle metabolites and improved acid-base balance after sprint 1 resulting in enhanced power, but this was counterbalanced by enhanced lactate clearance.

However, based simply on an examination of the blood lactate concentrations during recovery, the present training regimen did not appear to have any impact on lactate
removal (fig. 5.8). This finding is in agreement with a number of studies employing serial or cross-sectional models to examine lactate removal after sub-maximal intensity exercise (Evans and Cureton, 1983; Bassett et al., 1991; Taoutaou et al., 1996; Oosthuse and Carter, 1999; Bret et al., 2003); but equally conflicts with the findings of some studies (Oyono-Enguelle et al., 1990; Freund et al., 1992; Messonnier et al., 2001). Lack of more frequent sampling did not allow the use of mathematical models to examine lactate kinetics (Freund et al., 1992; Messonnier et al., 2001). Nonetheless, it is not clear whether bioexponential models are appropriate for the study of high lactate concentrations (Freund et al., 1986). Lactate disappearance is inversely related with previous work rate (Freund et al., 1986; Freund et al., 1990) and thus in the present study higher lactate production as a result of higher MPO (Cheetham et al., 1986) may have impaired faster lactate removal from the circulation. Thus the lack of enhanced lactate removal may have been due to inhibition of lactate efflux (Hirche et al., 1975; Mainwood and Worsley-Brown, 1975) and/or utilization (Sahlin et al., 1976; Tesch et al., 1978) during recovery due to extremely low pH after sprinting (fig. 5.9, Nevill et al., 1989).

This is the first training protocol that has examined plasma ammonia kinetics during recovery. Recovery of plasma ammonia from highest concentrations post-sprint to lowest concentrations during recovery was of a similar pattern and magnitude before and after training in the training group in comparison with control group. Liver, resting muscle and lungs are the sites of ammonia uptake from the plasma with the lungs perhaps the most significant site (Graham et al., 1990). In theory, an endurance training-induced more efficient pulmonary blood flow (higher ammonia extraction from the lungs and/or increased blood flow to the lungs) during recovery from the sprints would ultimately result in a higher clearance rate. This did not appear to be the case in the present study, though. It is uncertain whether the moderate endurance adaptations found in the present study or some other mechanisms, which are not affected by this type of training, are responsible. The pathway(s) and the mechanisms of ammonia removal from the circulation are still tentative (Graham et al., 1990; Snow et al., 1992) and the present methodology is deficient in terms of blood flow and post-sprint ventilation measurements to highlight these factors. Moreover, the only other investigation which examined plasma ammonia kinetics after sprinting as a result of training (cross-sectional model) did not comment on the apparent 11%
(estimation of their graphs) higher clearance rates for ammonia for the endurance trained athletes (Nevill et al., 1996a).

Finally, estimated percentage change in plasma volume was unaltered in response to both sprints after training, although there was a small shift (ns) in the training group towards higher fluid losses. Estimated resting absolute plasma volume was also unchanged denoting either inefficiency of the training regimen or methodological shortcomings inherent with the Hct, Hb technique (Johansen et al., 1998).

One of the confounding factors when analysing results involving female subjects is the effect of menstrual cycle phase. However, in chapter 4 we reported that both sprint performance and blood metabolites were unaffected by menstrual cycle phase. It remains the possibility though, that perimenstrual pain may have influenced to some extent the present findings (Giacomoni et al., 2000). However, subjects did not report pain prior to any test as indicated from the pre-test health status questionnaire given to the volunteers (data not shown). Moreover, the menstrual cycle does not have any effect on \( \dot{V}O_{2\text{max}} \) (De Souza et al., 1990; Bemben et al., 1995; Beidleman et al., 1999; Suh et al., 2002) or on blood lactate response to exercise intensities above 65 \% \( \dot{V}O_{2\text{max}} \) (Jurkowski et al., 1981; Hessemmer and Bruck, 1985; Lamont, 1986; Nicklas et al., 1989; Kanaley et al., 1992; Cambell et al., 2001; Suh et al., 2002).

Similarly, oxygen consumption is unaffected at higher (> 70 \% \( \dot{V}O_{2\text{max}} \)) sub-maximal intensities (Lamont, 1986; Nicklas et al., 1989; Hackney et al., 1991; Kanaley et al., 1992; Beidleman et al., 1999; Bailey et al. 2000; Cambell et al., 2001; Suh et al., 2002) with only one report to the contrary (Williams and Krahenbuhl, 1997). As shown in table 5.4, only two volunteers (one from each group) were tested in the mid-luteal phase (both post-training). Thus, it seems that neither \( \dot{V}O_{2\text{max}} \) nor \% \( \dot{V}O_2 \text{max} @ 4 \text{ mmol·L}^{-1} \) and sprint performance were substantially influenced by menstrual cycle phase in the present study.

On the other hand the effects of oral contraceptives on performance and metabolism in the present study are extremely difficult to assess since for this study subjects oral contraceptive users had been taking the contraceptives for at least two years and there are no previous reports of the long-term impact of oral contraceptive use.
Nevertheless, due to the short period of the study the influence, if any, of the oral contraceptives on the variables measured should be considered minimal (Bemben, 1993).

In summary, this is the first study to demonstrate that short-term endurance training can be beneficial to non-endurance athletes to enhance recovery from sprinting. Improved acid-base balance and quite possibly PCr resynthesis, even though it was not measured, appear the prevailing underlying mechanisms. More sophisticated methodology including muscle biopsies and expired air collections are necessary to clarify these issues.
CHAPTER 6: THE EFFECTS OF SHORT-TERM ENDURANCE TRAINING ON PERFORMANCE AND MUSCLE METABOLIC RECOVERY AFTER SPRINTING

6.1. INTRODUCTION

Skeletal muscle phosphocreatine (PCr) is the most immediate reserve for the rephosphorylation of adenosine triphosphate (ATP) during exercise. It is this characteristic that makes PCr an essential energy substrate for activities which require high energy demands such as sprinting. The availability of PCr is even more important when multiple sprints are to be performed, as in many game sports such as soccer (Mayhew and Wenger, 1985), hockey (Reilly, 1992) and rugby (McLean, 1992). Thus the rapid recovery of PCr in between sprints is of paramount importance (Tomlin and Wenger, 2001).

There is currently general consensus (Nevill et al., 1996a; Greenhaff and Timmons, 1998) that the recovery process of PCr is oxygen (O2) dependent as research has shown that PCr resynthesis does not occur when the circulation is occluded (Quistorf et al., 1992). Furthermore, studies in rats (Pagganini et al., 1997) and in humans (Jansson et al., 1990) have demonstrated significant positive relationships between citrate synthase activity and PCr restoration after exercise ($r = 0.84, P < 0.01$ and $r = 0.69, P < 0.05$, respectively). This association between PCr resynthesis and oxygen availability has also attracted attention from the medical sciences, as examination of PCr recovery is becoming an increasing important diagnostic tool for muscular disorders such as severe mitochondrial myopathy (Radda et al., 1982; Arnold et al., 1984).

Subsequently, research with a cross-sectional design using sprint cycling as the exercise mode has suggested that there is a link between PCr recovery, power output and endurance fitness (Bogdanis et al., 1996a). Faster power/force output and/or speed recovery in endurance fitter individuals has also been reported by other researchers, therefore reinforcing the view that endurance training might be beneficial for power/explosive sports (Hakkinen and Myllyla, 1990; Hamilton et al., 1990;
Dawson et al., 1993; Caiga and Doherty, 1995; Hoffman, 1997; McMahon and Wenger, 1998; Tomlin and Wenger, 2002).

It has been well established that endurance training improves mitochondrial number and density (Holloszy and Coyle, 1984), enhances aerobic enzymes' activity (Gollnick et al., 1973), increases capillarisation (Andersen and Henriksson, 1977a; Ingjer, 1978) and thus facilitates oxygen extraction (Rowell, 1993). Muscle blood flow also increases during maximal exercise after endurance training and the expansion of blood volume (Sawka, 2000) further facilitate oxygen delivery. However, to date no study has examined directly the effect of endurance training on the recovery of power output and muscle metabolites after sprinting. Recent evidence suggests that endurance adaptations can take place within just a few weeks of training (< 6 weeks, e.g. Green et al., 1991b; Green et al., 1992), but the impact of any such adaptations on sprint performance is unknown.

Thus, it was the purpose of the present investigation to test the hypothesis that six weeks of endurance training will enhance the recovery of performance during 2 x 30-s treadmill sprints and that this enhanced recovery of performance may be due to a faster resynthesis of PCr and/or other changes in muscle metabolism during the first sprint or subsequent recovery.

6.2. METHODS
6.2.1. SUBJECTS
Seventeen female athletes volunteered to participate in the present investigation. All the individuals participated in various game sports such as rugby, basketball, hockey and lacrosse. Some of them were members of England national team U23 during the course of the study or in the recent past in their individual sports. Due to an injury (n = 1) and lack of compliance with the experimental procedures (n = 2) 3 subjects were excluded (1 from the training group and 2 from the control group) from the analysis of the results. The physical characteristics of the remaining 14 volunteers, for age, body mass and stature were (mean ± SD) 22 ± 3 y, 1.66 ± 0.07 m, 60.2 ± 11.7 kg. The study was approved by Loughborough University Ethical Committee. Subjects received information about the purpose of the study, the potential risks and benefits in
both oral and written form and then signed an informed consent which clearly stated that they could withdraw from the study at any point without explanation.

6.2.2. ERGOMETERS
The non-motorised treadmill as described in the general methods (section 3.4.1), was used in this investigation. For the \( \dot{V}O_2 \ max \), speed-\( \dot{V}O_2 \) and speed-lactate tests the motorised treadmill, as described in section 3.4.2, was used.

6.2.3. FAMILIARISATIONS
A thorough habituation was undertaken by the subjects as described in the general methods (section 3.3).

6.2.4. \( \dot{V}O_2 \ max \) AND SPEED-LACTATE TEST
A detailed description of these procedures is given in the general methods chapter and more particularly in sections 3.5.3-3.5.5.

6.2.5. PROTOCOL AND EXPERIMENTAL DESIGN
Volunteers reported to the laboratory after a standardised diet (24 h) and light exercise (24 h) and after having performed the \( \dot{V}O_2 \ max \) (48 h) and speed-lactate test (24 h) prior to the sprint protocol. Participants were also advised to abstain from alcohol (24 h) and caffeine (12 h) prior to the main trials. This procedure was followed at the same time and day after the 6 weeks training period in both training (n = 9) and control group (n = 5). Figure 6.2 illustrates the time plan of the experimental procedures. The procedures for anthropometry, were as described in the general methods (section 3.6).

The sprint protocol consisted of two tests 48 h apart. The first test involved two 30 s sprints with 2 min of passive recovery on an experimental couch which was identical to the sprint protocol of the first training study (chapter 5) as described in section 3.5.2 of the general methods. However, for the purpose of this investigation participants’ expired air was collected by the Douglas bag method for both sprints and during the recovery from the sprint 1. After a 5 min period with the mouthpiece and nose clip in place, while lying in a semi-reclined position on the experimental couch.
expired air was collected for 1 min period. Expired air was also collected during the 30-s sprints and immediately after sprint 1 for 55 s, interrupted for 10 s for the change of Douglas bag, and the remaining 55 s of the recovery between the sprints. For the purpose of this study two Douglas bags were connected together with a manual grip so that consecutive air samples could be taken during sprint 1 and the first 55 s of recovery. Figure 6.1 provides a schematic representation of the test 1. For the calculation of the oxygen consumed during the recovery period the value obtained during the resting sample was subtracted (i.e. recovery – rest).

Figure 6.1. The schematic representation of the protocol.

During the test 2 an identical protocol to test 1 was performed with the exception of only one sprint and the lack of blood and air samples. In addition, muscle biopsies taken at rest (after at least 20 min rest in semi-reclined position on the experimental couch) and at ~ 10 s and 2 min after the sprint (all the samples were taken in the semi-reclined positions). Exact timing of the biopsy samples from the point of the
termination of the sprint until the insertion of the biopsy sample in the liquid nitrogen was recorded by the same experimenter. Plates 3.3 and 3.4 in the general methods (chapter 3) depict the needle biopsy technique for two of the present participants. The complete description of the biopsy procedures is included in section 3.14.

Figure 6.2. The schematic representation of the sprint test 2 (biopsy test).

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Figure 6.3. Time plan for the present experimental design. Where F indicates familiarisations as described in paragraph 3.3.1. VO₂max-1: \( \dot{V} O₂ \text{ max} \) test before training; VO₂max-2: \( \dot{V} O₂ \text{ max} \) test after training; SPLA-T 1: speed-lactate test before training; SPLA-T 2: speed-lactate test after training.
6.2.6. MENSTRUAL CYCLE INFORMATION
Menstrual cycle information was initially obtained from menstrual cycle phase questionnaires [Appendix G]. Verification of menstrual cycle phase was obtained later by hormonal analysis of estradiol and progesterone.

6.2.7. CLINICAL BIOCHEMISTRY
Collection, treatment, storage and analysis of blood and muscle samples were performed as mentioned in detail in the general methods (section 3.13 and 3.14, respectively).

6.2.8. STATISTICAL ANALYSIS
A three-way analysis of variance for mixed subjects design -within (before and after training) and between subjects (between training and control group)- was used to assess whether there were any differences in performance variables before and after training (main effect: training), between the first and second sprint (main effect: sprint) and between training and control group (main effect: group). Two-way analysis of variance for mixed subjects design –within (before and after training) and between subjects (between training and control group)- was used to examine if any differences existed in recovery of performance data before and after training (main effect: training) and between groups (main effect: group). Three-way analysis of variance for mixed subjects design - within (before and after training) and between subjects (between training and control group)- was also employed to ascertain any variations in metabolic responses to endurance training (main effect: training), the response to each group with respect to time (main effect: time) and between training and control group (main effect: group). When a significant interaction (training by sprint by group, or, training by group, or, training by time by group) was found adjusted unpaired t-tests, corrected by the bonferroni method, were used. Relationships between variables were evaluated using the Pearson Moment Product Correlation Coefficient. Results are expressed as mean ± SD, unless otherwise stated.
6.3. RESULTS

6.3.1. ENDURANCE TRAINING ADAPTATIONS

6.3.1.1. Training volume

Participants in the training group ran on average, for 80 to 90 min per week. This time corresponded to a distance of 15133 m in the first week and 16803 m in the fifth week per subject. Distance during the last week of training was slightly lower as 2 subjects missed one training session. Figures 6.4 and 6.5 depict the quantitative aspects of the training performed.

![Figure 6.4. Treadmill running time (min) for each week of training for the training group (mean ± SD, n = 9).](image1)

![Figure 6.5. Distance run (m) for each week of training for the training group (mean ± SD, n = 9).](image2)
6.3.1.2. $\dot{V}O_{2\text{max}}$.

Maximum oxygen uptake was increased 2.3 % after training in the training group, while there was a 1.5 % decrease in the control group (ns). One control subject increased by 6 % in the post-test which was a clear outlier. Eliminating this individual from the analysis a significant interaction is revealed ($P = 0.042$). Figure 6.6 illustrates these findings.

The time to exhaustion for the incremental $\dot{V}O_{2\text{max}}$ test was 12.7 % longer after training for the training group while the control group was 4.1 % shorter after the six weeks experimental period (9.52 ± 1.12 vs 10.65 ± 1.49 and 10.30 ± 1.50 vs 9.80 ± 2.00 min before and after training in training and control group, respectively, $P = 0.095$, figure 6.7).

![Figure 6.6](image_url)

Figure 6.6. $\dot{V}O_{2\text{max}}$ test, before (pre) and after (post) training in training (TG, n = 9) and control (CG, n = 5) group. (mean ± SD).
The remaining physiological responses to the $\dot{V}O_{2}\text{max}$ test are presented at table 6.1. Maximum heart rate showed a training by group interaction reflecting lower maximal heart rate for the training group in comparison with the control group after training ($P < 0.05$).

Similarly, maximal ventilation demonstrated a strong tendency towards higher volumes after training in the training in comparison with the control group ($P = 0.069$).

Finally, maximal ventilatory equivalent for oxygen, did not change after training, although there was a tendency for higher values in the training group in comparison with the control group ($P = 0.056$).
Table 6.1. $\dot{V} O_{2\text{max}}$ (mL·kg$^{-1}$·min$^{-1}$) test physiological profile in training group (TG) and control group (CG), pre and post training. Where "C" main effect: training by group, $P < 0.05$. (mean ± SD, n = 9 for TG, n = 5 for CG).

<table>
<thead>
<tr>
<th></th>
<th>$\dot{V} O_{2\text{max}}$</th>
<th>MAX $\dot{V} E$</th>
<th>MAX $\dot{V} E/\dot{V} O_2$</th>
<th>Heart rate max$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG</td>
<td>49.2 ± 4.0</td>
<td>86.6 ± 6.6</td>
<td>28.5 ± 1.9</td>
<td>198 ± 7</td>
</tr>
<tr>
<td>CG</td>
<td>49.2 ± 5.7</td>
<td>90.6 ± 8.6</td>
<td>28.4 ± 2.0</td>
<td>192 ± 6</td>
</tr>
<tr>
<td>TG</td>
<td>50.3 ± 3.7</td>
<td>93.5 ± 8.0</td>
<td>30.3 ± 2.4</td>
<td>194 ± 7</td>
</tr>
<tr>
<td>CG</td>
<td>48.6 ± 7.6</td>
<td>90.7 ± 10</td>
<td>28.2 ± 1.3</td>
<td>196 ± 10</td>
</tr>
</tbody>
</table>

6.3.1.3. Speed lactate test

Table 6.2 displays the blood lactate responses during the speed-lactate test. Training resulted in a trend towards lower lactate concentrations in the training group in comparison with the control group (training by time by group interaction, $P = 0.063$). Figures 6.8 and 6.9 show these trends. From the graphs can be seen that the training was more effective in reducing blood lactate at the higher two speeds.

Figure 6.8. The speed-lactate test with an example of the speeds that correspond to 4 mmol·L$^{-1}$ before (PRE) and after (POST) training in the training group (TG, mean ± SD, n = 9).
Figure 6.9. The speed-lactate test with an example of the speeds that correspond to 4 mmol·L⁻¹ before (PRE) and after (POST) training in the control group (CG, mean ± SD, n = 5).

There was a 3.6 % (83.4 ± 5.3 vs 86.1 ± 4.2 %) improvement in % $\dot{V}O_2$ max @ 4 mmol·L⁻¹ in the training group after training while at the same time the control group showed a 1.9 % reduction (84.8 ± 5.5 vs 83.0 ± 4.3 %, ns). Nevertheless, close inspection of individual data reveals an outlier from the training group (16 % reduction after training). By taking this subject out of the analysis the improvement in $\dot{V}O_2$ max is statistically significant ($P = 0.028$).

Further physiological responses to the speed-lactate test are shown in table 6.2. All the physiological variables revealed a slight reduction in the post-training responses in both groups, which was more pronounced in the training group. Nevertheless, repeated measures of ANOVA did not show any significant training by time by group interaction.

6.3.2. SPRINT PERFORMANCE RESPONSES

Subjects' body mass was unaltered after six weeks of training in both training (62.2 ± 4.0 vs 61.3 ± 3.6 kg before and after training, respectively, ns) and control group (63.7 ± 6.6 vs 64.3 ± 6.9 kg before and after training, respectively, ns). Therefore performance parameters are presented in absolute terms (i.e. not divided by body mass).
Table 6.2. Speed-lactate data for the training group (n = 9) and control group (n = 5) before (PRE) and after training (POST). Where, 4, 8, 12 and 16 indicate collection min during the 16 min speed-lactate test. $\dot{V}O_2$, $\dot{V}E$, $\dot{V}CO_2$, $\dot{V}O_2/\dot{V}E$, is in mL·kg$^{-1}$·min$^{-1}$, while lactate in mmol·L$^{-1}$. (mean ± SD).

<table>
<thead>
<tr>
<th></th>
<th>TRAINING GROUP</th>
<th></th>
<th>CONTROL GROUP</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PRE</td>
<td>POST</td>
<td>PRE</td>
<td>POST</td>
</tr>
<tr>
<td>$\dot{V}O_2$</td>
<td>29.4</td>
<td>34.1</td>
<td>39.9</td>
<td>44.7</td>
</tr>
<tr>
<td>±</td>
<td>2.8</td>
<td>± 3.4</td>
<td>± 4.1</td>
<td>± 4.3</td>
</tr>
<tr>
<td>$\dot{V}CO_2$</td>
<td>24.1</td>
<td>29.5</td>
<td>35.9</td>
<td>43.0</td>
</tr>
<tr>
<td>±</td>
<td>2.4</td>
<td>± 3.3</td>
<td>± 4.7</td>
<td>± 5.1</td>
</tr>
<tr>
<td>$\dot{V}E$</td>
<td>36.8</td>
<td>46.1</td>
<td>56.5</td>
<td>69.7</td>
</tr>
<tr>
<td>±</td>
<td>3.2</td>
<td>± 4.7</td>
<td>± 7.3</td>
<td>± 7.4</td>
</tr>
<tr>
<td>$\dot{V}O_2/\dot{V}E$</td>
<td>20.2</td>
<td>21.7</td>
<td>22.7</td>
<td>25.0</td>
</tr>
<tr>
<td>±</td>
<td>1.6</td>
<td>± 1.6</td>
<td>± 1.6</td>
<td>± 1.6</td>
</tr>
<tr>
<td>$R$</td>
<td>0.82</td>
<td>0.87</td>
<td>0.90</td>
<td>0.96</td>
</tr>
<tr>
<td>±</td>
<td>0.04</td>
<td>± 0.03</td>
<td>± 0.04</td>
<td>± 0.04</td>
</tr>
<tr>
<td>RPE</td>
<td>9</td>
<td>11</td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td>±</td>
<td>1</td>
<td>± 1</td>
<td>± 1</td>
<td>± 1</td>
</tr>
<tr>
<td>Heart rate</td>
<td>143</td>
<td>161</td>
<td>178</td>
<td>190</td>
</tr>
<tr>
<td>±</td>
<td>11</td>
<td>± 8</td>
<td>± 7</td>
<td>± 6</td>
</tr>
<tr>
<td>lactate</td>
<td>1.8</td>
<td>2.0</td>
<td>3.5</td>
<td>6.3</td>
</tr>
<tr>
<td>±</td>
<td>1.2</td>
<td>± 0.5</td>
<td>± 0.8</td>
<td>± 1.8</td>
</tr>
</tbody>
</table>
Table 6.3. Sprint Performance parameters (2 x 30 s sprints) for training group (mean ± SD, n = 9) and control group (mean ± SD, n = 5) before (pre) and after (post) training. Where "c": training by group interaction (P < 0.05). (mean ± SD, n = 9 for TG, n = 5 for CG).

