INDIVIDUAL VARIABILITY OF EFFORT-BASED TRAINING PRESCRIPTIONS IN COMPETITIVE CYCLISTS

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Declaration

No part of this thesis has been submitted in support of an application for any degree or other qualification of the University of Kent, or any other University or Institution of learning.

Dedication

This thesis is dedicated to Freya, my Rocket.

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Abstract

The aim of this thesis was to investigate the use of effort-based intensity prescriptions as a method to reduce individual variability and the occurrence of training non-response. Specifically, this thesis explores the physiological, psychological, and metabolomic responses to the interaction of training duration and effort-based intensity on a single-bout basis, whole session basis, and during chronic training intervention.

The first experimental study (Chapter 4) investigated individual variability during self-paced exercise bouts at a rating of perceived exertion (RPE) of either 9, 13, or 17, conducted for either 1, 4, or 8 min. The study found that effort-based intensity prescriptions at higher RPEs and shorter durations result in lower levels of individual variability. The second study (Chapter 5) investigated individual variability during maximal isoeffort training sessions in either short interval (30 sec), long interval (5 min), or continuous exercise matched for total training duration. Long intervals displayed lowest variability in both how the session was performed as well as physiological response compared to short intervals and continuous sessions. The third study (Chapter 6) comprised a 6-week training intervention using maximal isoeffort intensity prescriptions using short (30 sec) or long interval (5 min) session formats, as well as a control group. Whilst short intervals resulted in higher levels of individual variability, a greater training response was found following this session format compared to both the long interval and control groups. The fourth study (Chapter 7) investigated the metabolomic differences between session formats, and between variable and consistent responding participants from Chapter 5. Distinct metabolomic differences were found between all session formats, and key metabolites were found relating to energy turnover, purine metabolism, and amino acid metabolism based on whether individuals were consistent or variable in session performance. The fifth study (Chapter 8) investigated the chronic changes in the urinary metabolome following the training interventions as described in Chapter 6. Several metabolomic markers differentiated between training responders and non-responders, in addition to metabolites associated with increased MMP or $\dot{V}O_{2max}$ across all training groups.

The main finding of this thesis was that the use of higher intensity effort-based targets and shorter interval durations has potential in reducing the occurrence of non-response to training.

Key Words: Training, Effort, Variability, Prescription, Exercise, VO_2max, Cycling, Individualisation, HIIT, Individualised training, Between-athlete variability, Within-athlete variability, Training response.

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List of Abbreviations and Symbols

$[Ca^{2+}]$	Intramuscular calcium concentration
$\%\dot{V}O_{2max}$	Percentage of maximal oxygen uptake
ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
AMPK	Adenosine monophosphate activated protein kinase
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
BAV	Between-athlete variability
B_f	Breathing frequency
BLa^{-1}	Blood lactate
BPI	Base peak intensity
bpm	Beats per minute
CaMK	Calcium-calmodulin kinase
C_aO_2	Arterial oxygen content
$C_{av}O_2$	Venous oxygen content
CI	Confidence interval
CO_2	Carbon dioxide
CP	Critical power
cQC	Conditioning quality control
CS	Citrate synthase
CV	Coefficient of variation
DALDA	Daily Analyses of Life Demands for Athletes
EIC	Extracted ion chromatogram
ESI	Electrospray ionisation
ESI^-	Negative ionisation mode
ESI^+	Positive ionisation mode
ETC	Electron transport chain
GC	Gas chromatography
GE	Gross efficiency
GLUT4	Glucose transporter 4
h	Hour
HHb	Deoxygenated haemoglobin
HIIT	High-intensity interval training
HMDB	Human metabolome database
HPLC	High-performance liquid chromatography
HPLC	High-performance liquid chromatography
HR	Heart rate
HR_{max}	Maximal heart rate
HRR	HR reserve
IMP	Inosine monophosphate
Kg	Kilogram
kJ	Kilojoules
LC	Liquid chromatography

