Measuring Gene Expression in Endurance Athletes as a Novel Technique for Determining training Response to Sprint Interval Training (SIT)

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Abstract

The fundamental aim of an endurance athlete is to improve their ability to physically perform at a high intensity for a prolonged period of time. Furthermore, this high performance area of sport can be metabolically demanding, requiring high rates of oxidative (aerobic) and non-oxidative (anaerobic) metabolism with precise regulation and control. To meet these requirements, monitoring of training is an essential component of a successful training plan, as manipulation of training volume, frequency, intensity and recovery is vital for optimal results. Due to the long-distance nature of endurance events, high-volume and low-intensity training has traditionally been the main focus for endurance athletes. However, this high-volume load can often lead to overtraining and/or a performance plateau. More recently, a shift in training volume and intensity involving low volume but high intensity sessions (termed high-intensity interval training; HIT), has become more commonly used by endurance athletes. While endurance athletes have long appreciated the role of HIT as part of a comprehensive training program, the recent surge in HIT popularity is mainly due to the efficiency of the training and the recent evidence that it causes gene expression and phenotypic performance changes that resemble those of endurance training. Studies have suggested that in young healthy persons of average fitness, intense interval training is therefore a time-efficient strategy with results comparable to traditional endurance training. However, despite recent research advancements, the fundamental question remains regarding the minimum volume of exercise necessary to improve performance.

The aim of the proposed study was to investigate two different regimes of high intensity exercise (low-volume/high-frequency and high-volume/low-frequency) in 26 well-trained endurance cyclists, measure their effect on both physiological changes and gene expression changes, and determine whether the changes caused by HIT are due to total work or are regime-dependent. The training intervention consisted of nine bouts of 30-second sprints per week for two weeks at different volumes per group: one low-volume/high-frequency group with 3 repetitions 3 times per week (9 subjects), one high-volume/low-frequency group with 9 repetitions once per week (9 subjects), and one control group without training intervention (8 subjects). The physiological measures of interest related to the metabolic
changes from the endurance capacity test, VO_{2}\text{max} test, and Wingate test, which were measured on all subjects at time-points before (baseline) and after the intervention period. To achieve a full understanding of the biochemical adaptation, regulation and markers associated with HIT, an examination of the gene expression changes in vivo was performed by taking blood samples from participants before training (baseline), immediately after the final training session (acute response), and 72 hours after the final training session (delayed response), extracting RNA from white blood cells, and undertaking a genome-wide microarray analysis to identify genes differentially expressed after high-intensity exercise. The aim was also to investigate the changes in expression levels in response to varying levels of exercise (frequency of weekly sessions and number of bouts per session), and which of these training regimes would achieve a higher performance outcome.

Some significant differences were found in the physiological traits measured between training intervention groups and the control group, as well as between the groups themselves. While there were no significant changes in ventilation threshold (VT1) between the two training groups themselves, a significant difference was found between both of the training groups and the control group post-training. Furthermore, a significant increase from baseline (p = 0.006) was found in the post-training intervention endurance capacity test (ECT) for the high-volume/low-frequency group, while no significant change was seen in the low-volume/high-frequency group or the control group. These findings suggest that the high-volume/low-frequency regime may be more effective at improving endurance performance in relation to the endurance capacity test.

In relation to gene expression changes, data was only available for 6 subjects in the low-volume/high-frequency group and 4 subjects in the high-volume/low-frequency group. Microarrays were performed for these subjects at three time-points (baseline, acute post-training and delayed post-training); however, after normalisation, quality control and statistical analysis of participant data, no significant changes in gene expression were found between either of the two post-training time-points compared to baseline, or between different training regime groups.
Overall, however, this study allowed researchers to obtain an increased understanding of the physiological and gene expression adaptations that can result from high intensity training and determined that training regime influences performance outcome. By implementing the minimum training regime necessary to obtain performance improvements it may be possible to optimise athlete response to training whilst avoid overtraining and illness. Additionally, no alteration in gene expression was detected after the training intervention, possibly due to that the effect size investigated being smaller than anticipated. Further research in this area may provide sport coaches, exercise physiologists, sport scientists, and athletes another tool to optimise training prescription for athletes as well as evaluating and monitoring an individual’s biological response to exercise.
Declaration of Originality

This thesis is submitted to Bond University in fulfilment of the requirements of the degree of Doctor of Philosophy. This thesis represents my own original work towards this research degree and contains no material which has been previously submitted for a degree or diploma at this University or any other institution, except where due acknowledgement is made.

Signed: Siri Lauluten Selezak

Siri Lauluten Selezak

August 2015
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“For better it is to dare mighty things, to win glorious triumphs, even though checkered by failure, than to take rank with those poor spirits who neither enjoy much nor suffer much, because they live in the gray twilight that knows not victory nor defeat.”