<table>
<thead>
<tr>
<th></th>
<th>TRAINING GROUP</th>
<th></th>
<th>CONTROL GROUP</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PRE</td>
<td>POST</td>
<td>PRE</td>
<td>POST</td>
</tr>
<tr>
<td>PPO (1st sprint) (W)</td>
<td>450 ± 58</td>
<td>443 ± 71</td>
<td>397 ± 67</td>
<td>386 ± 67</td>
</tr>
<tr>
<td>PPO (2nd sprint) (W)</td>
<td>349 ± 46</td>
<td>480 ± 49</td>
<td>341 ± 38</td>
<td>387 ± 46</td>
</tr>
<tr>
<td>REC OF PPO (%)</td>
<td>78 ± 8</td>
<td>87 ± 9</td>
<td>87 ± 9</td>
<td>88 ± 7</td>
</tr>
<tr>
<td>MPO (10 s) (1st sprint) (W)</td>
<td>371 ± 39</td>
<td>354 ± 46</td>
<td>329 ± 53</td>
<td>326 ± 69</td>
</tr>
<tr>
<td>MPO (10 s) (2nd sprint) (W)</td>
<td>301 ± 41</td>
<td>292 ± 29</td>
<td>298 ± 37</td>
<td>285 ± 46</td>
</tr>
<tr>
<td>REC of MPO (10 s) (%)</td>
<td>82 ± 9</td>
<td>83 ± 5</td>
<td>91 ± 7</td>
<td>88 ± 7</td>
</tr>
<tr>
<td>FLPO (1st sprint) (%)</td>
<td>56 ± 7</td>
<td>56 ± 10</td>
<td>48 ± 7</td>
<td>50 ± 8</td>
</tr>
<tr>
<td>FLPO (2nd sprint) (%)</td>
<td>50 ± 10</td>
<td>54 ± 8</td>
<td>52 ± 3</td>
<td>46 ± 7</td>
</tr>
<tr>
<td>MPO (1st sprint) (W)</td>
<td>296 ± 23</td>
<td>287 ± 27</td>
<td>274 ± 46</td>
<td>269 ± 59</td>
</tr>
<tr>
<td>MPO (2nd sprint) (W)</td>
<td>226 ± 19</td>
<td>240 ± 28</td>
<td>236 ± 26</td>
<td>237 ± 41</td>
</tr>
<tr>
<td>REC of MPO (%)</td>
<td>77 ± 7</td>
<td>84 ± 5</td>
<td>87 ± 8</td>
<td>89 ± 8</td>
</tr>
<tr>
<td>Peak speed (1st sprint) (m·s⁻¹)</td>
<td>5.70 ± 0.34</td>
<td>5.59 ± 0.42</td>
<td>5.26 ± 0.36</td>
<td>5.26 ± 0.34</td>
</tr>
<tr>
<td>Peak speed (2nd sprint) (m·s⁻¹)</td>
<td>5.04 ± 0.46</td>
<td>5.21 ± 0.34</td>
<td>4.98 ± 0.37</td>
<td>4.91 ± 0.31</td>
</tr>
<tr>
<td>REC of peak speed (%)</td>
<td>94.2 ± 2.3</td>
<td>97.6 ± 2.5</td>
<td>92.0 ± 5.3</td>
<td>93.0 ± 2.5</td>
</tr>
<tr>
<td>mean speed (10 s) (1st sprint) (m·s⁻¹)</td>
<td>5.45 ± 0.29</td>
<td>5.34 ± 0.36</td>
<td>5.05 ± 0.37</td>
<td>5.02 ± 0.41</td>
</tr>
<tr>
<td>mean speed (10 s) (2nd sprint) (m·s⁻¹)</td>
<td>4.72 ± 0.41</td>
<td>4.90 ± 0.32</td>
<td>4.78 ± 0.35</td>
<td>4.70 ± 0.35</td>
</tr>
<tr>
<td>REC of peak speed (10 s) (%)</td>
<td>86.5 ± 5.7</td>
<td>91.7 ± 2.4³</td>
<td>94.7 ± 4.4</td>
<td>93.6 ± 3.9</td>
</tr>
<tr>
<td>FLSP (1st sprint) (%)</td>
<td>30 ± 4</td>
<td>26 ± 4</td>
<td>23 ± 4</td>
<td>28 ± 4</td>
</tr>
<tr>
<td>FLSP (2nd sprint) (%)</td>
<td>30 ± 7</td>
<td>28 ± 6</td>
<td>30 ± 1</td>
<td>28 ± 4</td>
</tr>
<tr>
<td>Mean speed (1st sprint) (m·s⁻¹)</td>
<td>4.87 ± 0.24</td>
<td>4.86 ± 0.26</td>
<td>4.61 ± 0.45</td>
<td>4.53 ± 0.46</td>
</tr>
<tr>
<td>Mean speed (2nd sprint) (m·s⁻¹)</td>
<td>4.16 ± 0.31</td>
<td>4.39 ± 0.25</td>
<td>4.25 ± 0.30</td>
<td>4.20 ± 0.37</td>
</tr>
<tr>
<td>REC of mean speed (%)</td>
<td>85.50 ± 4.99</td>
<td>90.50 ± 3.27³</td>
<td>92.40 ± 5.92</td>
<td>92.99 ± 4.64</td>
</tr>
</tbody>
</table>

All the performance data pertinent to the sprints are presented at tables 6.3 and table 6.4 (biopsy sprints). Endurance training induced improvements in recovery of MPO, recovery of peak speed, recovery of mean speed (10 s) and recovery of mean speed (P < 0.05, in all cases) for the training group in comparison with the control group.
Finally, statistical analysis indicated that performance profile in the first sprint was not changed due to the experimental period in any of the groups (main effect: training by group interaction, ns, table 6.3).

<table>
<thead>
<tr>
<th>Table 6.4. Sprint Performance variables for the training group (mean ± SD, n = 9) for the single (biopsy) trial.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TRAINING GROUP</strong></td>
</tr>
<tr>
<td><strong>PRE</strong></td>
</tr>
<tr>
<td>PPO (W)</td>
</tr>
<tr>
<td>MPO&lt;sub&gt;10&lt;/sub&gt;</td>
</tr>
<tr>
<td>F&lt;sub&gt;Po&lt;/sub&gt; (%)</td>
</tr>
<tr>
<td>MPO (W)</td>
</tr>
<tr>
<td>Peak speed (m·s&lt;sup&gt;-1&lt;/sup&gt;)</td>
</tr>
<tr>
<td>Peak speed (10 s) (m·s&lt;sup&gt;-1&lt;/sup&gt;)</td>
</tr>
<tr>
<td>F&lt;sub&gt;ISP&lt;/sub&gt; (%)</td>
</tr>
<tr>
<td>Mean speed (1&lt;sup&gt;st&lt;/sup&gt; sprint) (m·s&lt;sup&gt;-1&lt;/sup&gt;)</td>
</tr>
</tbody>
</table>

**6.3.3. MUSCLE METABOLIC RESPONSES TO THE SPRINT AND RECOVERY**

Table 6.5 shows muscle metabolite concentrations at rest, post-sprint 1 and 2 min into recovery after sprint 1, before and after training. All the muscle metabolites changed over time ($P < 0.01$).

Before training during the 30-s sprint glycogen degradation was $100 ± 17$ mmol glucosyl units kg dry muscle<sup>-1</sup>, while after training the glycogen degradation decreased to $90.0 ± 2$ ($P = 0.062$). Similarly, PCr degradation was reduced after training ($P < 0.05$). As a result, total ATP turnover from anaerobic resources had a tendency to be lower as well ($P = 0.098$). The percentage contribution of PCr degradation and glycolysis to energy supply was the same before and after training. Table 6.6 summarises these findings.
Table 6.5. Muscle metabolites (mmol·kg dry muscle\(^{-1}\)) in vastus lateralis before and after training for the training group (mean ± SD, n = 9). Where, PS = post-sprint; REC = 2 min recovery; U = uncorrected concentrations for TCr; C = corrected for TCr; N/A = not applicable; G6P = glucose 6 phosphate.

<table>
<thead>
<tr>
<th></th>
<th>BEFORE TRAINING</th>
<th>AFTER TRAINING</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rest</td>
<td>Rest</td>
</tr>
<tr>
<td>U</td>
<td>19.3 ± 2.1</td>
<td>19.4 ± 2.2</td>
</tr>
<tr>
<td>PCr</td>
<td>84.3 ± 8.9</td>
<td>82.6 ± 6.8</td>
</tr>
<tr>
<td>Creatine</td>
<td>50.7 ± 11.3</td>
<td>49.5 ± 9.8</td>
</tr>
<tr>
<td></td>
<td>± 11.3 ± 9.8</td>
<td>± 11.3 ± 11.3</td>
</tr>
<tr>
<td>Total Cr</td>
<td>135.0 ± 14.9</td>
<td>N/A</td>
</tr>
<tr>
<td>Glycogen</td>
<td>333 ± 52</td>
<td>N/A</td>
</tr>
<tr>
<td>G6P</td>
<td>0.7 ± 0.7</td>
<td>± 0.7 ± 17.8</td>
</tr>
<tr>
<td>Lactate</td>
<td>8.1 ± 5.2</td>
<td>N/A</td>
</tr>
<tr>
<td>Calculated</td>
<td>7.18 ± 0.03</td>
<td>N/A</td>
</tr>
<tr>
<td>pH</td>
<td>0.03</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Table 6.7 demonstrates the calculated anaerobic glycogenolytic and glycolytic rates as well as the measured rate of glycogen degradation. Glycogenolytic and glycolytic rates were similar after training. However, the measured rate of glycogen degradation tended to be lower after training (\(P = 0.054\)) as did the rate of PCr degradation (\(P = 0.051\)) and the total ATP rate from anaerobic metabolism (\(P = 0.098\), table 6.7).
Table 6.6. ATP provision (mmol· kg dry muscle\(^{-1}\)) from the anaerobic resources before and after training in the training group (means ± SD, n = 9). Where, “\(^{*}\)” = \(P < 0.05\) from before training.

<table>
<thead>
<tr>
<th>ATP FROM ATP DEPLETION</th>
<th>BEFORE TRAINING</th>
<th>AFTER TRAINING</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP FROM PCr DEGRADATION</td>
<td>58.8 ± 3.6</td>
<td>53.2 ± 16.0*</td>
</tr>
<tr>
<td>ATP FROM GLYCOLYSIS</td>
<td>121.0 ± 15.0</td>
<td>108.5 ± 24.1</td>
</tr>
<tr>
<td>TOTAL ATP PRODUCTION</td>
<td>184.1 ± 15.5</td>
<td>164.6 ± 39.1</td>
</tr>
<tr>
<td>% ATP FROM ATP DEPLETION</td>
<td>2.2 ± 2.0</td>
<td>1.7 ± 0.8</td>
</tr>
<tr>
<td>% ATP FROM PCr DEGRADATION</td>
<td>32.4 ± 0.7</td>
<td>32.6 ± 3.6</td>
</tr>
<tr>
<td>% ATP FROM GLYCOLYSIS</td>
<td>65.3 ± 2.3</td>
<td>65.6 ± 4.2</td>
</tr>
</tbody>
</table>

Table 6.7. Rates of ATP provision from the various anaerobic resources before and after training in the training group (means ± SD, n = 9).

<table>
<thead>
<tr>
<th>RATES</th>
<th>BEFORE TRAINING</th>
<th>AFTER TRAINING</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLYCOGENOLYTIC (mmol glucosyl·kg dry muscle(^{-1})·s(^{-1}))</td>
<td>1.9 ± 0.4</td>
<td>1.8 ± 0.4</td>
</tr>
<tr>
<td>GLYCOLYTIC (mmol glucosyl·kg dry muscle(^{-1})·s(^{-1}))</td>
<td>1.3 ± 0.3</td>
<td>1.2 ± 0.3</td>
</tr>
<tr>
<td>RATIO</td>
<td>1.46</td>
<td>1.50</td>
</tr>
<tr>
<td>RATE OF GLYCOGEN (mmol glucosyl·kg dry muscle(^{-1})·s(^{-1}))</td>
<td>3.3 ± 0.5</td>
<td>3.0 ± 1.0</td>
</tr>
<tr>
<td>RATE OF PCr (mmol· kg dry muscle(^{-1})·s(^{-1}))</td>
<td>2.0 ± 0.2</td>
<td>1.8 ± 1.3</td>
</tr>
<tr>
<td>TOTAL ATP (mmol· kg dry muscle(^{-1})·s(^{-1}))</td>
<td>6.1 ± 1.0</td>
<td>5.5 ± 1.3</td>
</tr>
</tbody>
</table>

Recovery of PCr and lactate was unaltered after training (8.4 and 1.7 % faster recovery for uncorrected and corrected PCr concentrations and 2.7 % for lactate) (figures 6.10 – 6.12). Likewise, the remaining metabolites did not show any trend of changes in recovery patterns after training (table 6.5).

Finally, muscle lactate and calculated muscle pH (table 6.5) tended to be of lower post-sprint after training (t-test, \(P = 0.095\), in both occasions).
Figure 6.10. Recovery of PCr before and after training for concentrations corrected for total creatine Where REC = recovery; Pre = pre-training; Post = post-training (means ± SD, n = 9).

Figure 6.11. Recovery of PCr before and after training for concentrations not corrected for total creatine (n = 9). Where REC = recovery; Pre = pre-training; Post = post-training (means ± SD, n = 9).
6.3.4. BLOOD METABOLIC RESPONSES TO SPRINT

All the blood metabolic responses changed over time ($P < 0.01$).

Figures 6.13 to 6.16 show the blood metabolic responses for a repeated sprint. There was a training by time by group interaction ($P < 0.05$) for blood lactate, reflecting lower lactate concentrations after the sprints in the training group in comparison with the control group after training (figure 6.13).
Figure 6.13. Blood lactate concentrations at rest, immediately post warm-up (PWP), immediately post-sprint 1 (PS1) and 2 (PS2) and during the recovery from the sprints (numbers indicate minutes of blood sampling after the 2nd sprint) before (pre) and after (post) training in training (TG, n = 9) and control (CG, n = 5) group, respectively. Where, "b" = main effect: time, \( P < 0.01; \) "c" = main effect: training x time x group interaction, \( P < 0.05. \) (mean ± SD).

On the other hand blood pH was similar after training in both groups (figure 6.14). Similarly, no alterations in calculated blood buffering capacity were present as a result of training in the first sprint (32.93 ± 8.47 vs 38.35 ± 4.98 and 30.74 ± 4.26 vs 34.50 ± 3.51 μmol·L\(^{-1}\)·pH unit\(^{-1}\) before and after training, in the training and control group, respectively). Similarly, blood buffering capacity did not change after the 2nd sprint (43.97 ± 10.61 vs 49.17 ± 2.37 and 44.59 ± 7.76 and 47.80 ± 3.41 μmol·L\(^{-1}\)·pH unit\(^{-1}\), before and after training, in the training and control group, respectively).
Figure 6.14. Blood pH at rest, immediately post warm-up (PWP), immediately post-sprint 1 (PS1) and 2 (PS2) and during the recovery from the sprints (numbers indicate minutes of blood sampling after the 2nd sprint) before (pre) and after (post) training in training (TG, n = 9) and control (CG, n = 5) group, respectively. Where, "b" = main effect: time, \( P < 0.01 \). (mean ± SD).

Similarly, plasma ammonia did not show any significant changes after training (figure 6.15).

Figure 6.15. Plasma ammonia concentrations at rest, immediately post warm-up (PWP), immediately post-sprint 1 (PS1) and 2 (PS2) and during the recovery from the sprints (numbers indicate minutes of blood sampling after the 2nd sprint) before (pre) and after (post) training in training (TG, n = 9) and control (CG, n = 5) group, respectively. Where, b = main effect: time, \( P < 0.01 \). (mean ± SD).
Estimated percentage changes in plasma volume due to sprinting were unaltered after training (figure 6.16). Estimated absolute plasma volume at rest was unchanged as well (2633 ± 179 and 2639 ± 194 vs 2592 ± 315 and 2618 ± 307 mL·kg⁻¹, before and after training in the training and control group, respectively).

Figure 6.16. Estimated plasma volume changes at rest, immediately post warm-up (PWP), immediately post-sprint 1 (PS1) and 2 (PS2) and during the recovery from the sprints (numbers indicate minutes of blood sampling after the 2nd sprint) before (pre) and after (post) training in training (TG, n = 9) and control (CG, n = 5) group, respectively. Where, "a" = main effect: time, P < 0.01. (mean ± SD).

6.3.5. PHYSIOLOGICAL RESPONSES TO SPRINT

Table 6.8 summarises the oxygen uptake at rest, during the sprint 1, during the initial phase of the recovery from sprint 1 (first 50 s), during the second phase of the recovery from sprint 1 (second 50 s) after the 10 s break for changing the Douglas bags, total oxygen uptake during recovery and during the sprint 2. There was a training by time interaction (P < 0.01), reflecting lower oxygen uptakes after training in both groups as a total, however no significant interaction for training by time by group was revealed indicating the lack of influence of endurance training on this variable.
Table 6.8. Oxygen uptake (mL· kg\(^{-1}\)· min\(^{-1}\)) at rest, during sprint 1, during the initial 50 s (REC 1) of the 2 min recovery after the sprint 1, during the second 50 s (REC 2), during the total recovery period (TOTAL REC) and during sprint 2 before (PRE) and after (POST) training in training and control groups (mean ± SD, n = 9 and n = 5 for training and control group, respectively).

<table>
<thead>
<tr>
<th></th>
<th>TRAINING GROUP</th>
<th></th>
<th>CONTROL GROUP</th>
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<tbody>
<tr>
<td></td>
<td>REST SPRINT</td>
<td>TOTAL SPRINT</td>
<td>REST SPRINT</td>
</tr>
<tr>
<td></td>
<td>1 1 2 2 2 2 2</td>
<td>1 1 2 2 2 2</td>
<td>1 1 2 2 2 2</td>
</tr>
<tr>
<td>PRE</td>
<td>4.0± 38.3± 28.2 13.7 37.9± 40.4± 3.7± 38.1± 30.1 12.8 38.1± 39.9±</td>
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<tr>
<td>(mL· kg(^{-1})· min(^{-1}))</td>
<td>0.5 3.0 ± 2.8 ± 1.6 4.2 2.6 0.3 ± 3.3 ± 2.9 2.7 2.5</td>
<td></td>
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<tr>
<td>POST</td>
<td>3.8± 34.3± 27.5 13.1 36.9± 38.1± 3.7± 36.1± 28.3 11.6 36.2± 38.7±</td>
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<tr>
<td>(mL· kg(^{-1})· min(^{-1}))</td>
<td>0.3 3.8 ± 2.1 ± 1.8 3.4 2.7 0.4 ± 3.4 ± 2.4 4.0 3.0</td>
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</table>

6.3.6. MENSTRUAL CYCLE INFORMATION

The resting hormonal concentrations for estradiol and progesterone prior to the repeated sprints for the training and control group before and after training are shown in table 6.9. The individual concentrations for estradiol and progesterone show that the majority of the subjects were tested at the same phase of their menstrual cycle before and after training for the repeated sprints. Only subject No 2 and No 8 from the training group were tested in different phases of their menstrual cycle before and after training (follicular vs luteal and follicular vs late luteal for subject No 2 and No 8, before and after training, respectively). For subject No 8 the circumstances were more complicated since perimenstrual problems were mentioned. Nonetheless, her testing days in the pre-training sprint trial coincided with the first 2 days of her menstrual cycle, where the perimenstrual symptoms remained (that is, similar conditions). From the control group there was one subject (No 3 in table 6.9) with low progesterone and high estradiol concentrations for the pre-training sprint trials and high progesterone and estradiol concentrations for the post-trials. For the biopsy sprint as well as the speed-lactate test and \(\dot{V}O_2\) max test, the menstrual cycle phase was calculated based on the sprint trials’ hormones and menstrual cycle diary (data not shown). Apart from the subjects mentioned above all the other volunteers were tested in an identical phase of their menstrual cycle before and after training in the rest of the tests. For subject No 2 the rest of the tests were estimated to have been conducted at similar hormonal concentrations before and after training. For subject No 8 it was estimated to be late
luteal phase before and after training for the speed-lactate and $\dot{V}O_2$ max tests while it would have been the late luteal and early follicular phase (beginning of the menses) for the biopsy trial. For the subject No 3 for the control group the $\dot{V}O_2$ max test and speed-lactate test were performed during the follicular phase before training while after training during the luteal phase.

It should be noted here that participants with perimenstrual problems (apart from subject No 8) did not perform any test during the days of this discomfort.

Finally, all subjects using oral contraceptives use had done so for at least 2 years prior to commencement of the experimental procedures and all used MICROGYNON monophasic oral contraceptives (which contained 30 µg ethinyl-oestradiol and 0.15 mg levonorgestrel). Subject No 5 from the controls reported that she had stopped taking oral contraceptives 4 months prior to her participation in the study.

Table 6.9. Individual values for progesterone and estradiol before (PRE) and after (POST) training in training (n = 9) and control (n = 5) group. Where +: individuals with perimenstrual problems; *: individuals using oral contraceptives.

<table>
<thead>
<tr>
<th>SUBJECTS</th>
<th>TRAINING GROUP</th>
<th>CONTROL GROUP</th>
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<tbody>
<tr>
<td></td>
<td>Estradiol (pg·mL⁻¹)</td>
<td>Progesterone (ng·mL⁻¹)</td>
</tr>
<tr>
<td></td>
<td>PRE</td>
<td>POST</td>
</tr>
<tr>
<td>1</td>
<td>3*</td>
<td>39</td>
</tr>
<tr>
<td>2</td>
<td>77+</td>
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<td>47+</td>
<td>81</td>
</tr>
<tr>
<td>9</td>
<td>123</td>
<td>40</td>
</tr>
</tbody>
</table>
6.3.7. CORRELATIONS

From the performance variables that improved after training (recovery of MPO, recovery of peak speed, recovery of mean speed during the first 10 s and recovery of mean speed) \( \dot{V}O_2 \text{ max} @ 4 \text{ mmol L}^{-1} \) did not show any association with recovery of power output. However, \( \dot{V}O_2 \text{ max} \) was positively correlated with all the above variables before and after training, association that reached significance only after training in two of them (\( r = 0.54, P < 0.05 \) for MPO; \( r = 0.54, P < 0.05 \) for peak speed). \( \dot{V}O_2 \text{ max} \) was also significantly correlated with the recovery of MPO and peak speed during the first 10 s of the second sprint after training (\( r = 0.53, P < 0.05 \) and \( r = 0.68, P < 0.01 \), respectively). It should be noted here though, that these relationships were found only when pooled data from both groups were analysed.

For the training group, peak speed, mean speed, PPO and MPO were all correlated with ATP provision from anaerobic resources before training (\( r = 0.60-0.66, \text{ns} \)). Interestingly, these associations had disappeared after training (\( r = 0.0-0.11 \)).

Taking the group as a whole \( \dot{V}O_2 \text{ max} \) was significantly correlated with a) \( \dot{V}O_2 \) during the sprint 1 (\( r = 0.70, P < 0.01 \)), reflecting higher aerobic contribution to the sprint for the individuals who possess high \( \dot{V}O_2 \text{ max} \), b) with initial recovery \( \dot{V}O_2 \) (\( r = 0.76, P < 0.01 \)), indicating that the higher the \( \dot{V}O_2 \text{ max} \) the higher the initial oxygen uptake after the sprint, and c) with the sprint 2 (\( r = 0.53, P < 0.05 \)). After training using pooled data these associations were still present for the first sprint (\( r = 0.67, P < 0.01 \)) and initial recovery \( \dot{V}O_2 \) (\( r = 0.71, P < 0.01 \)), but only for the first sprint (\( r = 0.70, P < 0.05 \)) when the training group data were analysed without the control group.

Phosphocreatine at 2 min into recovery after training was correlated with recovery of MPO (\( r = 0.77, P < 0.05 \)), showing that MPO recovery was best in individuals with a high PCr prior to sprint 2.
6.4. DISCUSSION

The purpose of the present study was to examine the effect of short-term endurance training on the recovery of selective muscle metabolites and power output restoration during a repeated 30-s treadmill sprint in female non-endurance athletes. It was revealed that moderate changes in endurance fitness could evoke considerable improvements in performance restoration as reflected by the faster recovery of MPO ($P < 0.05$), peak speed ($P < 0.05$), mean speed during the first 10 s ($P < 0.05$) and mean speed ($P < 0.05$) during the second sprint after training in the training group in comparison with the control group. PCr resynthesis and muscle lactate removal were not significantly altered after training, although both PCr degradation ($P < 0.05$) and, glycogen utilisation ($P = 0.062$) were reduced after training. Thus, there was an 11 % (ns) lower ATP provision from anaerobic energy resources during the first sprint after training which may have had an impact on the restoration of power in the second sprint.

Participants in the training group ran on average 15000 m (1st wk) to almost 17000 m (5th wk) per week (figure 6.4), or, 5000 m per training session at an intensity that elicited 80-85 % $\dot{V} O_2$ max. This training distance was equivalent to a duration of 84 min every week (figure 6.5), or, alternatively 28 min every session. The participants training compliance was excellent and only 5 training sessions were missed in total and no more than 2 from the same subject.

Although maximum oxygen uptake was similar before and after training when one outlier was removed from the control group the increase of 2.3 % in the experimental group became statistically significant in comparison with the control group ($P = 0.042$), clearly reflecting the positive impact of this training regimen on $\dot{V} O_2$ max. That there were only five subjects for the control group for this variable was possibly another reason for the difficulty in observing statistically significant differences between the two groups in the training state. This 2.3 % increase in maximum oxygen uptake in the present study is similar in magnitude to the increase for the training group in chapter 5 and for published studies with similar designs (Williams and Nute, 1986; Callister et al., 1988). Other investigations have shown greater improvements in $\dot{V} O_2$ max after similar training programmes (Gaesser and Rich, 1984; Hardman et
CHAPTER 6 ENDURANCE TRAINING AND POWER RECOVERY

al., 1986; Mayes et al., 1987). It is possible that in these studies where there was a relatively large increase in \( \dot{V}O_2 \text{max} \) subjects were further from their genetic potential at the start of the training programme so that the increases when expressed as a percentage were large (Pollock, 1973; Mayes et al., 1987). The participants in the present training study were well-trained (based on training status questionnaire, Appendix G) and in addition, their pre-training \( \dot{V}O_2 \text{max} \) (49.2 mL· kg\(^{-1}\)· min\(^{-1}\) for both groups) is consistent with this statement as indicated from the ACSM rankings for women of 22 years of age (American College of Sports Medicine, 2000).

Maximal ventilation showed a clear training effect \((P < 0.05)\) as manifested with the 7.9 % higher volume in the training group in contrast with the minimal 0.3 % elevation of the control group after training. This finding probably reflects the higher requirements for oxygen and the analogous need to eliminate carbon dioxide when a higher \( \dot{V}O_2 \text{max} \) appears after training (McArdle et al., 2001).

Likewise, maximal heart rate was reduced \((P < 0.05)\) in the training group in comparison with the control group after training. Although this is a surprisingly adaptation for only six weeks of training it agrees with other published investigations (Hurley et al., 1984; Williams and Nute, 1986). The training probably increased the tonic activity of parasympathetic depressor neurons and decreased the tonic activity of the sympathetic accelerator resulting in a lower maximal heart rate after training (Goldsmith et al., 1997).

The exercise time to exhaustion at the highest work load during the \( \dot{V}O_2 \text{max} \) test has been used previously as an endurance performance test (Schoene et al., 1981; Jurkowski et al., 1981). Due to the already heavy programme of the subjects during the course of these experimental perturbations (44 visits in the laboratory each) it was decided to use time to exhaustion as an endurance performance test and not employ any additional test. Subjects in the training group were able to run for longer as shown by a 12.7 % \((P = 0.095)\) longer time at the highest work load during the \( \dot{V}O_2 \text{max} \) test in comparison with 4.1 % decline in the control group. This tendency to run longer during the \( \dot{V}O_2 \text{max} \) test was possibly the result of metabolic adaptations within the skeletal muscle and more particularly with the sparing effect of endurance training on
skeletal muscle glycogen and greater dependency towards noncarbohydrate sources namely oxidation of fats and more particularly muscle triglyceride oxidation (Romijn et al., 1993). The significant positive relationship ($r = 0.55$, $P < 0.05$, data not shown) between time to exhaustion and blood lactate concentration at the 4th (highest lactate concentration) speed after training (pooled data) supports this notion in the context that lactate concentration at a given speed reflects endurance adaptations at the cellular level (Sjodin et al., 1982; Weltman, 1995). In addition, reduced glycolysis in the present study would have been accompanied by a lower production of lactic acid and thus lower H+ which in turn would have lessened the predisposition to fatigue (Sahlin, 1992). In addition, improved muscle buffering capacity after training can not be excluded (Weston et al., 1997).

The influence of training on sub-maximal exercise responses during the speed-lactate test is reflected by the strong tendency ($P = 0.063$) for lower blood lactate concentrations during the speed-lactate test in the training group in comparison with the control group (table 6.2 and figure 6.8). This finding is in agreement with numerous studies in the literature (Yoshida et al., 1982b; Hardman et al., 1986; Williams and Nute 1986; Mayes et al., 1987) and probably reflects a lower rate of lactate production in the training state (Constable et al., 1987; Green et al., 1991b; Coggan and Williams, 1995). The suggested drop in glycolysis in the present study probably reflects the increased contribution of other pathways to supply energy with the best likely explanation being an increase in fat oxidation (Henriksson, 1977; Holloszy and Coyle, 1984; Hurley et al., 1986). After training a higher energy supply from fat oxidation would probably be via greater oxidation of muscle triglycerides (Hurley et al., 1986). Muscle triglycerides are the major energy substrate from fat oxidation at an intensity which elicits 85 % $\dot{V}O_2$ max in both men (Romijn et al., 1993) and women (Romijn et al., 2000) and it was the intensity of the training programme adopted here. Greater oxidation of muscle triglycerides can be achieved with unchanged oxygen uptake at the same absolute work load following training (Henriksson, 1977), as was the case in this study, and is a function of improved muscle oxidative capacity (Henriksson, 1977; Hurley et al., 1986). The large increase in activity of the mitochondrial enzymes cytochrome oxidase and succinate
dehydrogenase in a similar training study (Henriksson and Reitman, 1977) is consistent with the above idea.

Nevertheless, neither muscle triglycerides nor plasma free fatty acids or plasma glycerol were measured in the present study in order to verify the above suggestion. In addition, respiratory exchange ratio (R) as an indirect measure of the percentage contribution of fat and carbohydrates has inherent limitations (Livesey and Elia, 1988) when high values are obtained, as was the case with this study (table 6.2.).

There was a 3.6 % increase in the % \( \dot{V}O_2 \max @ 4 \text{ mmol} \cdot \text{L}^{-1} \) in the training group in comparison with the 1.9% fall in the control group after training (ns). However, it would not be entirely appropriate to interpret the data in this context since close inspection of the individual values revealed that one volunteer of the training group demonstrated a 16 % drop after training. If this participant was eliminating from the statistical analysis the training group improvement was increased 6.1 % after training and the probability value dropped to \( P = 0.028 \). This 6.1 % improvement agrees favourably with the 7 % enhancement in % \( \dot{V}O_2 \max @ 4 \text{ mmol} \cdot \text{L}^{-1} \) after training in a study with a much more severe training protocol (6 times per week for 12 weeks) by Hurley and co-workers (1984) and supports further the suggestion of an increased oxidative capacity after training in the training group probably via increases in the number, size and surface area of skeletal muscle mitochondria (Hurley et al., 1984; Holloszy and Coyle, 1984) and/or mitochondrial enzymes such as cytochrome oxidase and succinate dehydrogenase (Henriksson and Reitman, 1977).

Although some indexes of the endurance training adaptations did not reach statistical significance after training in the training group in comparison with the control group (i.e. \( \dot{V}O_2 \max, P > 0.05 \), lactate concentrations during the sub-maximal speed-lactate test, \( P = 0.063 \)) adaptations at the cellular level (i.e. muscle oxidative capacity) might be more sensitive to the training stimulus of the present study. This view is supported by a study employing a much shorter length of continuous endurance training which was accompanied by reductions in glycogen and PCr utilization and lower muscle lactate concentrations during sub-maximal exercise after training (Green et al., 1991a). These adaptations were independent from the initial fitness status of the
subject as indicated by $\dot{V}O_2\max$ (Green et al., 1999), changes in $\dot{V}O_2\max$ per se (Green et al., 1991c; Green et al., 1992; Green et al., 1999) enzyme activity (Green et al., 1991a; Green et al., 1992) or skeletal muscle mitochondrial capacity (Green et al., 1992). In addition, it has been experimentally shown that the time course of endurance adaptations in some enzymes such as cytochrome oxidase and succinate dehydrogenase is much quicker that the respective time course for changes in $\dot{V}O_2\max$ (Henriksson and Reitman, 1977).

Moreover, the experimental design of the present study was very robust in that a control group was employed in contrast to many other studies in the literature (Henriksson and Reitman, 1977; Yoshida et al., 1982; Hurley et al., 1984; Hurley et al., 1986; Hardman et al., 1986; Williams and Nute 1986; Mayes et al., 1987; Green et al., 1991a; Green et al., 1992; Green et al., 1999). This inclusion of the control group made the attempt to reject the null hypothesis even harder especially when considering the high initial fitness status of the present study volunteers and the short length of the training programme. For example examining the training group alone (as in most of the earlier studies), blood lactate concentrations during the speed-lactate test would have been clearly statistically lower ($P < 0.01$).

Thus, in summary this training programme did result in a marked increase in endurance training volume and intensity for these subjects. In addition, the programme did facilitate some improvements in endurance fitness and thus provides a suitable physiological framework to assess the effects of endurance training on repeated sprint performance and metabolism in non-endurance athletes.

Before training the PPO and MPO during the 2 sprints were similar in magnitude to the PPO and MPO reported by Cheetham et al. (1986) and Nevill et al. (1996a) who employed an identical design. Similarly, the majority of muscle metabolites (mmol·kg dry muscle$^{-1}$), at rest and immediately after the sprint are consistent with those values published previously for female and male subjects when the muscle samples were taken from the vastus lateralis muscle using the needle biopsy technique (Cheetham et al., 1986; Nevill et al., 1989; Bogdanis et al., 1995). However, ATP concentration at rest was somewhat lower than expected (19.4 mmol·kg dry muscle$^{-1}$). This is not a
unique finding, though as there are several published reports in the literature with similar resting ATP concentrations (Hellsten et al., 1993; Stathis et al., 1994). Although speculative, it seems that the low ATP concentrations observed in the present study may be due to the fact, at least in part, that the muscle biopsy sprint trial was only 48 h after the first repeated trial and consequently ATP may not have fully recovered (Hellsten et al., 1993; Stathis et al., 1994).

Pre-training PCr degradation during the first 30-s sprint was similar to that reported previously after treadmill sprinting (Cheetham et al., 1986; Nevill et al., 1989), but it was somewhat less pronounced when compared with sprint cycling (15-20 % of the resting concentration at post-sprint sample, Bogdanis et al., 1995; Bogdanis et al., 1996a). There are two possible explanations for these findings. Firstly, it has been suggested that only elite sprinters can deplete high energy resources maximally or to a greater extent in comparison with sub-elite sprinters (Hirvonen et al., 1987). The participants in the present study could by no means be considered elite sprinters. Some of them were national calibre athletes (England U19 and U23) but they were all games players. A second possible explanation for the lower degradation of PCr during sprint running in comparison with sprint cycling is the fact that the vastus lateralis is not recruited to the same extent in sprint running as in sprint cycling (Howald et al., 1985; Clarys and Cabri, 1993). The time delay from the cessation of the sprint to the taking the biopsy is also a possibility, but seems rather unlikely as an explanation since all the samples in the present study were collected (from cessation of exercise to the placement in liquid nitrogen) 14.1 ± 1.3 s and 13.8 ± 2.5 s before and after training, respectively. Cheetham et al. (1986) reported a similar PCr degradation to that described here and reported an average biopsy time of 7.2 ± 0.9 s.

Oxygen uptake during a 30 s whole-body running sprint has not been previously reported and thus the oxygen uptakes during treadmill sprinting are a novel finding in the present study. Pre-training oxygen uptake during the first 30-s sprint was 38.3 ± 3.0 mL· kg⁻¹· min⁻¹ and 38.1 ± 3.3 mL· kg⁻¹· min⁻¹ for the training and control group, respectively (table 6.8). This was equivalent to about 78 % \( \dot{V}O_{max} \) for both groups and it is 17 % higher than the reported oxygen uptake (using the same Douglass bag method) for a 30 s cycle ergometer sprint (Bogdanis et al., 1996a). The higher oxygen
uptake during treadmill sprinting in comparison with cycling probably reflects the larger muscle mass recruited during whole body sprinting in the present study in contrast to sprint cycling (Bogdanis et al., 1996a). In the second sprint the oxygen uptake was even higher (table 6.8) as has been described earlier for cycle ergometer sprinting (Bogdanis et al., 1996a). These findings should be treated with caution though as the Douglas bag technique has its limitations when expired air samples are collected for only 30 s (Taylor et al, 1955; Howley et al., 1995 and discussion below).

A key finding of the present study was that endurance training did not affect performance during the first 30-s sprint, which is important for the subsequent examination of the effect of endurance training on the recovery of performance. This lack of impact on the first sprint is in agreement with an earlier cycling study where 6 weeks of endurance training at 85 % $\dot{V}O_2$ max did not compromise performance in the first 30-s cycle ergometer sprint (Hardman et al., 1986). Moreover, the resting concentrations of all muscle metabolites were similar before and after training which is consistent with previous research (Karlsson et al., 1972; Saltin and Gollnick, 1983).

However, after training some of the muscle metabolic responses immediately after the first sprint were attenuated. The ATP generated from PCr degradation during the first 30-s sprint declined by 10 % after training in the training group ($P < 0.05$) whilst ATP depletion was 53 % less (ns) and, glycogen utilisation was 10 % lower ($P = 0.062$). This resulted in 11 % reduction of ATP provision from anaerobic resources ($P = 0.098$), but the percentage contribution from each anaerobic energy source did not change after training. This slight weakening in the association between anaerobic energy supply and performance was also shown by the drop in their positive correlations after training ($r = 0.60-0.66$ before training and $r = 0.00$ to 0.11 after training between PPO, MPO, mean speed, peak speed and ATP provision from anaerobic metabolism).

As performance in the first sprint was unchanged after training, but anaerobic energy supply tended to decrease, possibly there was an improvement either in the aerobic contribution to the sprint and/or in the mechanical efficiency. There was a significant positive correlation between $\dot{V}O_2$ max and oxygen uptake during sprint 1 after
training \((r = 0.70, P < 0.05)\), but as mentioned earlier oxygen uptake during sprinting and recovery from sprinting, measured with Douglas bag method, was unchanged. However, the Douglas bag method may not be sensitive enough to detect small changes in oxygen uptake \((Taylor \textit{et al.}, 1955; Howley \textit{et al.}, 1995)\), thus it remains a possibility that there was greater energy supply from aerobic metabolism to the sprint after training.

The reduction in PCR degradation and the tendency for the reduction in glycogen utilisation during the first sprint after training could also be explained by some sort of transformation of fast twitch \(\text{I}b\) fibres to fast twitch \(\text{IIa}\) or slow twitch fibres \(I\) as previously shown in a similar training design \((Andersen \textit{and Henriksson}, 1977b)\). It has also been reported that cycling exercise at an intensity requiring 85 \% \(\dot{V}O_2\max\) (identical as in the present study) recruits all three types of muscle fibres \((Andersen \textit{and Sjøgaard}, 1975)\) which further supports the above view. Therefore, this shift in metabolic response could be explained, at least in part, by the increases in oxidative capacity of mixed skeletal muscle as a consequence of enhancement in oxidative capacity of type \(\text{IIa}\) fibres and a transformation of some \(\text{I}b\) fibres into \(\text{IIa}\). However, performance was unchanged during the first sprint and had the number of \(\text{I}b\) fibres had been reduced, a parallel impact in PPO (reduced) should appear. It seems that although previously histochemical analysis has revealed some transformation of type \(\text{I}b\) fibres to type \(\text{IIa}\) and \(I\) \((Andersen \textit{and Henriksson} 1977b, Baumann \textit{et al.}, 1987; Howald \textit{et al.} 1985), all employing similar training programmes, both type \(\text{IIa}\) and \(\text{I}b\) fibres could be identical in protein composition after training containing the same fast variety of myosin light chains and heavy chains as well as troponin-I thereby their interconversion could not be seen at the cellular level \((Baumann \textit{et al.}, 1987)\). This view agrees with the conceptual framework of Pette \(1984\) that skeletal muscle fibres respond in a concerted way in order to adapt their function to varying physiological demands: a) intracellular regulation of activity by handling of the free calcium concentration, b) energy metabolism, and, c) the contractile machine itself. It is also consistent with previous research that short-term endurance training with similar type of training increases the oxidative capacity of all fibre types as indicated with the significant increases in mitochondrial volume \((Howald \textit{et al.}, 1985)\). Furthermore, in these early studies \((Andersen \textit{and Henriksson}, 1977b; Howald \textit{et al.} 1985; Baumann \textit{et al.}, 1987)\).
et al., 1987) muscle fibre types were identified with histochemical analysis which is no longer considered as accurate as the SDS gel electrophoresis and in recent years another type of fast twitch fibre has been discovered, the type IIx, an intermediate fibre between IIa and IIb (Fitts and Windrick, 1996). Thus, for the present study at least, the training regimen adopted seems to have increased the oxidative potential of all muscle fibres without compromising their contractile characteristics and thereby power output.

The second possibility for this reduction in energy supply from anaerobic energy resources is the enhanced mechanical efficiency after training. This suggestion is supported by the unaltered oxygen uptake during the sprint 1 and recovery from the sprint, considering of course, the limitations of the Douglas bag technique mentioned in the previous paragraphs. In maximal intensity whole body exercise the determination of efficiency (E) is complex because it is difficult to determine the energy production during this type of exercise (Bangsbo, 1996). In sprint running the problems are even more complicated since the external mechanical work can not be easily measured (Bangsbo, 1996). With respects to the nonmotorised treadmill ergometer used in these series of experiments this limitation is overcome for the first time by enabling the measurement of the horizontal work done during sprinting (Lakomy, 1987) which can be considered adequate to describe the external mechanical work. The horizontal work done is considered adequate to reflect the external mechanical work as the vertical component of the work done is independent of the running speed (Fukunaga et al., 1978). However, judging from the equation 1 with regards to E during maximal intensity exercise:

\[ E = W_e \cdot (E_t - E_r)^{-1} \]  

Where \( W_e \) = external mechanical work, \( E_t \) = total energy consumption and \( E_r \) = energy consumption at rest.

as given by Bangsbo (1996) the possibility of accurately measuring E is difficult from the present data. However, the fact that external work did not change after training coupled with the similar oxygen uptake and the reduction in PCr degradation and tendency for the reduction in glycogen utilisation suggests that an improvement in mechanical efficiency was certainly a possibility. At the cellular level this may be
explained by the fact that endurance training may enhance $\dot{V}O_2\text{ max}$ and the capacity for oxidative metabolism in muscle (possible adaptation as in the present study), but at the same time have little influence on the enzymes in glycolysis (Saltin and Golnick, 1983, glycolytic and glycogenolytic rate the same after training in the present study, table 6.7.) and the contents of high energy phosphates in the muscle (present study, Karlsson et al., 1972). Bangsbo (1996) suggests, based in in vitro animal data (Crow and Kushmerick 1982), that improved E may be due to increased oxidative potential of single fibres (discussed above) due to their ability to recover quicker (Casey et al., 1996; Growther and Gronka, 2002). In addition, improved thermoregulation after endurance training (Baum et al., 1976) would mean that less energy is consumed to regulate muscle temperature during sprinting which has been shown to increase by 2.1 °C (Allsop et al., 1990). This in turn will reduce further the denominator in equation 1 resulting in further increases in mechanical efficiency after training.

An attractive alternative hypothesis for the lower PCr degradation could be that the initial phase of PCr resynthesis was higher after training resulting from the improved oxidative capacity of fast twitch fibres after training as mentioned above. This suggestion matches well with enhanced PCr recovery during the initial 12 s of recovery (similar with the timing of the post-sprint biopsy in the present study) findings based on $^{31}$P-NMR spectroscopy technique (Walter et al., 1997).