Theodore Roosevelt
# Table of Contents

Abstract................................................................................................................................................................. iii
Declaration of Originality ...................................................................................................................................... vii
Acknowledgments ................................................................................................................................................. ix
Table of Contents ................................................................................................................................................ xi
List of Abbreviations .......................................................................................................................................... xix
Publications Arising from Thesis ........................................................................................................................ xxiii
Contribution to jointly published work .............................................................................................................. xxv

## Chapter 1 - Introduction

1.1 Aims and hypotheses ......................................................................................................................................... 13

## Chapter 2 – Review of the Literature

2.1 Exercise Physiology ........................................................................................................................................... 17
2.2 Energy systems and adaptations to exercise ..................................................................................................... 20
  2.2.1 Metabolic pathways - ATP -Phosphocreatine Pathway ............................................................................. 21
  2.2.2 Metabolic pathways - Glycolysis .................................................................................................................. 22
  2.2.3 Metabolic pathways - Beta Oxidation .......................................................................................................... 25
  2.2.4 Metabolic pathways - TCA Cycle ................................................................................................................. 26
  2.2.5 Electron Transport Chain ............................................................................................................................ 27
2.3 Endurance training ............................................................................................................................................ 30
  2.3.2 Endurance training for performance and competition ................................................................................. 31
2.4 Assessment of performance .............................................................................................................................. 33
  2.4.1 Oxygen consumption (VO$_2$) .................................................................................................................... 34
  2.4.2 VO$_2$max limitations .................................................................................................................................... 35
  2.4.3 VO$_2$max protocols ..................................................................................................................................... 36
  2.4.4 Ventilation thresholds ................................................................................................................................. 39
  2.4.5 Endurance capacity test (ECT) .................................................................................................................... 40
  2.4.6 Wingate – The peak and average power test ............................................................................................. 42
2.5 Training monitoring .......................................................................................................................................... 45
2.6 Endurance capacity and mode of training ......................................................................................................... 48
  2.6.1 Interval principle and mechanism of effect ................................................................................................. 51
  2.6.2 Interval training ......................................................................................................................................... 53
  2.6.3 High intensity and sprint interval training ................................................................................................. 57
Chapter 3 - Methodology .............................................................. 85
3.1 Training intervention .......................................................... 85
3.2 Subject characteristics ....................................................... 87
    3.2.1 Subjects .................................................................................. 87
    3.2.2 Dietary and Exercise Control ................................................. 88
3.3 Protocols, equipment and software ....................................... 88
    3.3.1 Cycle ergometer ................................................................. 88
    3.3.2 Cardio-pulmonary testing equipment and software .............. 88
    3.3.3 Wingate - Anaerobic Test ....................................................... 89
    3.3.4 Maximum oxygen consumption - VO2 max test .................. 92
    3.3.5 Endurance Capacity Test (ECT) ............................................. 93
3.4 Global Gene Expression Changes – protocols and equipment .... 95
    3.4.1 Blood Collection and Phlebotomy ......................................... 95
    3.4.2 Cell Isolation: Density Gradient Centrifugation .................... 95
    3.4.3 Total RNA Purification: Solid Phase Extraction ...................... 97
    3.4.4 Reverse transcription to make cDNA ................................... 98
    3.4.5 Genome-wide Microarray ................................................... 99
    3.4.6 Statistical analysis of Microarray .......................................... 100
    3.4.7 Reverse Transcription- PCR ............................................... 101

Chapter 4 - Physiological Changes caused by Sprint Interval Training Intervention ............. 110
4.1. Introduction ............................................................................. 110
4.2. Methods ................................................................................... 112
    4.2.1 Optimal ECT apparatus sub-study: Mouthpiece or facemask? 113
4.3. Results ...................................................................................... 117
4.4 Discussion ................................................................................ 122
4.5 Conclusions ............................................................................. 127
Chapter 5 - Global Gene Expression Changes caused by Sprint Interval Training Intervention ..... 129

5.1 Introduction ........................................................................................................................................... 129
5.2 Methods ............................................................................................................................................... 131
5.3 Results ............................................................................................................................................... 139
5.4 Discussion ......................................................................................................................................... 144
5.5 Conclusion ........................................................................................................................................ 156

Chapter 6 – Discussion and Future Directions ......................................................................................... 158

6.1 Improved athletic performance in trained cyclists after SIT ............................................................. 158
6.2 Global Gene Expression Changes caused by SIT ............................................................................. 159
6.3 Overall discussion ............................................................................................................................. 160

Chapter 7 – Overall Conclusions ............................................................................................................ 162

References ................................................................................................................................................ 164