The main finding of the present study was that MPO, peak speed, peak speed at 10 s and mean speed during the second sprint were increased (from 3-7 %, $P < 0.05$) in the training group after training (table 6.3). It was suggested that such improvements in performance during the second sprint may be due to enhanced PCr resynthesis. However, other than the possible faster resynthesis during the first 12 s of recovery there is no evidence from muscle metabolites that PCr resynthesis was any faster after training. Thus other explanations must be sought for the improvement in the recovery of performance after training.

One possible explanation could be that after endurance training an increased reliance on aerobic energy resources and/or improved mechanical efficiency in sprint 1, via
the mechanisms already mentioned in the previous paragraphs, would reduce the consumption of high energy phosphates (ATP, PCr) especially in fast twitch fibres which in turn would result in higher energy stores just prior to the sprint thereby better performance in sprint 2. This suggestion is supported by the 4 % higher PCr concentration after training (ns) just prior to the 2nd sprint (table 6.5) and explains half of the improvements in the recovery of MPO. There was also a significant positive correlation between the restoration of MPO and PCr just prior to sprint 2 (r = 0.77, \( P < 0.05 \)). Similar elevations of PCr after creatine supplementation just prior to a second bout of maximal exercise have resulted in even higher % restoration of MPO (10 %, Casey et al., 1996a).

If subjects were relying to a greater extent on aerobic metabolism after training anaerobic glycolysis could be reduced in the first sprint as well which in turn would reduce lactate production (Constable et al., 1987) resulting in lower accumulation of \( \text{H}^+ \) prior to the second sprint and therefore better resistance to fatigue (Hermansen 1982). Muscle lactate and muscle pH tended to be lower post-sprint 1 after training (t-test, \( P = 0.095 \), repeated measures of ANOVA, ns).

The second possibility for improved MPO recovery could be simply that improved mechanical efficiency was also enhanced in sprint 2 with similar mechanisms as mentioned for sprint 1. The reduced blood lactate concentrations post-sprint 1 and sprint 2 in the training state in the training group in comparison with the control group (\( P < 0.05 \), figure 6.13) are in the same direction.

One important question that emerges from these results is the lack of quicker PCr resynthesis after training even though the endurance fitness of the subjects was enhanced. From the theoretical point of view enhanced oxidative capacity and oxygen delivery in the skeletal muscle after training (Holloszy and Coyle, 1984) would result in faster PCr recovery since both of these endurance adaptations have been shown to enhance PCr recovery (Quistorf et al., 1992; Walter et al., 1997) as originally established by Harris et al. (1976).

One possible explanation for the lack of change in PCr after 2 min recovery is that it is believed that PCr resynthesis in later stages of the recovery process (> 1) does not
differ among individuals with variation in their endurance fitness when severe intramuscular acidosis (low pH) is present (McMahon and Jenkins, 2002). This view derives from studies utilising a small muscle mass in combination with $^{31}$P-NMR spectroscopy technique (Yoshida and Watari, 1993; Petersen and Cooke, 1994; Takahashi et al., 1995; Cooke et al., 1997) and is based on the curvilinear relationship between PCr and lactate content in the muscle which is independent of duration, intensity and type of exercise (Harris et al., 1977). According to this theory high accumulation of $\text{H}^+$ will shift the creatine kinase equilibrium (equation 2) towards PCr breakdown.

$$\text{PCr} + \text{MgADP}^- + \text{H}^+ \leftrightarrow \text{MgATP}^2^- + \text{creatine} \text{ [equation 2]}$$

In the present investigation despite the fact that muscle pH was not measured calculated pH from muscle lactate (Sahlin et al., 1976) was very low which agrees favourably with the above view. Low pH may also be the dominant factor for the absence of statistically significant lactate disappearance from the muscle after training in the present study (Sahlin et al., 1976; Tesch et al., 1978).

This view does not agree with the lack of association between muscle pH and PCr recovery and between muscle pH and performance in previous research (Bogdanis et al., 1996a). However, muscle pH with the needle biopsy technique measures both intra and extracellular pH in contrast with $^{31}$P-NMR spectroscopy technique where the true intracellular pH is measured (Wilson et al., 1988).

It is also known that PCr resynthesis exhibits a biphasic profile (Harris et al., 1976) with half-time of $\approx 56$ s after a 30-s sprint (Bogdanis et al., 1995). Bearing in mind the suggestion that PCr resynthesis is not prohibited during the initial stage of recovery (Walter et al., 1997; McMahon and Jenkins, 2002), then a faster initial PCr restoration after training in the training group could be an alternative possibility in this investigation. The higher PCr concentration 14 s post-sprint 1 supports this view. The fact that intramuscular acidosis does not play a major role during this initial stage is probably due to the fact that according to the creatine kinase equilibrium (equation
2) PCr resynthesis throughout the recovery behaves as a function of both intracellular pH and net ATP flux (Walter et al., 1997).

In addition, it has been reported that minimum muscle pH is not reached till 1 min into the recovery (Taylor et al., 1983) when muscle pH is at its maximum which in turn might explain why PCr is not impaired during the initial phase of recovery following maximal exercise. Further studies (with due attention to ethical concerns) would benefit from more biopsies in the recovery as a more detailed study of PCr restoration is required.

Another plausible mechanism could be that even though blood flow (and thus oxygen delivery), remains elevated following exercise (Bangsbo and Hellsten, 1998) perhaps it was not high enough to overcome the intramuscular acidosis. This might explain why in the Graham and colleagues (1995) preliminary report, where active recovery was employed, endurance-trained athletes possessed superior recovery of PPO in comparison with sprint-trained athletes even though after the two groups were matched for the same work for sprint 1 (2 sprints protocol). It seems that since PCr resynthesis is a function of both oxidative capacity (Walter et al., 1997) and oxygen delivery (Quistorf et al., 1992) in the skeletal muscle, elevated blood flow by utilising active recovery, thereby better oxygen delivery, would be able to overcome the obstacle of intramuscular acidosis by removing faster H⁺ and thus forcing backwards the creatine kinase equilibrium (equation 2) facilitating PCr resynthesis.

Two preliminary reports that examine PCr resynthesis before and after endurance training support the above speculations. PCr (measured by means of ³¹P-NMR) recovery was quicker after endurance training following a bout of plantar flexion exercise able to reduce PCr to 50 to 60% of its initial concentration while the end pH remained at 7.0-7.1 (McCully et al., 1991). It seems that on this occasion lack of severe intramuscular acidosis did not prevent PCr resynthesis after training according to the physiological mechanisms mentioned above. In a different experimental perturbation Bishop and colleagues (2003) used a time to fatigue cycle test (at an intensity that elicited peak $\dot{V}O_2$) before and after 8 weeks of endurance training that increased by 20% both peak $\dot{V}O_2$ and the lactate threshold (experimenters did not
define the last term), however, PCr resynthesis and lactate disappearance from the muscle were unaltered. In the latter study biopsies were performed before and at 10 s and 3 min after the test, while end pH was 6.88 and 6.87 before and after training. It appears that intramuscular acidosis inhibited PCr recovery and/or the experimenters missed the opportunity to see initial faster PCr resynthesis due to delay of the muscle biopsy (3 min post-exercise).

The blood metabolic responses to endurance training have been discussed in chapter 5 and are not considered further here as the responses in the two studies were very similar.

With respects to the menstrual cycle, in chapter 4 it was shown that menstrual cycle phase does not affect performance and possibly metabolic responses as suggested by blood metabolites. However, if perimenstrual pain is present some impact on performance may occur (Giacomoni et al., 2000). As mentioned in the results section of this chapter only one subject tested reported perimenstrual problems. However, this subject was tested in similar conditions (of perimenstrual pain) before and after training. Only one subject (from the control group) was tested in a different cycle phase for the speed-lactate test (for further discussion the reader is referred to chapter 5). On the other hand the effects of oral contraceptives on performance and metabolism in the present study are extremely difficult to assess since for this study subjects oral contraceptives users had been taking the contraceptives for at least 2 years and the impact of long-term oral contraceptive use is unknown. Nevertheless, due to the short period of the study the influence, if any, of the oral contraceptives on the variables measured should be considered minimal (Bemben, 1993).

In conclusion, the present study showed that short-term endurance training enhanced the recovery of performance in female subjects during 2 maximal 30 s treadmill sprints with a 2 min recovery between sprints. The enhanced performance during the second sprint could be explained by a faster initial PCr resynthesis (first min of recovery) whilst mechanical efficiency and a tendency towards greater reliance on aerobic energy resources in both sprints could be a contributory factor.
CHAPTER 7: GENERAL DISCUSSION.

7.1. INTRODUCTION

The aim of this chapter is to summarise the findings of the experimental studies of this thesis in order to provide a better insight into the physiological mechanisms that are involved in the recovery process during repeated sprints after endurance training. In addition, a brief overview of the findings with regards to the four methodological studies completed will be presented. Finally, the limitations of the work in the thesis, directions for future research and practical recommendations are also considered.

7.2. MAIN FINDINGS

1. It is clear from the experimental chapters 5 and 6 that the recovery of sprint performance is improved as a result of endurance training. This is mainly reflected by the 7 % better restoration of MPO during sprint 2 in the training group in comparison with the control group ($P < 0.05$) after training.

2. In the second training study where muscle biopsies were taken a 16 % higher ($P < 0.05$) post-sprint PCr concentration may reflect faster initial PCr resynthesis as a consequence of the endurance training. However, due to the strong tendency towards lower glycogen utilisation ($P = 0.062$) and a general tendency for a lower dependence on anaerobic energy resources ($P = 0.098$) an enhanced mechanical efficiency and a greater reliance on aerobic energy resources cannot be excluded.

3. The significant improvements in recovery of sprint performance were achieved with modest improvements in $\dot{V}O_2\text{max}$ and $\% \dot{V}O_2\text{max} @ 4\text{ mmol} \cdot \text{L}^{-1}$ (2-3 % higher after training).

4. From chapter 4 it is concluded that the menstrual cycle does not influence sprint running performance as reflected by the unchanged performance during the follicular phase, mid-cycle phase (just prior to ovulation) and the luteal phase. In addition, metabolic responses are also unaffected by menstrual cycle phase as suggested by the similar blood metabolic responses.
5. From the methodological study 1 it is clear that if the subjects are well familiarised the non-motorised treadmill is a very reproducible exercise model for measuring sprint performance.

6. The second methodological study demonstrated that whole blood lactate concentrations following sprinting may differ when different sampling sites are used. It also clearly shows that there are considerable differences when lactate is measured in different portions of blood (e.g. whole blood vs plasma).

7. In the third methodological study the principal finding was that whole body maximal exercise reduces plasma volume in addition to that of the posture change alone as reflected by the 10 % higher estimated plasma volume loss in comparison with change in posture.

8. Finally, prolonged freezing of whole blood lactate at −20 °C is possible for at least 3 months as long as defrosting does not occur (methodological study 4).

7.3. EFFECTS OF SHORT-TERM ENDURANCE TRAINING ON PERFORMANCE AND MUSCLE METABOLISM DURING REPEATED SPRINTS IN FEMALES

In these series of experiments the main interest was on the effects of endurance training on recovery of performance. The lack of similar studies in the literature makes any comparison impossible. However, the findings of the present study support the bulk of published reports which have used cross-sectional designs to examine the effects of endurance training on performance recovery (Hakkinen and Myllyla, 1990; Hamilton et al., 1990; Dawson et al., 1993; Caiga and Doherty, 1995; Hoffman, 1997; McMahon and Wenger, 1998; Tomlin and Wenger, 2002).

In both training studies reported in the present thesis, six weeks of endurance training resulted in 7 % better performance restoration ($P < 0.05$) as reflected by MPO recovery for the training group in comparison with the control group. These improvements in performance were accompanied by 2.3-3 % increases in $\dot{V} O_2 \text{max}$ and 3.0 (chapter 5) to 3.6 % (chapter 6) improvements in % $\dot{V} O_2 \text{max} @ 4 \text{ mmol·L}^{-1}$. 
Thus, it seems that these two training studies induced similar changes in endurance fitness. However, correlation analysis revealed that for the first training study (chapter 5) there was a significant association between MPO recovery and % $\dot{V}O_2 \text{ max @ 4 mmol-L}^{-1}$ ($r = 0.64$, $P <0.05$), but not between $\dot{V}O_2 \text{ max}$ and MPO recovery. Opposingly, in the second training study (chapter 6) there was a significant association between $\dot{V}O_2 \text{ max}$ and MPO recovery ($r = 0.54$, $P <0.05$), but not between MPO recovery and % $\dot{V}O_2 \text{ max @ 4 mmol-L}^{-1}$ (although a small positive, but not significant correlation appeared after training, $r = 0.4$). Thus, the direction of the correlations was similar in the two studies, but the magnitude of the correlation and thus statistical significance varied, possibly simply due to subject differences between the two studies.

The % $\dot{V}O_2 \text{ max @ 4 mmol-L}^{-1}$ was used as an index of endurance fitness in the present study because the % $\dot{V}O_2 \text{ max}$ at a given blood lactate concentration has been shown to increase with endurance training (Hurley et al., 1984; Williams and Nute, 1986) and to be related to endurance capacity as reflected by the running or cycling time to exhaustion at 90 % and 88 % $\dot{V}O_2 \text{ max}$, respectively (Williams and Nute, 1986; Coyle et al., 1988). In addition, Bogdanis and co-workers (1996a) have demonstrated a relationship between % $\dot{V}O_2 \text{ max @ 4 mmol-L}^{-1}$, power output recovery and PCR resynthesis suggesting that % $\dot{V}O_2 \text{ max @ 4 mmol-L}^{-1}$ reflects the peripheral adaptations to endurance training. On the other hand the most common endurance fitness index reported in the literature, maximum oxygen uptake, has also been associated with power output recovery (Dawson et al., 1993; McMahon and Wenger, 1998). $\dot{V}O_2 \text{ max}$ is the maximal rate at which an individual can take up and utilise oxygen while breathing air at sea level (Astrand and Rodahl, 1986) and it is therefore the product of both oxygen transport and oxygen utilisation. This relationship can be expressed as (equation 1):

$$\dot{V}O_2 \text{ max} = \dot{Q} \text{ max} \times (a-\bar{v})O_2 \text{ max} \quad \text{[equation 1]}$$

Equation 1 also denotes that $\dot{V}O_2 \text{ max}$ is a function of two components, one central [cardiac output ($\dot{Q}$)] and one peripheral [arteriovenous difference for $O_2 ((a-\bar{v})O_2)$],
Despite the fact that there is continuing debate as to which of these components limit $\dot{V}O_2\max$ (Basset and Howley, 2000). Thus, $\dot{V}O_2\max$ indicates both peripheral and central endurance adaptations. Therefore it seems that in the first training study (chapter 5) training adaptations were more pronounced in the periphery (positive correlation between MPO recovery and $\% \dot{V}O_2\max$ @ 4 mmol·L$^{-1}$) whilst in the second training study (chapter 6) training adaptations were more pronounced centrally (positive correlation between MPO recovery and $\dot{V}O_2\max$).

Regardless of whether the adaptations were more pronounced centrally or peripherally recovery of PCr resynthesis is a function of both central (Quistorf et al., 1992) and peripheral (Walter et al., 1997) factors and both $\dot{V}O_2\max$ and $\% \dot{V}O_2\max$ @ 4 mmol·L$^{-1}$ increased.

From these series of training studies it appears that power output recovery is not purely a result of faster PCr resynthesis, but also is probably pH dependent while improved mechanical efficiency and greater reliance on aerobic energy sources could also be important factors. PCr resynthesis during 110 seconds recovery from sprint 1 was not significantly higher after training, a finding which agrees with the notion that PCr resynthesis does not improve after endurance training when exercise results in severe intramuscular acidosis. Equation 2 describes this relationship:

$$\text{PCr} + \text{MgADP}^- + \text{H}^+ \leftrightarrow \text{MgATP}^2^- + \text{creatinine} \quad \text{[equation 2]}$$

However, the higher PCr concentration 14 s ($P < 0.05$) into recovery from sprint 1 after training perhaps indicates not only greater reliance on aerobic energy resources and better mechanical efficiency but also faster initial PCr resynthesis (Walter et al., 1997). Initial PCr resynthesis ($< 1 \text{ min}$) is not inhibited by high $\text{H}^+$ accumulation due to the fact that equation 2 (creatine kinase equilibrium) is a function of both intracellular pH and net ATP flux (Walter et al., 1997). It seems possible that the muscle biopsy 110 seconds into recovery was too late in order to demonstrate the initial faster PCr resynthesis. Similar findings were shown by Bishop et al. (2003) where at 3 min into recovery no changes in PCr resynthesis appeared. Had the biopsy had been taken at 1 min post-sprint 1, there is possibly a higher chance that faster PCr
restoration would have been observed. Alternatively, faster power output could result from a higher mechanical efficiency in the first and second sprint after endurance training or greater reliance on aerobic energy resources. ATP provision from anaerobic energy resources had a tendency to be lower post-sprint 1 after training ($P = 0.098$) supporting the latter view. It is also possible that the combination of all these factors (faster initial PCr resynthesis, mechanical efficiency and greater contribution from aerobic energy resources) equally or in various percentages cannot be excluded.

It is unclear whether a different training programme would result in different findings with respects to PCr recovery. It is known that the cellular changes inherent to any type of exercise are restricted to the muscle fibres recruited to perform this exercise task (Fitts and Windrick, 1996). Thus, it is possible that a training programme with a higher intensity (interval training) would recruit to a different extent the type II fibres by improving even more their oxidative capacity thereby resulting in faster power output recovery. Tesch et al. (1989) found that 30 s maximal voluntary knee extensions resulted in identical PCr depletion, but 60 s following exercise PCr replenishment was quicker in type I fibres in comparison with type II. Similar findings were presented by Casey et al. (1996b) where PCr resynthesis was complete within 4 min of passive recovery in type I fibres.

Nevertheless, it is currently inconclusive as to what it is the optimal intensity and duration of training to induce adaptations in various metabolic (i.e. enzyme activities) and other physiological mechanisms (capillarisation in the various fibre types) in response to endurance training (Henriksson and Hickner, 1998).

The effect of the subjects' habitual training (additional to the imposed training programme) on metabolic adaptations. In all these series of experiments subjects continued their routine training programmes on the top of the endurance training for the experimental procedures. Data to date is limited, but one study has demonstrated that short-term concurrent sprint and endurance training does not compromise the sprint or endurance training performance although these performances are slightly (ns) better when sprint or endurance training are performed independently (Callister et al., 1988).
One of the principal findings of this thesis was the lack of influence of menstrual cycle phase on repeated sprint performance. This finding is in agreement with the majority of the studies to date (De Bruyn-Prevost et al., 1984; Quadagno et al., 1991; Miskec et al., 1997; Lynch and Nimmo, 1998; Giacomoni et al., 2000) and when subjects did not present with premenstrual or menstrual syndrome (Giacomoni et al., 2000). It seems that earlier postulations with respects to the effects of high estradiol concentrations on inorganic phosphate during the cross-bridges kinetics in the absence of the anti-estrogen effects of progesterone (period just prior to ovulation) do not apply in maximal dynamic exercise (Philips et al., 1996; Sarwar et al., 1996). During maximal isometric contraction (designs of Philips et al., 1996 and Sarwar et al., 1996) during which the length of the muscle does not change while it is contracting type I and II fibres produce exactly the same amount of force (power). Oppositely in dynamic muscle action when the muscle fibre is either shortening or lengthening (eccentric/concentric) a type II fibre produces more force than the type I fibre (Fitts, 1997). It is also known, that animal muscle type II fibres are considerably less sensitive to inorganic phosphate (Altringham and Johnson, 1985) which may explain the lack of influence of estradiol on these fibres. Alternatively, this lack of influence could be due to the noticeably lower estrogen receptors in type II fibres (Saartok, 1984). It may be for these reasons in dynamic as opposed with isometric exercise, that the menstrual cycle phase does not have a detrimental effect.

7.4. METHODOLOGICAL STUDIES
The methodological studies reported in this thesis showed that there was a 17.7 % loss in plasma volume after a single 30 s sprint and that 11.7 % of this loss is attributable to the sprint per se. Thus, the concentration of a specific metabolite in the plasma (i.e. lactate) could be 17.7 % less than the initial measured. This can be easily seen with the data from the second methodological study where plasma lactate was measured. The 16.4 mmol·L⁻¹ concentration for plasma lactate found 5 min post-sprint (table 3.11) could be 13.5 mmol·L⁻¹ after the correction for plasma volume shifts. It is clear from this example that lack of information of sprint on fluids shifts can limit the interpretation of these data, as does the lack of information concerning the posture during which the blood samples are taken. Thus these results challenges the early
perceptions that running exercise can not exert further hemoconcentration after moving from the supine position (Harrison, 1985).

The methodological studies also showed that there is 5.6 % \( (P < 0.05) \) higher blood lactate concentration in capillary blood in comparison with blood from an arm vein. Regardless of the underlying metabolic and/or physiological mechanisms of these differences these data illustrate the need for careful consideration of the methods when comparing studies that employ different sampling sites for blood lactate concentrations (e.g. capillary in comparison with venous) or when there is variation in the sampling site between pre- and post-trial.

One of the novel characteristics of these investigations was the use of 95 % limits of agreement (Bland and Altman, 1986), or, when heteroscedasticity was present, the ratio limits of agreement (Nevill, 1996). Applying this statistical methodology in the first methodological study (and in the second) it was possible to examine for the first time the reliability of power output during a 30-s sprint on the sprint treadmill with appropriate statistical technique. It was revealed that sprint treadmill produces high repeatability as reflected with the almost identical MPO (276 ± 29 vs 273 ± 27 W, in trial 1 vs trial 2, respectively) which is in agreement with previous research (Cheetham, 1987). However, analysis with 95 % limits of agreements found PPO more variable (still reproducible) (means: 427 vs 422 W, absolute limits of agreement: 5 ± 42 W) indicating the robustness of this technique.

Finally, blood lactate can be stored for prolonged periods without substantial influence on its concentration whilst repeated defrosting may alter these concentrations (methodological study 4).

### 7.5. LIMITATIONS

While the muscle biopsy technique provides a wealth of information at the cellular level as reported in chapter 6 of this thesis, the procedure does not give information about the "true" intracellular pH or ADP (Sapega et al., 1987; Wilson et al., 1988). Both intracellular pH and ADP, considered key factors for PCr kinetics during
recovery (Tomlin and Wenger, 2001) and thus the inability to measure such metabolites must be considered a limitation of the last study reported in the thesis.

Another factor that may have influenced the findings of the last study was the timing of the post-exercise biopsy at $14.1 \pm 1.3$ and $13.8 \pm 3.5$ s, before and after training respectively. Even though the surgeon was highly skilled and experienced this delay in the post-sprint period may have had an impact on PCr concentration for the post-sprint values due to the quick initial kinetics of PCr after sprinting (Bogdanis et al., 1995). Nevertheless, the immediate post-sprint PCr responses of this study match well with values reported from previous investigators using the same experimental model highlighting the fact that all research groups experience the same problem with the muscle biopsy technique (Cheetham et al., 1986; Nevill et al., 1989).

The decision not to take biopsies from the control group and the decision not to take a biopsy post-sprint 2 (4th biopsy) did reduce the amount of information available to explain possible mechanisms involved. Nonetheless, the reported experiments and protocols represent a compromise between obtaining the most information and causing the least discomfort to the volunteers with the least number of tests or invasive experimental perturbations.

As already reported in chapter 6, use of the Douglas bag technique for only 30-s collection of expired air may have led to inaccuracies in describing the oxygen uptake during sprinting.

The studies in this thesis only addressed the impact of one particular type and duration of endurance training on the recovery of performance and metabolites after sprinting. Other intensities and durations of training may have resulted in different findings. However, this is the first longitudinal attempt to assess the effects of endurance training on recovery from sprinting and the studies reported in chapter 6 and 7 still make a very useful contribution to the literature. In fact there is currently no conclusive data indicating the interdependency between exercise intensity and duration on a number of metabolic adaptations at the cellular level due to endurance training (Henriksson and Hickner, 1998).
Due to the lack of permanent personnel in the maximal intensity exercise group it was impossible to guarantee the presence of the same experimenters (apart from the main author of this thesis) for each participant in these series of experiments. This may have influenced slightly the performance of some volunteers occasionally. Furthermore, all studies of this type rely on a maximal effort from the subjects and there is no way of knowing for sure that each subject gave a maximal effort on each occasion. However, based on comparisons of performance during familiarisations (including short sprints) and main trials it would seem that any such influences on performance were negligible.

All the studies in the present work were compromised (with respects to the information) by the lack of the actual absolute plasma volume values. This was a result of the lack of an appropriate technique. Evans blue dye dilution technique is not appropriate when repeated measurements are required since it takes some time before the dye is completely mixed (Johansen et al., 1998). It could be argued though that absolute resting values could be possibly measured with the Evans blue dye dilution technique. Although this was a possibility, ethical committee restrictions, cost, the invasive nature of this technique (arteries are required) and practical issues such as the availability of a specialist to perform the method meant that it was not possible to use this technique. As already mentioned in chapter 3 the methodological error using Hct/Hb can bring about an underestimation of 30 to 50% in changes in plasma volume (Johansen et al., 1998; Lundvall and Lingren, 1998). The use of albumin and total protein presents an alternative option in estimating plasma volume since they do give more accurate information than the Hct/Hb method (Lundvall and Lingren, 1998), but still are much inferior to the Evans blue dye dilution technique and thus do not provide a good cost (time and money)- effect ratio. However, according to Harrison (1985) when exercise is being considered “it is the change relative to some steady-state control condition that is of primary interest”. Furthermore, there is not a general consensus with respects to the usefulness or not, for the corrections of metabolites according to plasma volume changes (Kargotich et al., 1998).

The effects of natural fluctuations of estradiol and progesterone due to menstrual cycle phase on performance and the metabolic responses were a major area of concern in this thesis. Nevertheless, this study and previous studies, examining this topic in
various types of exercise, suffer from the fact that estrogen receptors in the human skeletal muscle have only recently been discovered (Lemoine et al., 2003) and progesterone receptors are still to be found in human skeletal muscle. Due to this brand-new discovery of the estrogen receptor in human skeletal muscle our understanding of the metabolic impact of such receptors in human skeletal muscle is still immature and probably far behind the information obtained from animal models.

It has been suggested that bioavailability (free and non-specifically bound) of hormones rather than total concentrations reflect more accurately the clinical situation (Vermeulen et al., 1999). To date estradiol and progesterone bioavailability has not been evaluated together with performance variations and only few studies to date have reported this issue at all (Elliot et al., 2003). The main study 1 (chapter 4) does not provide information for bioavailability of progesterone and estradiol.

Concern has also arisen with regards to the responses of estradiol and progesterone to exercise. Although a number of studies do report some changes as a consequence of exercise (Nicklas et al., 1989) their findings cannot be considered conclusive because they lack information for plasma volume and posture changes across menstrual cycle phases.

Finally, the methodological studies presented in chapter 3 have the slight disadvantage of a small sample size (methodological study 1 and 2). According to Bland and Altman (1999) reliability studies (methodological study 1) or method comparison (methodological study 2) demand 40 subjects for clear cut information to be obtained.

7.6. DIRECTIONS FOR FUTURE RESEARCH

Based on the experimental findings we believe that the present work does provide a step forward in understanding the physiology of sprinting and more particularly in furthering understanding of the effects of endurance training on recovery of power output after sprinting. In addition, these series of experiments clarify the potential effects of menstrual cycle phase on sprint running performance and provide
information with respects to a number of methodological issues pertinent to the metabolic responses to sprinting.

It was mentioned in the main study 3 (chapter 6) that it is possible that PCr recovery had recovered quicker during the initial phase (≤ 1 min) of the recovery after training due to the lack of influence of intramuscular acidosis on initial PCr kinetics (Walter et al., 1997). Thus, a study with a similar protocol to that employed for chapter 6, but with the addition of a biopsy after 1 min of recovery may clarify if indeed PCr replenishment is faster during the initial phase of recovery post-sprint after endurance training.

Future studies should focus on the effects of different types of endurance training protocols as well as endurance, in comparison with sprint, training programmes on recovery of power output and muscle metabolites after sprinting. However, with sprint training studies PPO and/or MPO of the first sprint are very likely to be increased and thus mask any improvements in recovery of PPO and/or MPO (see section 2.2.2 in chapter 2).

It is also unclear whether training intensities should be based on percentage of $\dot{V}O_2$ max or on specific lactate concentrations (McLellan et al., 1989; Gass et al., 1991; Weltman, 1995). A study examining the peripheral ($\% \dot{V}O_2$ max @ 4 mmol·L$^{-1}$) and central ($\dot{V}O_2$ max) determinants of recovery of power output after sprinting may elucidate this issue.

It would be interesting to know the acute responses of estradiol and progesterone due to sprinting and whether, if any, these responses change across menstrual cycle. Nevertheless, in the context of the lack of knowledge concerning the different ratios of estradiol and progesterone on performance (chapter 4) these data can only provide general information. Bioavailability of estradiol and progesterone during the menstrual cycle would also provide interesting information since the free and non-specifically bound plasma hormone levels generally reflect the clinical situation more accurately than total plasma hormone levels (Vermeulen et al., 1999). However,
preliminary data for estradiol suggests a lack of such influence of menstrual cycle phase (early follicular vs mid-luteal) on bioavailability (Elliott et al., 2003).

It is certain that future studies should examine with more detail the effects of oral contraceptives on performance and more particularly the effects of different time lengths of oral contraceptive use on various performance and metabolic parameters. In addition, any differences of monophasic in comparison with triphasic oral contraceptives on the same variables would be of great interest. Finally, male contraception (Handelsman, 1999) would bring about a new era in oral contraceptives research and future studies should examine the potential effects of such hormonal regulation on performance and metabolic responses in males.

With new technological developments, in the future it may be possible to accurately assess absolute plasma volume and the subsequent changes due to exercise protocols. Therefore, it will also possible to correct plasma metabolites for changes in plasma volume thereby uncovering the true metabolic responses due to exercise. However, it is currently debatable whether metabolic responses should be corrected for plasma volume shifts (Kargotich et al., 1998 plus discussion in page 138 in chapter 3).

Future reliability (methodological study 1) or method comparison (methodological study 2) studies should be performed with 40 subjects/samples according to Bland and Altman (1999). Finally, where possible, sample size estimation in order to find significant differences in a particular study should be performed.

It is of great practical importance for any experimenter to know for how long, serum/plasma samples for growth hormone, progesterone and insulin can be stored at -70 °C, and whether and to what extent or defrosting can influence these values. Further work is needed in this area.

### 7.7. PRACTICAL RECOMMENDATIONS

Although endurance training enhanced recovery of power output after sprinting in the present series of experiments for sprint/power trained athletes, this does not mean that
endurance training will improve sprint performance. It would be not sensible to make such a statement. The present data cannot, and do not, suggest that endurance training should be the main “ingredient” of a training programme throughout a training period of, for example, one year. Appropriate manipulation of the training programme to include periods of endurance training may, however be advisable with the timing varying with the nature of the competitive activity, the endurance fitness of the athlete and their genetic potential.

Since endurance training is not specific for sprint/power events it would be more realistic to be placed in the pre-season training period with maintenance considerations during the in-season training. Training experts have already suggested this pattern (Plisk, 1991; Hedrick, 1999).

The advantage of endurance training is clearer in team sports where repeated bouts of high-intensity exercise interspersed with various durations of relief periods are involved and athletes should be educated as to the value of endurance-training in the pre-season period for enhanced game-play and repeated sprint training during the season. Endurance training out season may also be advantageous for individual sports (e.g. sprinters). In this context, training sessions with high intensity exercise require high quality repetitions and thus faster recovery between repetitions (such as track 200 m sprints would have a positive impact on both the quality and quantity of work which can be completed in one session.

In terms of female athletes, chapter 4 indicates that performance is not affected by menstrual cycle phase and therefore, no particular alterations in a training regimen should occur for a female athlete as a result of menstrual cycle phase. However, as mentioned earlier when perimenstrual problems are present, performance in sport activities involving eccentric exercise is reduced (Giacomoni et al., 2000) accompanied by high risk of injury (Liu et al., 1997). When certain female athletes demonstrate such problems, mainly in the last 2-3 days of the menstrual cycle and first 2 days of the onset of the menses, athletic trainers should either reduce the volume of the training load or omit high risk exercises such as plyometrics and flat out sprints. When these days coincide with a major event (100 m race, or a soccer/rugby/hockey game) consideration could be given the possibility of athletes
not taking part, but the coach could be guided by the athlete and by the significance and importance of the event in making these decisions.
REFERENCES.


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ANTONIS TSAMPOUKOS

Ph.D. THESIS


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ANTONIS TSAMPOUKOS 295 Ph.D. THESIS
REFERENCES


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# APPENDIXES

<table>
<thead>
<tr>
<th>Appendix</th>
<th>Page</th>
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</thead>
<tbody>
<tr>
<td>A</td>
<td>316</td>
</tr>
<tr>
<td>B</td>
<td>319</td>
</tr>
<tr>
<td>C</td>
<td>327</td>
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<td>D</td>
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APPENDIX A1: FIRST AID AND DRAWING BLOOD FROM THE CANNULA CERTIFICATE

East Midlands Ambulance Service
NHS Trust

Certificate of Attendance

This is to certify that

Antonis Tsampoukos

Attended a course in

Cardio Pulmonary Resuscitation (CPR)

Held On

9th February 2000

Conducted by

East Midlands Ambulance Service
NHS Trust

Signed: [Signature]
APPENDIX A2: VENIPUNCTURE CERTIFICATE

GLENFIELD HOSPITAL
NHS TRUST
THIS IS TO CERTIFY THAT
ANTONIS TSAMPOUKOS

has completed a course of
instruction in taking venous
blood samples for
pathological testing and is
now competent to work
without supervision.

Date:- 18th May 01
Signed: -

Phlebotomy Trainer
A3: UNDERTAKING WORK IN RADIOCHEMISTRY

CERTIFICATE

RADIATION PROTECTION SERVICE

CERTIFICATE OF ATTENDANCE

[Signature]

attended a training course in Radiation Safety

on

3rd July 20xx

[Signature]

Radiation Protection Officer
APPENDIX B1: ETHICAL COMMITTEE APPROVAL

EXAMPLE DOCUMENT

Ref No: R00/P4

LOUGHBOROUGH UNIVERSITY
ETHICAL ADVISORY COMMITTEE

HUMAN BIOLOGICAL INVESTIGATION
RESEARCH PROPOSAL

Title: Effect of endurance training on recovery of power output during repeated sprints.

Applicants: Dr M E Nevill
Mr A Tsampoukos

Departments: PE, SS & RM

Date of clearance: 18 May, 2000

Comments of the Committee:
The Committee was content to issue clearance after receiving a satisfactory response to their comments.

Investigators: Dr M E Nevill
Mr A Tsampoukos
APPENDIX B2: ETHICAL COMMITTEE PROPOSAL

EXAMPLE

LOUGHBOROUGH UNIVERSITY OF TECHNOLOGY

ETHICAL ADVISORY COMMITTEE

RESEARCH PROPOSAL FOR HUMAN BIOLOGICAL INVESTIGATIONS

This application should be completed after reading the Code of Practice paying particular attention to the advice given in Section 6.3.

(I) APPLICANTS:

Dr Mary E. Nevill, Mr Antonis Tsampoukos & MSc students.

(II) PROJECT TITLE:

Effect of endurance training on recovery of power output during multiple sprints.

(III) AIMS AND OUTLINE OF THE PROJECT:

During recovery from maximal repeated exercise the prime concern of the recovering energy system is the resynthesis of phosphocreatine (PCr) and acid-base balance, thereby preparing the exercising muscles for the next rise in demand for ATP. There is great interest in the literature concerning the recovery of muscle metabolites and acid-base balance during repeated sprinting exercise and the effects of these metabolites on subsequent sprints (Sahlin et al., 1989; Allsop et al., 1990; Bogdanis et al., 1995; Bogdanis et al., 1996).

Some of these studies suggest that endurance fitness (as indicated from the % \(\dot{V}O_2\) max at a given blood lactate concentration) may have beneficial effects on recovery of power output (Bogdanis et al., 1995; Bogdanis et al., 1996). This suggestion comes from the fact that there has been found a relationship between recovery of power output and the resynthesis of PCr, and, between the recovery of power output and endurance fitness (Bogdanis et al., 1995; Bogdanis et al., 1996; McMahon and Wenger, 1998).

In spite the fact, that, preliminary cross-sectional studies have confirmed this relationship (Hamilton et al., 1991; Graham et al., 1994; McMahon and Wenger, 1998) longitudinal studies involving power-trained athletes have not been as yet reported in the literature. In our first attempt to directly investigate whether long-term endurance training has positive impact on PCr resynthesis and subsequent performance, it was found that six weeks endurance type training routine (three times a week for 20-30 min at 85% \(\dot{V}O_2\) max) improved performance (Mean Power Output, Mean Speed) and significant relationships were revealed between recovery of power output and endurance fitness (Tsampoukos and Nevill, 2000-data to be published). However, the major drawback of this study was the absence of muscle metabolic data, and, thus direct examination of the relationship between PCr resynthesis (as well as other metabolites) and performance profile.
In addition, aerobic contribution during and after (that is recovery) each sprint was not measured. Thus, it was the purpose of this study to directly investigate if the implementation of six weeks endurance training improves endurance fitness (as reflected with $\dot{V}O_2\text{max}$ and $\%\dot{V}O_2\text{max}$ at a given blood lactate concentration) and the influence of such training routine in the recovery of muscle metabolites, power output and oxygen kinetics, during repeated/multiple sprints in power-trained (sprinters and game players) athletes.

(IV) NAMES AND STATUS OF THE EXPERIMENTERS:

<table>
<thead>
<tr>
<th>Name</th>
<th>Status</th>
<th>Institution</th>
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<tbody>
<tr>
<td>Dr Mary E. Nevill</td>
<td>Senior lecturer, Dept. of PESSRM, Loughborough. Univ</td>
<td></td>
</tr>
<tr>
<td>Mr Antonis Tsampoukos</td>
<td>Ph.D student, Dept. of PESSRM, Loughb. Univ</td>
<td></td>
</tr>
<tr>
<td>Three MSc students</td>
<td>Postgraduate students, Dept. of PESSRM, Loughb. Univ</td>
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(V) SUBJECTS:

The subjects will be physically active female volunteers (who normally undertake 2 or more training sessions per week, e.g. aerobics, running, swimming or team sports), aged 18-35 years, recruited by general notice, although it is anticipated that most will be either students or research staff within the department of PESSRM. It is anticipated that 16-30 subjects will be recruited for this investigation. All subjects will have the study explained to them in both written and verbal form, complete a health questionnaire and be asked to sign a voluntary consent form, which clearly stipulates that they may withdraw at any stage without giving a reason. Prior to the main trials, the subjects will undergo a thorough period of familiarisation in order to become acquainted with equipment and procedures of the study. During both the familiarisation and main trials because subjects are female, a female assistant will be present.

(VI) LOCATION (ANY SPECIAL FACILITIES TO BE USED):

The work will be carried out in the Sports Science Research Laboratories within the Department of Physical Education, Sports Science and Recreation Management at Loughborough University.
(VII) DURATION (INCLUDING DEMAND ON SUBJECT'S TIME):

Prior to the main trials, subjects will attend at least three to five familiarisation sessions, each lasting approximately 30-40 minutes, so that the study can be fully explained to them and in order to practise the procedures to be employed in the main study. The main trials will require the subjects to visit the laboratory on four separate occasions before and after training. Each visit will be of a 30-60 min duration. During the training period the experimental group will be asked to attend 3 sessions per week for 6 weeks. Each visit will be of 30-45 min duration.

(VIII) REASONS FOR UNDERTAKING THE STUDY (E.G. CONTRACT, STUDENT RESEARCH):

This study is part of a Ph.D thesis that is broadly concerned with the effect of endurance fitness on recovery of power output after maximal exercise bouts in women.

(IX) METHODOLOGY (A BRIEF OUTLINE OF THE RESEARCH DESIGN):

The subjects will report to the laboratory, after prior familiarisation, on 3 separate occasions before and after training. After having finished the standardised warm-up the subjects rest for 5-min and will start sprinting (trial 1: sprint trial) from a rolling start of 2 m·s⁻¹. The participants will perform (on 2 separate occasions before and after 6 weeks of training) two 30 s sprints interspersed with 2-min passive recovery. This protocol is identical with the one has been used and described before (Tsampoukos and Nevill, 2000: “The effects of short-term endurance training on recovery of power output during repeated sprints”). During this trial blood samples will be taken from the antecubital vein. In a separate day, the experimental (training group) will perform a single 30-s sprint. In this occasion three muscle biopsies will be implemented: at rest, immediately after sprint one and 2 min after sprint 1 in order the recovery of muscle metabolites to be examined. This protocol is identical with the one has been used and described before (Bogdanis and Nevill, 1995: “Recovery of power output and muscle metabolites following 30 s of maximal sprint cycling in man”). In separate days, after the sprint trial, two other trials will take place. The second trial (24 h at least after the sprint trial) will include a maximum oxygen uptake test with a protocol based on Taylor (1956) original progressive test. The third testing day an incremental sub-maximal trial (Speed Lactate Test) will be performed in order to establish the endurance fitness level of the female volunteers. This test will be performed 24-48 h after the second trial. Both of these tests (maximum oxygen uptake test, speed lactate test) has been used and prescribed before (Tsampoukos and Nevill, 2000: “The effects of short-term endurance training on recovery of power output during repeated sprints”).
The termination of the maximum oxygen uptake test will be terminated based on the volitional exhaustion of the volunteers and/or if the experimenter judges that the volunteer is not able to continue (i.e. grabbing the hand bars, not good balance). Finally, for extra safety for the volunteers, a health status questionnaire (please find enclosed) will be given to them, in the experimental days.

In addition, as part of their training regimen (experimental group only), volunteers will undertake three continuous/interval running training sessions on separate days.

(x) PROCEDURES AND MEASUREMENTS (FOR EXPERIMENTAL AND CONTROL SUBJECTS):

Performance variables will be measured with the aid of microcomputer interfaced to the treadmill. Blood samples will also be drawn (either by catheter or by finger-prick). During the familiarisation sessions the subjects’ height and weight will be recorded. The cannula will be placed by Dr Mary E. Nevill (or another fully qualified personnel) and the blood samples will be drawn by Mr Antonis Tsampoukos or another appropriately trained member of the research group. Approximately 104ml of blood per subject for each session (for the two main trials, before and after training). The blood samples will be drawn in order to measure the following metabolites and hormones: lactate, ammonia, hypoxanthine, pH, haematocrit, haemoglobin, progesterone, cortisol, growth hormone and catecholamines. Muscle biopsies will be employed in order to investigate the recovery of muscle metabolites such as PCr, glycogen, Pyruvate dehydrogenase (PDH), pH, ATP.

In order to ensure good status of health volunteers will be asked to complete a health status questionnaire (please find enclosed). In addition, during the tests (apart from the sprinting) heart rate recording and rate of perceived exertion (RPE) will be monitored so as to the safety of the subjects to be guaranteed. Full instructions for the optimal use of RPE will be given to the participants (please find enclosed). Finally, as already has been mentioned, participants will be asked to complete an extra health status questionnaire on each visit to the lab when heavy exercise is to be performed (please find enclosed).
(XI) POSSIBLE RISKS, DISCOMFORTS AND/OR DISTRESS (SEE SECTION 6.3k)

The experiment involves exercise of a very high intensity and is, thus, demanding. However, the subjects will be fully familiarised with the procedures. The cannula will be placed under local anaesthetic (lignocaine), and therefore discomfort will be minimised. Blood sampling via a cannula may cause minor bruising and carries an extremely small risk of plastic or air embolism. However, good practice minimises that risk.

A number of similar studies have previously been approved by the Ethical Committee whereby maximal intensity exercise is performed on a treadmill ergometer. Frequently, they have involved metabolic measurements, from blood and/or muscle samples, in addition to performance measures. The most recent similar application was made to the Ethical Committee by Nevill and Tsampoukos (2000) entitled the "Effects of short-term endurance training on recovery of power output during repeated sprints".

(XII) PROCEDURES FOR TAKING MEASUREMENTS AND FOR CHAPERONING AND SUPERVISION OF SUBJECTS DURING INVESTIGATIONS:

The experiment will involve female subjects and in addition to one of the investigators, Mr Antonis Tsampoukos being present at familiarisation and main trials, a female assistant will be present.