Appendices ............................................................................................................................................... 197

Appendix A: HIT training protocols and outcomes from the literature ........................................... 200
Appendix B: Gene expression in muscle tissue ..................................................................................... 207
Appendix C: Gene expression in Human White Blood Cells ............................................................... 208
Appendix D: Typical training session ..................................................................................................... 210
Appendix E: RNA isolation protocol .................................................................................................... 211
Appendix F: Diary ..................................................................................................................................... 213
Appendix G: Array overview .................................................................................................................. 214
List of Figures

Figure 1. Anaerobic threshold (AT) or VT1 and respiratory compensation threshold (RCT) or VT2 ..... 5
Figure 2 - Maximum oxygen consumption .................................................................................. 6
Figure 3 - The process of transcription and translation................................................................. 8
Figure 4 - Anaerobic metabolism of glucose – Glycolysis............................................................. 24
Figure 5 - TCA cycle .................................................................................................................... 26
Figure 6 - Electron transport chain ............................................................................................ 28
Figure 7 - Format of treadmill protocols..................................................................................... 38
Figure 8 – A traditional view of gene expression........................................................................ 70
Figure 9 - Training intervention schedule for G1 and G2............................................................ 86
Figure 10 – Separation of white blood cells from whole blood using Ficoll-Paque PLUS........... 96
Figure 11 - RT-PCR Melt Curve ................................................................................................. 105
Figure 12 - RT-PCR Cycle threshold........................................................................................ 106
Figure 13 - Physiological responses throughout each ECT ....................................................... 117
Figure 14 - Differences in VO_2 L.min^{-1} vs. mean value VO_2 L min^{-1} ................................. 119
Figure 15 - Performance test results.......................................................................................... 120
Figure 16 - Public calculator available for sample size calculation ......................................... 138
Figure 17 - Sliding scale of sample size calculations with 80% power ................................... 143
Figure 18 - Sliding scale of sample size calculations with 90% power ................................... 144
List of Tables

Table 1 - Training volume of the subjects during each of the three periods ........................................... 32
Table 2 - Training intensity of the subjects during each of the three periods ........................................... 33
Table 3 – Constants for comparisons of WAnT between subjects ............................................................. 43
Table 4 – Different types of interval training and their velocities ............................................................... 64
Table 5 - Studies investigating transcriptional responses using microarray ............................................. 76
Table 6 - Characteristics for participating subjects (N= 26) ........................................................................ 87
Table 7 - Constants for comparisons of WAnT between subjects ............................................................. 91
Table 8 - Characteristics for participating subjects (N=10) ....................................................................... 115
Table 9 – ECT study results ...................................................................................................................... 118
Table 10 - Physiological and performance measures ................................................................................. 121
Table 11 – Primer design for microarray validation of genes ILR, IL16, and CD69 ................................. 136
Table 12 - Top 20 genes, comparison of G1 vs. G2 at each time-point .................................................... 139
Table 13 - Top 20 genes, pairwise comparisons of time-points for group 1 ............................................. 140
Table 14 - Top 20 genes, pairwise comparisons of time-points for group 2 ............................................. 140
Table 15 – qRT-PCR Validation results and comparison with Microarray .............................................. 142
Table 16 – Regression analysis of qRT-PCR and Microarray data .......................................................... 142
## List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>2,3-DPG</td>
<td>2, 3-diphosphoglycerate</td>
</tr>
<tr>
<td>ADP</td>
<td>Adenosine diphosphate</td>
</tr>
<tr>
<td>AMP</td>
<td>Adenosine monophosphate</td>
</tr>
<tr>
<td>AMPK</td>
<td>5' adenosine monophosphate-activated protein kinase</td>
</tr>
<tr>
<td>AT</td>
<td>Anaerobic threshold</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>ATP-PC</td>
<td>Phosphagen system</td>
</tr>
<tr>
<td>a-vO₂</td>
<td>Arterio-venous oxygen difference</td>
</tr>
<tr>
<td>BTPS</td>
<td>Body temperature and pressure, saturated</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>Calcium (ionised)</td>
</tr>
<tr>
<td>CaO₂</td>
<td>Arterial oxygen content</td>
</tr>
<tr>
<td>CCD</td>
<td>Charge-coupled device</td>
</tr>
<tr>
<td>cDNA</td>
<td>Complementary deoxyribonucleic acid</td>
</tr>
<tr>
<td>CO₂</td>
<td>Carbon dioxide</td>
</tr>
<tr>
<td>CoA</td>
<td><em>Coenzyme A</em></td>
</tr>
<tr>
<td>COPD</td>
<td>Chronic obstructive pulmonary disease</td>
</tr>
<tr>
<td>CDT</td>
<td>Constant duration test</td>
</tr>
<tr>
<td>CPT</td>
<td>Constant power test</td>
</tr>
<tr>
<td>CWT</td>
<td>Constant-work test</td>
</tr>
<tr>
<td>CP</td>
<td>Creatine phosphate</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>CPT1B</td>
<td>Carnitine palmitoyltransferase 1B</td>
</tr>
<tr>
<td>CRAT</td>
<td>Carnitine O-Acetyltransferase</td>
</tr>
<tr>
<td>CvO₂</td>
<td>Mixed venous O₂ content</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>dsDNA</td>
<td>Double stranded DNA</td>
</tr>
<tr>
<td>ECG</td>
<td>Electrocardiogram</td>
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<tr>
<td>ECT</td>
<td>Endurance capacity test</td>
</tr>
<tr>
<td>EMG</td>
<td>Electromyography</td>
</tr>
<tr>
<td>ERK1/2</td>
<td>Extracellular-signal-regulated kinases</td>
</tr>
<tr>
<td>FAD</td>
<td>Flavin adenine dinucleotide (oxidised)</td>
</tr>
<tr>
<td>FADH₂</td>
<td>Flavin adenine dinucleotide (reduced)</td>
</tr>
<tr>
<td>FC</td>
<td>Fold change</td>
</tr>
<tr>
<td>GAPDH</td>
<td>Glyceraldehyde-3-phosphate dehydrogenase</td>
</tr>
<tr>
<td>GRP58</td>
<td>58 kDa glucose-regulated protein</td>
</tr>
<tr>
<td>GTP</td>
<td>Guanosine triphosphate</td>
</tr>
<tr>
<td>H₂O</td>
<td>Dihydrogen monoxide/water</td>
</tr>
<tr>
<td>Hb</td>
<td>Haemoglobin</td>
</tr>
<tr>
<td>HIT</td>
<td>High intensity interval training</td>
</tr>
<tr>
<td>HK</td>
<td>Hexokinase</td>
</tr>
<tr>
<td>HR</td>
<td>Heart rate</td>
</tr>
<tr>
<td>HRV</td>
<td>Heart rate variability</td>
</tr>
<tr>
<td>JNK</td>
<td>c-jun NH₂-terminal kinase</td>
</tr>
</tbody>
</table>
log₂