(XIII) NAMES AND INVESTIGATORS AND PERSONAL EXPERIENCE OF PROPOSED PROCEDURES AND/OR METHODOLOGIES:

Dr Mary Nevill 15 years experience of similar investigations in this laboratory.
Mr Antonis Tsampoukos 4 years experience of similar investigations in this laboratory.
3 MSc students 1 year experience in this laboratory as part of their course.

(XIV) DATA PROTECTION:

Although information will be stored on a computer, each subject will be entered as a number rather than by name will not be identifiable. This is in accordance with the Data Protection Act.
(XV) DETAILS OF ANY PAYMENT TO BE MADE TO THE SUBJECTS:

NONE.

(XVI) DO ANY INVESTIGATORS STAND TO GAIN FROM A PARTICULAR CONCLUSION OF THE RESEARCH PROJECT:

NONE

(XVII) WHETHER THE UNIVERSITY'S INSURERS HAVE INDICATED THAT THEY ARE CONTENT FOR THE UNIVERSITY'S PUBLIC POLICY TO APPLY TO THE PROPOSED INVESTIGATION (COMMITTEE USE ONLY):


(XVIII) WHETHER INSURANCE COVERS ADDITIONAL TO (XIV) HAS BEEN ARRANGED BY THE INVESTIGATOR (SEE SECTION 6.3):

NONE.

(XIX) IN THE CASE OF STUDIES INVOLVING NEW DRUGS OR RADIOSOTOPES, WRITTEN APPROVAL FOR THE STUDY MUST BE OBTAINED FROM THE APPROPRIATE NATIONAL BODY AND SUBMITTED WITH THE PROTOCOL. STATE IF APPLICABLE:

NOT APPLICABLE.

(XX) DECLARATION.
I have read the university's Code of Practice on Investigations on Human Subjects and completed this application.

Signature of the applicant: .................................................................

Signature of the Head of the Department: .............................................

Date: .................................................................
APPENDIX C: EXAMPLE OF RECRUITMENT ADVERT

ESPECIALLY FOR WOMEN

We are currently conducting a study investigating the effect of menstrual cycle phase on sprinting performance. It has been suggested that the female athlete during her reproductive years has a complex and ever-changing milieu of female steroid hormones resulting from the variations of estradiol and progesterone during a regular menstrual cycle. Both estrogens and progestins have individual, interactive and sometimes opposing physiological actions with potential effects on athletic performance.

We are looking for recreationally active females (from any sport and at any level, age 18-30 years old) who are willing to spend 60-min in the lab on three different occasions (main trials) plus some visits in order to get familiarised with running on a sprint treadmill. The test, on 3 occasions involves two 30-s sprints (flat-out) interspersed with 2-min of passive recovery.

If you think you might be interested in our study and would like more information (with no obligation), please ring Mr Antonis Tsampoukos on 228183 or on 0410 763 953 (any time) or Dr Mary Nevill on 223270 or e-mail Antonis: A.Tsampoukos@lboro.ac.uk

WHAT IS IN IT FOR YOU?

➢ Information about your sprint performance profile (peak power output, peak speed, fatigue index)
➢ Do you want to know about your endurance fitness? Information about your endurance training status are provided (maximal oxygen uptake test, intensity for optimal training as indicated by blood lactate accumulation)
➢ Have you ever imagined how many calories you take? Our full dietary analysis will provide you with a breakdown of your energy intake and the proportion of fat, carbohydrate and protein consumed
➢ Knowledge about various metabolic and hormonal variables important for health and/or training prescription (including lactate, pH, haematocrit, haemoglobin, reproductive hormones)
Find out if your performance varies during different phases of your menstrual cycle

And finally for those who are students in PESSRM this is a good opportunity for gaining experience with the laboratory procedures in exercise physiology
APPENDIX D: DESCRIPTION OF THE EXPERIMENTAL PROCEDURES (EXAMPLE)

SUBJECT INFORMATION

PROJECT TITLE: EFFECT OF SHORT-TERM ENDURANCE TRAINING ON RECOVERY OF POWER OUTPUT DURING REPEATED SPRINTS

INVESTIGATOR: ANTONIS TSAMPOUKOS + MSc STUDENTS.

SUPERVISOR: Dr MARY E. NEVILL.

Department of Physical Education, Sports Science and Recreation Management, Loughborough University, Loughborough, Leics, LE11 3TU.

Introduction.

The purpose of the present study is to examine whether short-term endurance training can improve the recovery of power output during multiple sprints.

It is well known that the energy to support high rates of muscular effort such as single sprints (100/200m in running) and sports that involve bursts of all-out effort (soccer, hockey, rugby, basketball, etc.) comes predominantly from anaerobic metabolism. Thus, at first glance it does not seem so important for athletes participating in these sports to do any endurance training to improve performance during these activities. However, both types of sports (sprint events and games) require a fast rate of recovery for performance (games) or training (athletics). In addition, it has been suggested that endurance training may enhance muscle lactate removal and the resynthesis of creatine phosphate by increasing the number of capillaries and providing more oxygen to the recovering muscles. Nonetheless, this hypothesis has not yet been directly tested.

Therefore, it is the purpose of the present study to test the hypothesis that short-term endurance training can improve recovery of power output during repeated sprints.
**Methods.**

**Familiarisation.** Prior to main trials you will perform at least 3-5 practice sessions in order to become fully familiarised with the equipment and the experimental protocol to be used. These sessions will also provide an opportunity for any questions to be answered. Height and weight will be recorded. So that the same diet may be followed before each trial, dietary record sheets and instructions will be given to you. Figure 1 and 2 shows the time required for the total experiment (including preliminary and main trials). Each session will last approximately 30-40 min (60 min for the main trials). For the experimental group, training will be conducted three times per week for 6 weeks (approximately 30 min per visit).

**Figure 1. Timetable for the experimental procedures (CONTROL GROUP).**

| VISIT 1 | Oral and written information for the purpose of the study, completion of the Health History Questionnaire, Menstrual Cycle Information, Informed Concern and Training Status Questionnaire. Explanations on how to record your diet. Weight recording. Practice session for sprinting on the non-motorised treadmill (20-30 min duration) |
| VISIT 2 | Height and weight recording, practice session (sub-maximal) for maximal oxygen uptake test (30-60 min duration, depending on your familiarity with typical treadmills), plus practice session for sprinting on the non-motorised treadmill (20 min duration) |
| VISIT 3 | Practice session for sprinting (20-30 min duration) |
| VISIT 4 | Practice session for sprinting (20 min duration) |
| VISIT 5 | Practice session for sprinting (20 min duration) |

**MAIN TRIALS**

| VISIT 1 | REPEATED SPRINTS |
| VISIT 2 | MAXIMUM OXYGEN UPTAKE TEST (30 MIN DURATION) |
| VISIT 3 | SPEED LACTATE TEST (30 MIN DURATION) |
| VISIT 4 | FIELD TEST |
SPECIAL NOTE: The visits in the lab for the practice sessions will be arranged according to the subject’s preference.

Figure 2. Timetable for the experimental procedures (TRAINING GROUP).

| VISIT 1 | Oral and written information for the purpose of the study, completion of the Health History Questionnaire, Menstrual Cycle Information, Informed Concern and Training Status Questionnaire. So that the same diet may be followed before each trial, dietary record sheets and instructions will be given to you. Weight recording. Practice session for sprinting on the non-motorised treadmill (20-30 min duration) |
| VISIT 2 | Height and weight recording, practice session (sub-maximal) for maximal oxygen uptake test (30-60 min duration, depending on your familiarity with typical treadmills), plus practice session for sprinting on the non-motorised treadmill (20 min duration) |
| VISIT 3 | Practice session for sprinting (20-30 min duration) |
| VISIT 4 | Practice session for sprinting (20 min duration) |
| VISIT 5 | Practise session for sprinting (20 min duration) |

MAIN TRIALS

| VISIT 1 | REPEATED SPRINTS |
| VISIT 2 | MAXIMUM OXYGEN UPTAKE TEST (30 MIN DURATION) |
| VISIT 3 | SPEED LACTATE TEST (30 MIN DURATION) |
| VISIT 4 | BIOPSY TRIAL: ONE 30 s SPRINT & BIOPSIES |
| VISIT 5 | FIELD TEST |

SPECIAL NOTE: The visits in the lab for the practice sessions will be arranged according to the subject’s preference.
Maximal Incremental Test (VO₂ max test).

In this test your maximum aerobic power will be identified. The procedure involves an incremental progressive run on a motor-driven treadmill with an anticipated duration of 9-15 min. The test begins with a warm-up that includes 3 min running at a low speed (which is going to be determined during the familiarisations) and 5-10 min stretching. After this standardised warm-up you will return to the treadmill and start the main part of the test. The test will start with a low speed (which, again, is going to be determined based on the practise sessions) while the grade will be at 3.5% for 3 min. After this stage the grade will be increased 2.5% every 3-min until volitional exhaustion. Figure 1 depicts the schematic representation of the experimental design.

Figure 1: Schematic representation of the experimental design. Where ↓: air sample.

Data collection.

During the final minute of each stage you have to breathe through a low-resistance respiratory valve in order for expired air to be collected. When you feel you
can only run for one more minute you must give an one-minute signal to the experimenter. Heart rate will be recorded throughout the test. Also, prior to each experiment a health status questionnaire is going to be given to you in order your health status to be assessed.

In addition, at the end of each stage and after the completion of the test you will be asked to rate your perceived exertion according to the Borg scale.

Finally, all these procedures will be introduced to you during the familiarisation session preceding the test.

**Sub-maximal Incremental Test (Speed Lactate Test).**

During this test the running speed equivalent to 4 mmol·l⁻¹ blood lactate concentration will be determined.

The test consists of 4 stages with different running speeds for each stage. These speeds will be determined after the VO₂ max test. At the end of each 4-min stage a finger-prick blood sample will be drawn (200μl in total). Moreover, as in maximal test an expired air sample will be collected during the last minute of each stage.

**COMMENTS**

1. Refrain from alcohol and caffeine 24-h before the tests.
2. For the Maximal and Sub-maximal Incremental Test you should not consume food for 12 h (overnight fast).
3. Abstain from strenuous physical activity the day before these preliminary trials.

**Repeated sprints.**

A schematic representation of the experimental protocol is shown in figure 2 that is identical for the two trials (before and after training). The present investigation involves the performance of two 30 s maximal bouts (“flat out” from the beginning of
the sprint, **NOT PANCED**) interspersed with 2 min passive recovery. Prior to two sprints a standardised warm-up (3 min jogging at 2 m·s⁻¹, 5-min stretch and 2 runs at 2.2 m·s⁻¹ and 2.8 m·s⁻¹ interspersed with 30-s, and finally, 5-min rest) will be performed. Blood samples will be collected with the aid of a cannula and will require 11.5 ml at each sampling point or 104 ml in total for every session. These samples will be drawn **a)** at rest (after resting on the couch for 20 min), **b)** post warm-up **c)** immediately after sprint 1, **d)** at the end of sprint 2, and, **e)** at 5, 10, 15, 20 and 30-min after sprint 2. At the end of sprint 2 and after the immediate post-sprint blood sample has been drawn you have to rest on the couch for 30 min.

![Figure 3. Schematic representation of the protocol. Where ↑: blood samples.](image)

In addition, during the sprint and the recovery after the sprint you will be asked to breathe through a low-resistance respiratory valve in order for expired air to be collected.

For the training group (ONLY), on a separate day (after the completion of the aforementioned trials), the muscle biopsy session will be performed. This trial will have the same procedure like the one just prescribed except of two differences: **a)** only one 30 s will be performed, and, **b)** three muscle biopsies will be taken (at rest, immediately after the sprint and approximately 2 min after the sprint). Figure 3 schematically represents the experimental protocol.
Field Test.

Eight days after the completion of the repeated sprints (control group) and biopsy trial (training group) you will perform a field test in which you will be requested to run two 50 m maximal sprints with 10 min rest between them. The better of the two trials will be retained for statistical analysis. Your performance will be measured with photocell system. This test is going to be performed again after the post-training trials.

Equipment

Exercise will take place on motorised (preliminary trials) and a non-motorised treadmill (main trials). A non-elastic belt will be placed around your waist of the in order to measure power output. Tips about how to sprint on the non-motorised treadmill are described in the following six steps:

STEP 1: Try to sprint in the middle of the treadmill belt. Look straight ahead and focus on a distant point. If for some reason you go very close to the sides of the treadmill a command "MOVE RIGHT" or "MOVE LEFT" will be given to you.

STEP 2: Perform the sprints as upright as possible (Ben Johnson technique) since this technique will help you have better co-ordination using this unique ergometer.
STEP 3: Just before the start of each sprint and while the experimenter counts 3, 2, 1 you must remain in the pre-determined rolling start speed and not accelerate until you hear the command “GO”.

STEP 4: You have to perform both sprints FLAT OUT from the beginning of the sprint. DO NOT PACE. It does not matter if at the end you are not able to sprint due to fatigue.

STEP 5: Throughout the sprint maintain pressure against the restraining belt.

Benefits from the study

• Information about your sprint performance profile (peak power output, peak speed, fatigue index)
• Do you want to know about your endurance fitness? Information about your endurance training status (maximal oxygen uptake, intensity for optimal training as indicated from blood lactate concentration of 4 mmol·l⁻¹ running speed)
• Knowledge about various metabolic and hormonal variables (including lactate, pH, haematocrit, haemoglobin, reproductive hormones)
• Knowledge about various muscle metabolites (i.e. glycogen, PCr) and muscle enzymes (PDH) before and after training (TRAINING GROUP ONLY).
• Make yourself fitter with a highly controlled endurance training (cardiovascular benefits)
• And finally for those who are students in PESSRM this is a good opportunity for gaining experience with the laboratory procedures in exercise physiology

Exercise Risks and Discomforts

Exercise during this study will be performed at a maximal intensity and thus requires that subjects should be motivated, in good health and of a good fitness level. Since blood sampling will be employed via cannulation it may cause minor bruising
and carries an extremely small risk of plastic or air embolism. Good practice minimises this risk. In addition the cannula will be placed under local anaesthetic (lignocaine), and therefore discomfort will be minimised.

There is some risk of bruising and stiffness the day after the muscle biopsy procedure. However, the risk is minimal when the procedure is performed by a trained and experienced person. Dr Boobis who will perform the biopsies, has carried out over 380 such biopsies during previous studies in the Department of PESSRM over the last 17 years.

Finally, due to the strenuous nature of sprinting some nausea and discomfort may be experienced.

**Restrictions necessary for the purpose of the study**

It is recommended that you should follow similar diet for 24 h prior to each trial (you are not required to weigh your food and eat exactly the same, but just to make sure that your diet is rich in carbohydrates and has similar proportions of carbohydrates), and that you should refrain from caffeine and alcohol consumption 24-h before each trial (including the preliminary trials).

In addition, the day before each main trial you should abstain from strenuous exercise and (IF YOU DID SO) complete the same amount of light exercise for the post training trial (for both the training and control group).

**Confidentiality**

Although, information will be stored on a computer, subjects will be entered as a number rather than by name and will not be identifiable. This is in accordance with the Data Protection Act.
Other information

All trials will take place in the Sports Hall Research laboratories. For further information please contact Mr Antonis Tsampoukos (07710 763 953) or e-mail him on: A.Tsampoukos@lboro.ac.uk whose office is located in room RR105 in the Sports Hall building or leave message for Mr Antonis Tsampoukos in his pigeon hole (John Hardie Building).
APPENDIX E: INFORMED CONSENT (EXAMPLE)

LOUGHBOROUGH UNIVERSITY, DEPARTMENT OF PHYSICAL EDUCATION, SPORTS SCIENCE AND RECREATION MANAGEMENT

Effect of short-term endurance training on recovery of power output during repeated sprints.

Statement of Informed Consent:

Your permission to take part in this study is voluntary. You are free to deny consent or to withdraw from the study at any point and without explanation, if you so desire.

I have read the information regarding this study and had the opportunity to ask questions of the investigators. I understand the procedures involved and consent to participate in this study.

Signature of the subject: Date:

Signature of witness:
**F1: HEALTH STATUS QUESTIONNAIRE**

**HEALTH SCREEN FOR STUDY VOLUNTEERS**  Name or Number........

It is important for volunteers participating in research studies are currently in good health and have had no significant medical problems in the past. This is to ensure (i) their own continuing well-being and (ii) to avoid the possibility of individual health issues confounding study outcomes.

Please complete this brief questionnaire to confirm fitness to participate:

1. **At present**, do you have any health problem for which you are:
   - (a) on medication, prescribed or otherwise
     Yes[ ] No[ ]
   - (b) attending your general practitioner
     Yes[ ] No[ ]
   - (c) on a hospital waiting list
     Yes[ ] No[ ]

2. **In the past two years**, have you had any illness which require you to:
   - (a) consult your GP
     Yes[ ] No[ ]
   - (b) attend a hospital outpatient department
     Yes[ ] No[ ]
   - (c) be admitted to hospital
     Yes[ ] No[ ]

3. **Have you ever had** any of the following:
   - (a) Convulsions/epilepsy
     Yes[ ] No[ ]
   - (b) Asthma
     Yes[ ] No[ ]
   - (c) Eczema
     Yes[ ] No[ ]
   - (d) Diabetes
     Yes[ ] No[ ]
   - (e) A blood disorder
     Yes[ ] No[ ]
   - (f) Head injury
     Yes[ ] No[ ]
   - (g) Digestive problems
     Yes[ ] No[ ]
   - (h) Heart problems
     Yes[ ] No[ ]
   - (i) Problems with bones or joints
     Yes[ ] No[ ]
   - (j) Disturbance of balance/coordination
     Yes[ ] No[ ]
   - (m) Ear/hearing problems
     Yes[ ] No[ ]
   - (n) Thyroid problems
     Yes[ ] No[ ]
   - (o) Kidney or liver problems
     Yes[ ] No[ ]

4. **Have you ever had** an allergic reaction to any substance or drug (such as lignocaine)?
   Yes[ ] No[ ]

If YES to any question, please describe briefly if you wish (e.g. to confirm problem was/is short-lived, insignificant or well controlled). ..................................

**Additional questions for female participants**

(a) are your periods normal/regular?
   Yes[ ] No[ ]

(b) are you on "the pill"?
   Yes[ ] No[ ]

(c) could you be pregnant?
   Yes[ ] No[ ]

(d) are you taking hormone replacement therapy (HRT)?
   Yes[ ] No[ ]
APPENDIX F2: HEALTH STATUS QUESTIONNAIRE PRIOR TO EVERY TEST

HEALTH QUESTIONNAIRE

Please complete the following brief questions to confirm your fitness to participate:

At present do you have any health problems for which you are:

1) On medication, prescribed or otherwise
   YES  NO  NO

2) Attending your general practitioner
   YES  NO  NO

Have you any common symptoms of ill health, such as those associated with a cold or other common infection?

   YES  NO

If you had answered yes to any of the above questions please give more details:

________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________

Signature: ____________________________  Date: ________________
G: MENSTRUAL CYCLE INFORMATION

Department of physical Education, Sports Science and Recreation Management

Effect of short-term endurance training on recovery of power output during repeated sprints.

MENSTRUAL CYCLE DETAILS

(All information is fully confidential)

Please circle the answer where appropriate.

Name:

Age and date of birth:

Date:

1) Have you had regular periods in the last two years? YES NO

2) How long in days is your menstrual cycle, from day 1 of menses/period to day of the next?

_________ DAYS

3) Is the above time the same between periods? YES NO

If the answer was NO, please state the irregularity:

_________________________________________________________________________

_________________________________________________________________________

4) How many days does your menstrual (blood) flow last?

_________ DAYS

5) Do you get pain during your period? YES NO

If YES, please state the symptoms and the days during the cycle when you suffer:

_________________________________________________________________________

_________________________________________________________________________

6) Do you avoid exercise during your period? YES NO
If YES, please state your reasons for avoiding exercise:

__________________________________________________

7) DO/DID you take contraceptive pills? YES NO

If YES, please state what, when and for how long?
What: ____________________________________________________
How long: ________________________________________________
When: __________________________________________________

8) If you are currently taking on the contraceptive pill, will you be continuing to do so for the next 3 months? YES NO

If NO, please state when you will stop taking contraceptive pills:

_______________________________________________________

9) Do you take any medication or hormones to regulate your menstrual cycle? YES NO

If YES, please state what you take and how often?

_______________________________________________________

10) When did you have your last period (day 1)?

_____________________________________________________
APPENDIX H: TRAINING STATUS QUESTIONNAIRE

TRAINING STATUS QUESTIONNAIRE

Name: ____________________________________________
Address: ____________________________________________
Phone number: ____________________________________________
Sport: ____________________________________________
Position/event: ____________________________________________
Level of performance: ____________________________________________
Current Training status: ____________________________________________
Other sports: ____________________________________________
Training sessions/week: ____________________________________________
Comments: ____________________________________________

Thank you for your co-operation!

ANTONIS TSAMPOUKOS
344 PH.D. THESIS
APPENDIX I: DIET RECORDING INFORMATION AND SHEETS

CONFIDENTIAL

NAME AND ADDRESS

DATE OF START OF DIET: 

FOOD RECORD DIARY

Please record everything you eat and drink during the next days. Instructions and an example are given inside.

Information about your diet will be treated in confidence and results will be returned to you as soon as possible.

Loughborough University
Department of Physical Education and Sports Science
Dietary Analysis Service
Loughborough
Leics LE11 3TU
INSTRUCTION FOR USING THE FOOD DIARY

Everything that you eat and drink one day prior to the main trials should be weighed and the weight and type of food or drink recorded.

For solid foods, the food should be placed on the scale on a plate or container. The plate or container must be weighed empty first and the scales can then be zeroed. Each item of food can then be added to the plate and weighed individually, returning the scales to zero between each item.

e.g. Plate 150g, zero scale.
     Roast Beef 100g, zero scale.
     Potato 150g, zero scale.
     Gravy 30g, zero scale.

For drinks, a cup or glass must first be weighed and then the scale can be returned to zero and the drink added. Please remember to record separately the weight of tea, milk and sugar put into a drink.

Do not forget to weigh and record second helpings and between meal snacks.

Any leftovers (e.g. apple cores) should also be weighed and recorded in the leftovers column.

Eating out-Most people eat foods away from home each day, please do not forget to record these. Take your diary and scales with you wherever it possible. If this is too inconvenient just record the type of food eaten with an estimated weight—but please say when a weight has been estimated.

Most snack foods will have the weight of the food on the packet so they do not need weighing if you eat the whole packet yourself.
Names and descriptions of foods should be as detailed possible, including the brand name and any other information available.

e.g. Cheese-is insufficient information.
Cheese, cheddar (Shape reduced fat)-is sufficient information.

Start a new page in your diary for each day, and record each item on a separate line. Record the time of day in the first column of each line.

e.g. 10.30 am Mcvities Biscuits (2) 50g Digestive

The space provided at the foot of each page for general comments is for you to give any further information about your diet for that day.

e.g. Missed lunch due to stomach pains.

A full example sheet is given over page showing how to record a day of food and how to fill in the comments section.

Please try to be as accurate as possible and try to choose a fairly typical day to record. For instance do not record a day when you are on holiday or when you are ill, if you feel that this would alter your normal diet or activities.
**APPENDIX I**

**DIET INFORMATION**

Date:............
Please use a separate line for each item eaten; live a line between different plate entries

<table>
<thead>
<tr>
<th>Time</th>
<th>Food eaten</th>
<th>Brand name of each item (except fresh food)</th>
<th>Full description of each item whether fresh, frozen, dried, canned; how cooked; what type of fat food fried in</th>
<th>Weight served</th>
<th>Weight leftover</th>
</tr>
</thead>
<tbody>
<tr>
<td>8am</td>
<td>x</td>
<td>Kellogg's cornflakes</td>
<td></td>
<td>50</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>whole milk</td>
<td>120</td>
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<td></td>
<td></td>
<td>Hovis sliced 2 slices brown bread</td>
<td></td>
<td>60</td>
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<td></td>
<td></td>
<td>Blue Band margarine</td>
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<tr>
<td>10am</td>
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<td>Nescafe coffee powder</td>
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<td>hot water</td>
<td>16</td>
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<td></td>
<td>milk whole</td>
<td>30</td>
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<td>sugar white</td>
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<tr>
<td>1pm</td>
<td>x</td>
<td>Hovis sliced bread 4 slices</td>
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<td>120</td>
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<tr>
<td></td>
<td></td>
<td>margarine</td>
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<td>20</td>
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<td></td>
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<td>tomato</td>
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<td></td>
<td></td>
<td>Cheddar cheese</td>
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<td></td>
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<td>apple granny smith</td>
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<td>sugar</td>
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<td>6pm</td>
<td>x</td>
<td>Sainsbury steak pie individual</td>
<td></td>
<td>160</td>
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<td></td>
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<td>Endus chips frozen</td>
<td></td>
<td>150</td>
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<tr>
<td></td>
<td></td>
<td>peas boiled</td>
<td></td>
<td>60</td>
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<td></td>
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<td>carrots boiled</td>
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<td>Mr Kipling apple pies individual</td>
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<td></td>
<td></td>
<td>Birds custard canned</td>
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<td>150</td>
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</table>

**GENERAL COMMENTS**

NO

Date:.............
Please use a separate line for each item eaten; live a line between different plate entries

<table>
<thead>
<tr>
<th>Time</th>
<th>Food eaten</th>
<th>Brand name of each item (except fresh food)</th>
<th>Full description of each item whether fresh, frozen, dried, canned; how cooked; what type of fat food fried in</th>
<th>Weight served</th>
<th>Weight leftover</th>
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**GENERAL COMMENTS**
**APPENDIX J: SPREAD SHEET FOR PERFORMANCE VARIABLES (EXAMPLE)**

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<td><strong>FORCE 1</strong></td>
<td><strong>POWER 1</strong></td>
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</tbody>
</table>

**EXAMPLE DATA**

- **Average (A2:A31)**
- **Average (B2:B31)**
- **Average (C2:C31)**
- **Average (D2:D31)**
- **Average (E2:E31)**
- **Average (F2:F31)**
- **Average (G2:G31)**
- **Average (H2:H31)**
- **Average (I2:I31)**
- **Average (J2:J31)**

**PH.D. THESIS**

ANTONIS TSAMPOUKOS

350
### APPENDIX K: SPREAD SHEET FOR OXYGEN CALCULATIONS (EXAMPLE)

#### COLLECTION TIME (min)

<table>
<thead>
<tr>
<th>Volume Sample (l/min)</th>
<th>O₂/CO₂</th>
</tr>
</thead>
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#### Average HR

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#### ANTONIS TSAMPOUKOS

351 Ph.D THESIS
### APPENDIX K

**GAS CALCULATION SPREAD SHEETS**

#### OXYGEN CALCULATIONS M22A

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## Oxygen Calculations

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### Notes

- The calculations are performed using Excel formulas: `SLOPE(G6:G7), INTERCEPT(G6:G7), 0 = ROUND([W6]*100)/100, 02 = ROUND([W7]*100).`
APPENDIX L1: COACHING DEGREE IN ATHLETICS

HELLENIC REPUBLIC
ARISTOTELIAN UNIVERSITY OF THESSALONIKI
DEPARTMENT OF SCIENCE OF PHYSICAL EDUCATION AND SPORTS

Cert. No: P.S.

CERTIFICATE

TO WHOM IT MAY CONCERN

It is certified that according to our files:

ANTONIOS TSAMPOUKOS son of CHARALAMBOS and VASSILIKI, during his
studies in 7th and 8th semester attended the speciality of
ATHLETICS.

The aforesaid after taking the prescribed examinations during the
examining period of February 1995, obtained the diploma/degree of
speciality of ATHLETICS

with grade 7.00 (seven and zero hundredth) - VERY GOOD.

Thessaloniki, 26 April 1995
The Registrar of the Department
sgd
Ioannis Baxevanos
(SEAL)

Bureau des Traductions du Ministère des Affaires Étrangères de la République
Hellénique, Athènes.
Hellenic Republic, Ministry of Foreign Affairs, Translation Office, Athens.

ANTONIS TSAMPOUKOS
Ph.D. THESIS
APPENDIX L2: STRENGTH AND CONDITIONING CERTIFICATE

January 4, 2000

Antonis Tsampoukos
John Hardle Building
Dept. of Physical Education
Loughborough University
Loughborough, LE11 3TU
United Kingdom

Dear Antonis:

Our testing service, Applied Measurement Professionals, has completed the grading of the recent Certified Strength and Conditioning Specialist examination. Your results by section and sub-category have already been sent.

It is my pleasure to personally congratulate you for having passed both sections of the certification examination and for becoming a Certified Strength and Conditioning Specialist (CSCS).

The NSCA Certification Commission is in the process of developing new certificates, which you will receive, along with your Continuing Education Unit (CEU) information, in late January. However, we would like to supply you with your certificate number and date of certification now. Your certification number is 200011743, and your certification date is October 24, 1999.

Your accomplishment of becoming a Certified Strength and Conditioning Specialist will be noted in an upcoming issue of the NSCA Bulletin. If you do not wish to have your name listed, please notify us – in writing – and we will withhold your name.

Antonis, congratulations on becoming a Certified Strength and Conditioning Specialist.

Best Regards,

Thomas R. Baechle
Executive Director of Certification, ACA

NSCA Certification Commission
1640 L Street, Suite G • Lincoln, NE 68508
Phone: 402-476-6669 • Toll Free: 888-746-CERT (2378) • Fax: 402-476-7141
E-mail: commission@nsca-cc.org • Web site: http://www.nsca-cc.org
TRAINING DIARY

SUBJECT: ___________
## WEEK 1

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### RPE

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**COMMENTS**

__________________________
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ANTONIS TSAMPOUKOS 357 Ph.D. THESIS
APPENDIX N: PERCEIVED EXERTION CHART

Rate Of Perceived Exertion

6 ________ Very Very Light
7 ________
8 ________
9 ________ Very Light
10 ________
11 ________ Fairly Light
12 ________ Fairly Hard
13 ________
14 ________ Hard
15 ________
16 ________
17 ________ Very Hard
18 ________
19 ________ Very Very Hard
20 ________ Maximum
APPENDIX O: RATE OF PERCIVED EXERTION GUIDE

INSTRUCTIONS FOR RPE CHART

Several times during the test, we will ask you to rate the work, according to this scale. You will be asked to choose a number to describe how hard the work is for you. A rating of “6” would correspond to those feelings and sensations you have during the easiest work you can imagine, not unlike sitting in a chair. A rating of “20” corresponds to the feelings and sensations you would have during the most difficult work you can imagine yourself doing, so exhaustive that you could not continue.
## APPENDIX P: HUMIDITY EXCELL SPREAD SHEET

![Excel Spreadsheet Image]

### APPENDIX P: HUMIDITY EXCEL SPREAD SHEET

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ANTONIS TSAMPOUKOS 360 Ph.D. THESIS
APPENDIX Q1: BLOOD LACTATE ASSAY

Principle:
The fluorimetric assay for lactate is based on that described by Maughan (1982).

\[
\text{NAD}^+ \xrightarrow{\text{Lactate dehydrogenase}} \text{NADH} + \text{H}^+ \xrightarrow{\text{Lactate}} \text{Pyruvate}
\]

Reagents:
Buffer: Hydrazine 1.1 mol·L\(^{-1}\), Ph 9.0 with 1 mmol·L\(^{-1}\) EDTA·Na\(_2\)
Cofactor: NAD
Enzyme: lactate dehydrogenase (LDH) 5500 U·mL\(^{-1}\) (undiluted)
Standard: L-Lactate 1 mol·L\(^{-1}\) (stock solution)
Diluent: 0.07 mol·L\(^{-1}\) HCL

Stock standards were prepared before each study and stored at -20 °C:

<table>
<thead>
<tr>
<th>L-Lactate 1 mol·L(^{-1}) (mL)</th>
<th>2</th>
<th>1.5</th>
<th>1</th>
<th>0.5</th>
<th>0.25</th>
<th>0.1</th>
<th>0.05</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4 mol·L(^{-1}) perchloric acid (mL)</td>
<td>0</td>
<td>0.5</td>
<td>1</td>
<td>1.5</td>
<td>1.75</td>
<td>1.90</td>
<td>1.95</td>
<td>2</td>
</tr>
<tr>
<td>Lactate concentration (mmol·L(^{-1}))</td>
<td>20</td>
<td>15</td>
<td>10</td>
<td>5</td>
<td>2.5</td>
<td>1</td>
<td>0.5</td>
<td>0</td>
</tr>
</tbody>
</table>

Reaction mixture (final concentration):
Buffer: 1 mL
NAD: 2 mg (2.98 mmol·L\(^{-1}\))
LDH: 10 μL (54.46 U·mL\(^{-1}\))

Procedure:
1. After being allowed to thaw at room temperature, samples were mixed (Whirlimix) and centrifuged (Eppendorf-Anderman Centrifuge 5414, Germany) for 3 min at 13000 rpm.
2. 20 µL of either standard or supernatant was pipetted into a glass fluorimeter tube and 200 µL of reaction mixture was added.

3. Then, the tubes were mixed thoroughly (Whirlmix), covered, and left to incubate at room temperature for 30 min.

4. 1.0 mL of lactate diluent (0.07 M HCL) was added to each tube and they were mixed thoroughly (Whirlmix) to stop the reaction.

5. The fluorescence of the blanks, standards and samples were measured (Locarte Model LF 8-9, Locarte, London, U.K.).

6. A linear regression plot of the standards was made and the lactate concentration of the samples was calculated.
APPENDIX Q2: PLASMA AMMONIA ASSAY

Blood collection:

When the blood was drawn 1.5 mL venous whole blood was dispensed into a calcium heparinised microcentrifuge tube (prepared using the method described below). Samples were centrifuged immediately for 3 min at 13000 rpm (Eppendorf-Anderman Centrifuge 5414, Germany). The plasma was then removed and pipetted into a plain microcentrifuge tube (Sarstedt Ltd., Leicester, U.K.) and immediately snap frozen until the end of the trial in liquid nitrogen and then stored at -70 °C until it was analysed, within 48 h. The time from blood being drawn to freezing was kept to a minimum in order to prevent contamination from external nitrogen sources and always less than 15 min.

Preparation of Ca\(^{2+}\)/Hep tubes:

⇒ The required amount of Ca\(^{2+}\)/Hep was calculated:

1 mg (or mL) of Ca\(^{2+}\)/Hep contains 183 units; 15 units are needed per mL of whole blood and each eppendorf tube would contain ≈ 1.5 mL of whole blood. Therefore, each microcentrifuge tube required ≈ 30 units of Ca\(^{2+}\)/Hep (rounded up to allow for wastage).

E.g. for each batch of 300 microcentrifuge tubes, 9000 units (300 x 30) would be required. This is equivalent to ≈ 50 mg (9000 ÷ 183) of Ca\(^{2+}\)/Hep which would be added to 1.2 mL (4 x 300) of distilled water.

⇒ 4 µL of this solution was then pipetted into each eppendorf tube.

Standards:

Each kit included an ammonia control solution at a concentration of 294 µmol· L\(^{-1}\). In addition control solutions at ammonia concentrations of 29.5 and 118 µmol· L\(^{-1}\) were used (Sigma Diagnostics).
Procedure:

1. A series of 1.5 mL disposable plastic cuvettes with a lightpath of 1 cm were set up for BLANK, STANDARD 1 (29.5 μmol·L⁻¹, in duplicate), STANDARD 2 (118 μmol·L⁻¹, in duplicate), STANDARD 3 (294 μmol·L⁻¹, in duplicate) and SAMPLES.

2. 0.5 mL of the reagent solution was added to each cuvette.

3. 150 μL of distilled water was added to the BLANK cuvette, 100 μL of distilled water and 50 μL of ammonia control solution to each of the STANDARD cuvettes and 150 μL of plasma to each of the SAMPLE cuvettes.

4. Cuvettes were covered with sealing film (Nescofilm, Bando Chemical Ind. Ltd., Kobe, Japan) and mixed by gentle inversion.

5. Cuvettes were allowed to equilibrate for approximately 3 min at room temperature.

6. INITIAL absorbance of cuvettes were read (Cecil Instruments) and recorded at 340 nm.

7. 5 μL of enzyme solution was added to each cuvette, cuvettes were mixed by gentle inversion and left to stand for approximately 5 min at room temperature for the reaction to complete.

8. FINAL absorbance of each cuvette was read and recorded at 340 nm.

9. The ammonia concentration was calculated using the following equations:

$$\Delta A = \text{INITIAL A} - \text{FINAL A}$$

$$\text{STANDARD Ammonia (μmol·L}^{-1}) = ((\Delta A_{\text{STANDARD}} - \Delta A_{\text{BLANK}}) \times 35.8) \times 58.8$$

$$\text{SAMPLE Ammonia (μmol·L}^{-1}) = ((\Delta A_{\text{SAMPLE}} - \Delta A_{\text{BLANK}}) \times 11.93) \times 58.8$$

Where factor 58.8 converts μg·mL⁻¹ to μmol·L⁻¹.

Where factors 35.8 and 11.93 are the output of volume of liquid in cuvet, weight (μg) of ammonia, millimolar absorptivity of NADPH at 340 nm and volume of specimen for standards (35.8) and samples (11.93)
APPENDIX Q3: HAEMOGLOBIN ASSAY

Principle (Cyanmethaemoglobin Method):

Haemoglobin + Cyanide + Ferricyanide → Cyanmethaemoglobin

(Van Kampen and Zijlstra, 1961)

Reaction mixture:

The reaction mixture ("Drapkins") was made from a kit (Boehringer Manheim) by diluting with distilled water (1000 mL) and contained phosphate buffer, potassium cyanide, potassium ferricyanide and detergent. It was then stored in a brown bottle at between +15 °C and +20 °C.

Procedure:

1. In duplicate, 20 µL of whole blood was added to 5.0 mL of the reaction mixture in a plastic tube and mixed.
2. The tubes were left to stand for at least 5 min but not longer than 24 h. The exact time varied between runs but in most cases samples were analysed at the end of a trial and the time samples were analysed at the end of a trial and the time samples were left to stand did not exceed 6h.
3. The absorbance of the sample was measured using a spectrophotometer (Cecil Instruments) at a wavelength of 546 nm in a cuvette with a 1.0 cm light path. The reaction mixture on its own was used as a blank in order to zero the spectrophotometer. Relative absorbance [(A) (absorbance of sample – absorbance of blank)] was therefore taken as the reading on the spectrophotometer.
4. The haemoglobin concentration of the sample was then calculated from the following equation:

\[
\text{Haemoglobin concentration (g·mL}^{-1}) = (37.2 \times A) + 0.06
\]

(Wintrobe, 1956)
APPENDIX Q: HAEMATOCRIT DETERMINATION

Procedure:

☑ In triplicate, Na-Hep microhaematocrit tube (Scientific Laboratory Supplies Ltd, Nottingham, U.K.) were approximately three-quarters filled with whole blood. The blood was then run up and down the tube to mix it.

☑ The tubes were sealed at one end with plasticine by placing them in a "miniseal" tray. They were then left for analysis at the end of the trial. Samples were not left for more than 7 h before they were analysed.

☑ Tubes were placed in the rotor of micro-haematocrit centrifuge (micro-haematocrit centrifuge, Hawksley and Sons Ltd, Lancing, U.K.) with sealed end towards the outside and centrifuged for 14 min.

☑ Percentage cell volume was calculated by placing each microhaematocrit tube into micro-haematocrit reader (Hawksley and Sons Ltd, Lancing, U.K.) where the base (bottom) line of the reader was aligned with the base of the red cells and the top line intersected the top of the plasma. The middle line was then adjusted so that it intersected the top of the red cells and the percentage cell volume read from the scale.
APPENDIX R1: PROGESTERONE ASSAY

Principle:
125I-labelled progesterone competes for a fixed time with progesterone within subjects sample for antibody sites. Because the antibody is immobilised to the wall of the polyprolene tube, simply decanting the supernatant permits the isolation of antibody-bound fraction of the radiolabeled progesterone. The radioactivity of progesterone was then determined with the aid of a Gamma Counter (Cobra II, Packard Instrument Company Inc., USA). The concentration of progesterone is inversely related to the counts and the exact concentration was given using a calibration curve.

Reaction mixture:
A commercially available kit (Coat-A-Count, DPC Ltd., Los Angeles, USA) was used to perform the assay which contained progesterone Ab-Coated Tubes (TPG1), 125I progesterone (TPG2).

Standards:
Progesterone (Lyophilized) calibrators (PGC3-9) in processed human serum provided with Coat-A-Count kit were utilised as standards.

Quality Control:
A tri-level, human serum-based immunoassay control (CON 6, DPC Ltd., USA), containing progesterone was used for the assay.

Procedure:
* Serum samples and quality controls were allowed to thaw at room temperature for approximately 60 min. They were mixed thoroughly but gently by inversions.
* Four plain (uncoated) polypropylene tubes T (total counts) and NSB (non-specific binding) were marked in duplicate.
* Pipet 100 µL of the zero calibrator A into the NSB and A tubes.
* Pipet 100 µL of each of the calibrators B through G into correspondingly labelled tubes in duplicate.
Pipet 100 µL of each quality control and subject sample into appropriately labelled antibody-coated tubes.

Add 1.0 mL of ¹²⁵I Progesterone (CLEAR) to every tube and vortex (whirlimixer).

Incubate for 3 hours at room temperature.

Decant thoroughly. At the end of the incubation period the iodine solution was poured from each tube except the “T” tubes and allowed to drain for 2-3 min. Then in order to shake off all the residual droplets the polypropylene tubes were stricken on absorbent paper for additional 2-3 min.

Count for 1 min in the Gamma Counter (Cobra II, Packard Instrument Company Inc., USA).

A software installed within the Gamma Counter (Cobra II, Packard Instrument Company Inc., USA) allowed programming a logit-log representation of the calibration curve which was used by the computer to determine the actual progesterone concentration of the subject’s samples and quality controls.

Notes:

i. Because progesterone has a tendency to absorb plastic, and even more so to glass, it is important to coat the pipette tip by rinsing a few times in the sample before making the transfer (dispensing the serum etc.).

ii. No more than 10 min should elapse between the dispensing of the samples and the addition of the tracer into the samples.

iii. Keep sealed the “T” tubes after adding the tracer.

iv. Use IODINE 129 to calibrate the Gamma Counter prior to the assay.
APPENDIX R2: ESTRADIOL ASSAY

Principle:
$^{125}\text{I}$-labelled estradiol competes for a fixed time with progesterone within subjects sample for antibody sites. Because the antibody is immobilised to the wall of the polyprolene tube, simply decanting the supernatant permits the isolation of antibody-bound fraction of the radiolabeled estradiol. The radioactivity of estradiol was then determined with the aid of a Gamma Counter (Cobra II, Packard Instrument Company Inc., USA). The concentration of estradiol is inversely related to the counts and the exact concentration was determined by comparing the counts to a calibration curve.

Reaction mixture:
A commercially available kit (Coat-A-Count, DPC Ltd., Los Angeles, USA) was used to perform the assay which contained estradiol Ab-Coated Tubes (TE21) and $^{125}\text{I}$ estradiol (TE22).

Standards:
Estradiol (Lyophilized) calibrators (TE23-9) in processed human serum provided with Coat-A-Count kit were utilised as standards.

Quality Control:
A tri-level, human serum-based immunoassay control (CON 6, DPC Ltd., USA), containing progesterone was used for the assay.

Procedure:

* Serum samples and quality controls were allowed to thaw at room temperature for approximately 60 min. They were mixed thoroughly but gently by inversions.

* Four plain (uncoated) polypropylene tubes T (total counts) and NSB (non-specific binding) were marked in duplicate.

* Pipet 100 $\mu$L of the zero calibrator A into the NSB and A tubes.

* Pipet 100 $\mu$L of each of the calibrators B through G into correspondingly labelled tubes in duplicate.
APPENDIX R

RADIOIMMUNOASSAYS

* Pipet 100 µL of each quality control and subject sample into appropriately labelled antibody-coated tubes.

* Add 1.0 mL of 125Ι estradiol (CLEAR) to every tube and vortex (whirlimixer).

* Incubate for 3 hours at room temperature.

* Decant thoroughly. At the end of the incubation period the iodine solution was poured from each tube except the “T” tubes and allowed to drain for 2-3 min. Then in order to shake off all the residual droplets the polyprolene tubes were stricken on absorbent paper for additional 2-3 min.

* Count for 1 min in the Gamma Counter (Cobra II, Packard Instrument Company Inc., USA).

* A software installed within the Gamma Counter (Cobra II, Packard Instrument Company Inc., USA) allowed programming a logit-log representation of the calibration curve which was used by the computer to determine the actual estradiol concentration of the subject’s samples and quality controls.