Logarithm to the base 2

LSD

Long slow distance training

LT

Lactate threshold

MAPK

Mitogen-activated protein kinases

MAPKAPK1

Mitogen activated protein kinase activated protein kinase 1

mRNA

Messenger RNA

MSK

Mitogen- and stress-activated protein kinase

NAD⁺

Nicotinamide adenine dinucleotide (oxidised form)

O₂

Oxygen (molecular)

OCTN2

Carnitine/organic cation transporter

PaO₂

Partial pressure of O₂ in arterial blood

PBMC

Peripheral blood mononuclear cell

PCO₂

Partial pressure of carbon dioxide

PCR

Polymerase chain reaction

PFK

Phosphofructokinase

PGC-1α

Peroxisome proliferator-activated receptor-gamma coactivator 1 alpha

Pᵢ

Inorganic phosphate

RCT

Respiratory compensation threshold

RER

Respiratory exchange ratio

R-I

Ratio-intensity

RNA

Ribonucleic acid

RPE

Rating of perceived exertion
RQ  Respiratory quotient
RT-PCR  Reverse transcription polymerase chain reaction
S6K  SK Kinase
SAGE  Serial analysis of gene expression
SaO2  Saturation level of oxygen in haemoglobin
SD  Standard deviation
SEM  Standard error of the mean
SIT  Sprint interval training
TCA cycle  Tricarboxylic acid cycle
TT  Time trial
VCO2  Volume of carbon dioxide
VE  Minute Ventilation
VEGF  Vascular endothelial growth factor
VO2max  Maximum oxygen consumption
VST  Variance-stabilizing transformation
VT1  Ventilation threshold 1
VT2  Ventilation threshold 2
WAnT  Wingate anaerobic test
WBC  White blood cells
Publications Arising from Thesis


Contribution to jointly published work


The idea of this project was generated by the candidate and B. Gray. The data collection procedures were undertaken by the candidate, G. Gass and A. M. Szlezak. The analysis of the data was undertaken by the candidate, G. Gass and A. M. Szlezak. The production of the manuscripts is undertaken by the candidate, B. Gray and A. M. Szlezak.


The idea of this project was generated by the candidate and B. Gray. The data collection procedures were undertaken by the candidate, J. Keane and A. M. Szlezak. The analysis of the data was undertaken by the candidate, the candidate and A. M. Szlezak. The production of the manuscripts is undertaken by the candidate, B. Gray and A. M. Szlezak and J. Keane.