Notes:

i. Pipet directly to the bottom of the polyprolene tubes.

ii. No more than 10 min should elapse between the dispensing of the samples and the addition of the tracer into the samples.

iii. Keep sealed the “T” tubes after adding the tracer.

iv. Use IODINE 129 to calibrate the Gamma Counter prior to the assay.
APPENDIX S1: ATP, PCr AND G-6-P ASSAYS

Principle:

\[
\begin{align*}
\text{Creatine kinase} & : \\
\text{PCr} + \text{ADP} & \rightarrow \text{Creatine} + \text{ATP} \\
\text{hexokinase} & : \\
\text{ATP} + \text{Glucose} & \rightarrow \text{ADP} + \text{Glucose-6-P} \\
\text{NADP}^+ & \rightarrow \text{NADPH} + \text{H}^+ \\
\text{glucose-6-dehydrogenase} & : \\
\text{Glucose-6-P} & \rightarrow \text{6-P-Gluconolactone}
\end{align*}
\]

Reagents:
Buffer: Tris-HCL (50 mmol· L\(^{-1}\)), pH 8.1 with 0.02 % Bovine Serum Albumin (BSA).
Cofactor: NADP (5 mmol· L\(^{-1}\)).
Enzymes: G6P-DH (14 U· mL\(^{-1}\)), HK (28 U· mL\(^{-1}\)), CK (1260 U· mL\(^{-1}\)).
Reagents: ADP (10 mmol· L\(^{-1}\)), Glucose (10 mmol· L\(^{-1}\)), Dithiothreitol (DTT, 50 mmol· L\(^{-1}\)), MgCl\(_2\) (100 mmol· L\(^{-1}\)).
Standards: ATP (2 mmol· L\(^{-1}\))
Diluent: Carbonate buffer (20 mmol· L\(^{-1}\), pH 10).

Working standards were performed immediately prior to the assays as follows:

\[
\begin{array}{ccccccc}
\text{ATP concentration (µmol· L}^{-1}) & 0 & 25 & 50 & 100 & 150 & 200 \\
\text{ATP [(2 mmol· L}^{-1}) (µL)]} & 0 & 25 & 50 & 100 & 150 & 200 \\
\text{Double distilled water} & 2000 & 1975 & 1950 & 1900 & 1850 & 1800 \\
\text{ATP concentration (µmol· L}^{-1}) & 0 & 25 & 50 & 100 & 150 & 200
\end{array}
\]

The reaction mixture (below) was prepared immediately prior to analysis.

**Reaction mixture (final concentrations):**

- **Buffer**: 1mL
- **NADP**: 10 µL (0.046 mmol· L\(^{-1}\))
APPENDIX S

MUSCLE METABOLITE ASSAYS

ADP 10 µL (0.091 mmol·L⁻¹)
Glucose 10 µL (0.091 mmol·L⁻¹)
DTT 10 µL (0.457 mmol·L⁻¹)
MgCl₂ 50 µL (4.566 mmol·L⁻¹)
G6P-DH 5 µL (0.064 mmol·L⁻¹)

Procedure:

1. **20 µL** of extract was pipetted into a fluorimeter tube and dilutes with 100 µL of double-distilled water (1:6 dilution). **10 µL** aliquots of the diluted extract were pipetted into 6 fluorimeter tubes resulting in 3 sets of duplicate samples. Three sets of double distilled water blanks and 1 set of standards were also pipetted (**10 µL** aliquots).

2. **200 µL** of the above reaction mixture was added to one set of tubes and blanks (G6-P determination, STEP 1)

3. **5 µL** of HK (0.127 U·mL⁻¹) was added per mL of remaining reaction mixture and **200 µL** of this reaction mixture was then added to the second set of tubes, blanks and ATP standards (ATP + G6P determination, STEP 2).

4. **10 µL** of CK (11.351 U·mL⁻¹) was added per mL of remaining reaction mixture and **200 µL** of this reaction mixture was then added to the third set of tubes and blanks (ATP + G6P + PCr determination, STEP 3).

5. After incubating for 30-40 min at room temperature (reaction completed), 1 mL of carbonate buffer was added to each tube using a Hamilton automatic dispenser (Hamilton microlab, Switzerland), and after thorough mixing fluorescence was read.

For ATP determination the relative fluorescence (sample fluorescence – respective blank fluorescence) of G6P (STEP 1) was subtracted from the relative fluorescence of ATP + G6P (STEP 2).

For PCr determination the relative fluorescence of ATP + G6P (STEP 2) was subtracted from the relative fluorescence of ATP + G6P + PCr (STEP 3).

The concentrations of ATP, PCr and G6P were determined using simple linear regression analysis of the standard concentrations (ATP) on fluorescence readings. Concentrations were corrected for dilutions during
the extraction procedure (1:125) and the 1:6 dilution during this assay:
Concentration (mmol·L⁻¹·kg dry muscle⁻¹) = regression value × 0.125 × 6

Notes:
* Enzymes G6P-DH, HK and CK were diluted using Tris-HCL 20 mmol·L⁻¹ (pH 8.1 with 0.02% BSA).
APPENDIX S2: CREATINE ASSAY

Principle:

Creatine kinase

\[
\text{Creatine} + \text{ATP} \rightarrow \text{P-Creatine} + \text{ADP}
\]

Pyruvate kinase

\[
\text{ADP} + \text{P-pyruvate} \rightarrow \text{ATP} + \text{Pyruvate}
\]

\[
\text{NADH} + \text{H}^+ \rightarrow \text{NAD}^+ \quad \text{Pyruvate} \rightarrow \text{lactate}
\]

\[
\text{lactate dehydrogenase}
\]

Reagents:

Buffer Imidazole-HCl (50 mmol·L\(^{-1}\), pH 7.5).

Cofactor CK (260 U·mL\(^{-1}\)), PK (75 U·mL\(^{-1}\), LDH (240 U·mL\(^{-1}\)).

Reagents ATP (10 mmol·L\(^{-1}\)), phosphoenol pyruvate [(PEP) (2 mmol·L\(^{-1}\))], MgCl\(_2\) (100 mmol·L\(^{-1}\)), KCl (3 mmol·L\(^{-1}\)), EDTA·Na\(_2\) (100 mmol·L\(^{-1}\)).

Standards Creatine (2 mmol·L\(^{-1}\))

Diluent Carbonate buffer (20 mmol·L\(^{-1}\), pH 10.0)

Working samples were prepared immediately prior to the assays as follows:

<table>
<thead>
<tr>
<th>Creatine [(2 mmol·L(^{-1})), (μL)]</th>
<th>0</th>
<th>50</th>
<th>100</th>
<th>150</th>
</tr>
</thead>
<tbody>
<tr>
<td>Double distilled water (μL)</td>
<td>2000</td>
<td>1950</td>
<td>1900</td>
<td>1850</td>
</tr>
<tr>
<td>Creatine concentration (μmmol·L(^{-1}))</td>
<td>0</td>
<td>50</td>
<td>100</td>
<td>150</td>
</tr>
</tbody>
</table>

The reaction mixture (below) was prepared immediately prior to analysis.

**Reaction mixture (final concentrations):**

<table>
<thead>
<tr>
<th>Buffer</th>
<th>1 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>MgCl(_2)</td>
<td>50 μL (4.413 mmol·L(^{-1}))</td>
</tr>
<tr>
<td>KCA</td>
<td>10 μL (26.478 mmol·L(^{-1}))</td>
</tr>
</tbody>
</table>
APPENDIX S

MUSCLE METABOLITE ASSAYS

ATP 20 μL (0.177 mmol·L⁻¹)
NADH 15 μL (13 μmol·L⁻¹)
PEP 25 μL (44 μmol·L⁻¹)
EDTA·Na₂ 1 μL (88 μmol·L⁻¹)
LDH 2 μL (0.424 U·mL⁻¹)
PK 10 μL (0.662 U·mL⁻¹)

Procedure:

20 μL of extract was pipetted into a fluorimeter tube and diluted with 100 μL double-distilled water (1:8 dilution). 10 μL aliquots of the diluted extract were pipetted into 4 fluorimeter tubes resulting in 2 sets of duplicate samples. Two sets of double distilled water blanks and 1 set of standards were also pipetted (10 μL aliquots).

200 μL of the above reaction mixture was added to one set of tubes and blanks (ADP + pyruvate determination, STEP 1).

10 μL of CK (11.024 U·mL⁻¹) was added per mL of remaining reaction mixture and 200 μL of this reaction mixture was then added to the second set of tubes, blanks and Creatine standards (Creatine + ADP + pyruvate determination, STEP 2).

After incubating for 50-60 min at room temperature (reaction completed), 1 mL of carbonate buffer was added to each tube using a Hamilton automatic dispenser, and after thorough mixing fluorescence was read.

For creatine determination the relative fluorescence of ADP + pyruvate (STEP 1) was subtracted from the relative fluorescence of Creatine + ADP + pyruvate (STEP 2).

The concentration of Creatine was determined using simple linear regression analysis of the standard concentrations (Creatine) on fluorescence readings. Concentrations were corrected for dilutions during the extraction procedure and the 1:8 dilution this assay: Concentration (mmol·L⁻¹·kg dry muscle⁻¹) = regression value x 0.125 x 8.

Notes:

Enzymes CK, PK and LDH were diluted using Tris-HCL 20 mmol·L⁻¹ (pH 8.1 with 0.02 % BSA).
APPENDIX S3: MUSCLE LACTATE ASSAY

Principle:

\[
\begin{align*}
NAD^+ & \quad \text{NADH} + \text{H}^+ \\
\text{lactate dehydrogenase} & \quad \text{Lactate} \quad \text{Pyruvate}
\end{align*}
\]

Reagents:

- Buffer: Hydrazine (1.1. mol· L\(^{-1}\), pH 9.0 with 1 mmol· L\(^{-1}\) EDTA· Na\(_2\))
- Cofactor: NAD (50 mmol· L\(^{-1}\))
- Enzyme: Lactate dehydrogenase [(LDH) 5500 U· mL\(^{-1}\) (undiluted)]
- Standard: L-Lactate (2 mmol· L\(^{-1}\))
- Diluent: Carbonate buffer (20 mmol· L\(^{-1}\), pH 10.0)

Working standards were prepared immediately prior to the assay as follows:

\[
\begin{array}{cccccccc}
\text{L-Lactate} & 2 \text{ mmol· L}^{-1} & (\mu L) & 0 & 50 & 75 & 100 & 150 & 400 & 750 & 1200 \\
\text{Double distilled water} & (\mu L) & 2000 & 1950 & 1925 & 1900 & 1850 & 1600 & 1250 & 800 \\
\text{La}^- \text{Concentration} & (\mu mol· L}^{-1}) & 0 & 50 & 75 & 100 & 150 & 400 & 750 & 1200 \\
\end{array}
\]

Reaction mixture:

- Buffer: 1 mL
- NAD: 10 \(\mu\)L (0.491 mmol· L\(^{-1}\))
- LDH: 8 \(\mu\)L (43.22 U· mL\(^{-1}\))

Procedure:

- **10 \(\mu\)L aliquots of the undiluted extract** were pipetted into a set of duplicate fluorimeter tubes. A set of double distilled water blanks and 1 set of standards were also pipetted (10 \(\mu\)L aliquots).
- **200 \(\mu\)L** of the above reaction mixture was added to each tube (mix well).
After incubating for 30-40 min at room temperature (reaction completed), 1 mL of carbonate buffer was added to each tube using a Hamilton automatic dispenser, and after thorough mixing (Whirlimixer), fluorescence was read.

The concentration of lactate was determined using simple linear regression analysis of the standard concentrations (lactate) on fluorescence readings.

Concentrations were corrected for dilutions during the extraction procedure (1:125): Concentration (mmol·L⁻¹·kg dry muscle⁻¹) = regression value x 0.125.
APPENDIX S4: GLYCOGEN ASSAY (mixed muscle method)

Preparation:

G5  0.1 M NaOH  
G6  0.1 M HCL  
G7  0.2 M Citric Acid  
0.2 M Na₂PO₄  
pH 5.0 (NaOH)

Amyloglucidase    200 mg·mL⁻¹ made up with G7 buffer. Prepare ~ 0.5 mL.

Ensure G6 neutralises G5 (Normally, 80 μL of HCL needed to neutralise 100 μL of NaOH).

The mix G6 and G7 in ratio 3 parts G7:1 part G6 (if above ratio correct, then 3.2 parts G7:0.8 part G6 i.e. for 10 mL solution you need 2 mL of G6 + 8 mL of G7).

Assay procedures:

- Spin powdered samples at 14 000 rpm for 3-4 min.
- Solubilise by adding G5 in the following quantities:

  0.50 – 2.00 mg muscle    100 μL G5  
  2.01 – 3.00 mg muscle    120 μL G5  
  3.01 – 4.00 mg muscle    160 μL G5  
  4.01 – 5.00 mg muscle    200 μL G5  
  > 5.01 mg muscle        200 μL G5

Vortex mildly at first and then vigorously until dissolved (a green colour will develop). (If sample is difficult to solubilise place sample at 80 °C for 5 min, vortex and then place at 80 °C for a further 5 min. See below).
• Incubate at 80 °C for 10 min (dry thermostat).
• Spin for 20-30 sec at 14000 rpm
• Add G7/G6 mixture buffer (4x the volume of G5 added) and vortex
• Add amyloglucosidase (use yellow positive displacement pipette):

  If 100 µL G5 used then 400 µL G7/G6 + 15 µL Amyloglucosidase
  If 120 µL G5 used then 480 µL G7/G6 + 15 µL Amyloglucosidase
  If 160 µL G5 used then 640 µL G7/G6 + 20 µL Amyloglucosidase
  If 200 µL G5 used then 800 µL G7/G6 + 25 µL Amyloglucosidase

• Vortex and then incubate at room temperature for 1 hour.
• Centrifuge for 2 min at 14 000 rpm
• Remove supernatant to a new screw-top Eppendorf tube (STEP Z)
• Assay on the same day or store at −80 °C until analysis

GLYCOGEN DETERMINATION:

Principle:

\[
\text{ATP + Glucose} \rightarrow \text{ADP + Glucose-6-P}
\]

\[
\text{NADP}^+ \rightarrow \text{NADPH + H}^+ \text{ glucose-6-dehydrogenase}
\]

\[
\text{Glucose-6-P} \rightarrow 6\text{-P-Gluconolactone}
\]

Reagents:

Buffer: Tris-HCL (100 mmol·L⁻¹), pH 8.1 with 0.02 % Bovine Serum Albumin (BSA).

Cofactor: NADP (5 mmol·L⁻¹).

Enzymes: G6P-DH (7 U·mL⁻¹), HK (28 U·mL⁻¹).
Reagents: ATP (200 mmol· L⁻¹), Dithiothreitol [(DTT), 50 mmol· L⁻¹], MgCl₂ (100 mmol· L⁻¹), EDTA· Na₂ (100 mmol· L⁻¹).

Standard: Glucose (0.505 mmol· L⁻¹).

Diluent: Carbonate buffer (20 mmol· L⁻¹).

Working standards were prepared immediately prior to the assay as follows:

<table>
<thead>
<tr>
<th>Glucose 0.505 mmol· L⁻¹ (µL)</th>
<th>0</th>
<th>20</th>
<th>50</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Double distilled water (µL)</td>
<td>505</td>
<td>485</td>
<td>455</td>
<td>405</td>
</tr>
<tr>
<td>Glucose concentration (µmol· L⁻¹)</td>
<td>0</td>
<td>20</td>
<td>50</td>
<td>100</td>
</tr>
</tbody>
</table>

Reaction mixture was prepared immediately prior to analysis for 2 sets of duplicate samples and quadruplicate double distilled water blanks, and a set of quadruplicate standards.

**Reaction mixture (final concentrations):**

- Buffer: 1 mL
- NADP: 6 µL (29 µmol· L⁻¹)
- ATP: 1.5 µL (0.29 mmol· L⁻¹)
- MgCl₂: 10 µL (0.966 µmol· L⁻¹)
- EDTA· Na₂: 5 µL (0.483 mmol· L⁻¹)
- DTT: 10 µL (0.483 mmol· L⁻¹)
- G6P-DH: 3 µL (0.02 U· mL⁻¹)

**Procedure:**

- 20 µL of the supernatant prepared in the previous procedures (STEP 2) was pipetted into 2 sets of duplicate samples. Two sets of double distilled water blanks and 1 set of standards were also pipetted (20 µL aliquots).
- 200 µL of the above reaction mixture was added to one set of samples and blanks (G6P determination; STEP 1).
- 5 µL of HK (0.135 U· mL⁻¹) was added per mL of remaining reaction mixture and 200 µL of this reaction mixture was then added to the second
set of samples, blanks and Glucose standards (Glucose + G6P determination, STEP 2).

After incubating for 30 min at room temperature (reaction completed), 1 mL of carbonate buffer was added to each tube using a Hamilton automatic dispenser, and after thorough mixing fluorescence was read.

The concentrations of Glycogen (glucosyl units) and G6P were determined using simple linear regression analysis of the standard concentrations (Glucose) on fluorescence readings.
### APPENDIX T1: SPREAD SHEET FOR PLASMA VOLUME

#### TABLE 1:

<p>| | | | |</p>
<table>
<thead>
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#### TABLE 2:

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</table>

### APPENDIX T2: SPREAD SHEET FOR PLASMA VOLUME

#### TABLE 3:

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### APPENDIX T3: SPREAD SHEET FOR PLASMA VOLUME

#### TABLE 4:

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ANTONIS TSAMPOUKOS  
Ph.D. THESIS
<table>
<thead>
<tr>
<th>No.</th>
<th>% Differences in BV</th>
<th>% Differences in CV</th>
<th>% Differences in PV</th>
<th>( \Delta V' )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>( \text{ROUND}(J5:$1 \times 100/100,1) )</td>
<td>( \text{ROUND}(K5:$1 \times 100/100,1) )</td>
<td>( \text{ROUND}(L5:$1 \times 100/0$1,1) )</td>
<td>( \text{ROUND}(A2+B2+C2/3,0.9) - \text{ABS}(PV' \text{CHANGES}) )</td>
</tr>
<tr>
<td>2</td>
<td>( \text{ROUND}(J6:$1 \times 100/100,1) )</td>
<td>( \text{ROUND}(K6:$1 \times 100/100,1) )</td>
<td>( \text{ROUND}(L6:$1 \times 100/0$1,1) )</td>
<td>( \text{ROUND}(A2+B2+C2/3,0.9) - \text{ABS}(PV' \text{CHANGES}) )</td>
</tr>
<tr>
<td>3</td>
<td>( \text{ROUND}(J7:$1 \times 100/100,1) )</td>
<td>( \text{ROUND}(K7:$1 \times 100/100,1) )</td>
<td>( \text{ROUND}(L7:$1 \times 100/0$1,1) )</td>
<td>( \text{ROUND}(A2+B2+C2/3,0.9) - \text{ABS}(PV' \text{CHANGES}) )</td>
</tr>
<tr>
<td>4</td>
<td>( \text{ROUND}(J8:$1 \times 100/100,1) )</td>
<td>( \text{ROUND}(K8:$1 \times 100/100,1) )</td>
<td>( \text{ROUND}(L8:$1 \times 100/0$1,1) )</td>
<td>( \text{ROUND}(A2+B2+C2/3,0.9) - \text{ABS}(PV' \text{CHANGES}) )</td>
</tr>
<tr>
<td>5</td>
<td>( \text{ROUND}(J9:$1 \times 100/100,1) )</td>
<td>( \text{ROUND}(K9:$1 \times 100/100,1) )</td>
<td>( \text{ROUND}(L9:$1 \times 100/0$1,1) )</td>
<td>( \text{ROUND}(A2+B2+C2/3,0.9) - \text{ABS}(PV' \text{CHANGES}) )</td>
</tr>
<tr>
<td>6</td>
<td>( \text{ROUND}(J10:$1 \times 100/100,1) )</td>
<td>( \text{ROUND}(K10:$1 \times 100/100,1) )</td>
<td>( \text{ROUND}(L10:$1 \times 100/0$1,1) )</td>
<td>( \text{ROUND}(A2+B2+C2/3,0.9) - \text{ABS}(PV' \text{CHANGES}) )</td>
</tr>
</tbody>
</table>
APPENDIX T2: SPREADSHEET FOR BLOOD BUFFERING CAPACITY (EXAMPLE)
APPENDIX U: IT COURSES

The following information technology courses were completed as part of the research training program:

- Sports Discus
- The library Catalogue, OPAC
- Tracing Completed and Ongoing Research for Science and Engineering Postgraduates
- Tracing Journal Articles for Science and Engineering Postgraduates
- Database Guide
- BIDS Service
- Embase
- Web of Science
- Science Citation Index
APPENDIX V: CALCULATION OF COEFFICIENT OF VARIATIONS

In the present thesis two type of coefficient of variation were used. The first of them was calculated according to Cohen and Holiday (1982) as follows:

\[ CV = \frac{SEM}{\bar{X}} \times 100 \]

The meaning for this coefficient is the maximum difference (as a percentage) between the observed value and the true score with a certainty of 68%.

The second coefficient, which was used only for the methodological study 1, is calculated as follows (Atkinson, 2003):

\[ CV = \left[ SD_{\text{diff}} \cdot (\sqrt{2})^{-1} \right] \cdot \left( \bar{X} \cdot 100 \right)^{-1} \]
APPENDIX W: POST-TRIAL QUESTIONNAIRE FOR MAIN STUDY 1

EFFECT OF MENSTRUAL CYCLE PHASE UPON SPRINT RUNNING PERFORMANCE

You have now completed the study, thank you very much for your help. We should be grateful if you would complete this questionnaire to help evaluate some of the procedures.

1. Do you have any comments regarding the ovulation kit, the scales and/or the syringe you were given—e.g. ease of use?

2. Generally, is there any particular time of the month, that is in relation to your menstrual cycle, when you feel your performance in physical activity is either impaired or enhanced?

3. Was there any time during the course of this study you felt, for reasons other than those related to the menstrual cycle, unable to perform your maximum (perhaps exam stress, illness, post-match tiredness, someone behaviour in the lab etc)?

4. Do you feel that during the time course of the study your activity level had declined/reclined? If yes could you please give more information?

5. How do you think your level of performance in the sprint tests over the course of the study has been?

   IMPROVED / DETERIORATED / ABOUT THE SAME

Thank you for your co-operation!