LT	Lactate threshold
LT_1	Primary lactate threshold
LT_2	Secondary lactate threshold
LTP	Lactate turn point
m/z	Mass-to-charge
MCT	Monocarboxylate transporter
MFC	Median fold change
Min	Minute
$mL.kg^{-1}.min^{-1}$	Millilitres of O_2, per kilogram of body mass, per minute
MLSS	Maximal lactate steady state
$mmol.L^{-1}$	Millimoles per litre
MMP	Maximum minute power
MS	Mass spectrometry
n	Sample size
NASA-TLX	National Aeronautics and Space Administration Task Load Index
NIRS	Near-infrared spectroscopy
NAD^+	Nicotinamide adenine dinucleotide
NMR	Nuclear magnetic resonance
O_2	Oxygen
O_2Hb	Oxygenated haemoglobin
OPLS-DA	Orthogonal partial least squares discriminant analysis
P	Significance level
PC	Principal component analysis
PC	Principal components
PGC-1α	Peroxisome proliferator-activated receptor-γ coactivator-1α
PL	Power law
PLS	Partial least squares
Q	Cardiac output
Q^2	Model predictive strength
QC	Quality control
qMS	Quadrupole-mass spectrometry
qTOF	Quadrupole-time-of-flight
r	Correlation coefficient
R^2	Coefficient of determination
R^2X	Variance in the X-matrix
R^2Y	Variance in the Y-matrix
RER	Respiratory exchange ratio
RPE	Rating of perceived exertion
RT	Retention time
s	Seconds
SD	Standard deviation
Session Δ	Difference between first interval and last interval
SNPs	Single-nucleotide polymorphisms
sRPE	Session rating of perceived exertion
TCA	Tricarboxylic acid

tHb	Total haemoglobin
TIC	Total ion chromatogram
TOF	Time-of-flight
TSI	Tissue saturation index
TT	Time trial
TTE	Time to exhaustion
TV	Total variability
UPLC	Ultra-performance liquid chromatography
UV	Unit variance
$\dot{V}CO_2$	Volume of carbon dioxide
$\dot{V}_E$	Minute ventilation
VIP	Variable importance to the projection
$\dot{V}O_2$	Volume of oxygen
$\dot{V}O_{2max}$	Maximal oxygen uptake
VT_1	Primary ventilatory threshold
VT_2	Secondary ventilatory threshold
W	Watts
WAV	Within-athlete variability
$\bar{X}$	Interval change
Δ	Delta; change in
η_p^2	Partial eta squared

Chapter 1 - Introduction

1.1 Background

Training to enhance exercise performance involves stressing numerous physiological systems in order to trigger facilitative adaptations (Borresen and Lambert 2008; Hawley and Burke 1998; Friel 2012; Rushall and Pyke 1991). For this training to be most effective, it needs to be structured according to training principles such as specificity, overload, and progression (Baechle, Earle and Wathen 2000; Rushall and Pyke 1991; Wilson *et al.* 1993). The various modalities, structures, and types of training that are most effective at improving endurance performance have been widely researched, such as low intensity endurance training, threshold training, and high-intensity interval training (Smith 2003; Seiler and Tønnessen 2009; Coyle *et al.* 1988; Bassett and Howley 2000; Joyner and Coyle 2008; Jacobs *et al.* 2011; Lundby and Robach 2015; Coyle *et al.* 1991; Bassett 2002).

Differences in response to exercise training and the associated physiological adaptations is termed individual variability and can be split into variation which is observed within the same individual (within-athlete variability), and variation which is observed between individuals (between-athlete variability; Hecksteden *et al.* 2015). The large between-athlete differences in training response are commonly observed throughout sports science literature makes prescribing optimal training from a scientific basis difficult (Hopker and Passfield 2014). Within scientific research investigating exercise training interventions, the most common method of reporting the efficacy of an intervention involves presenting a group mean change, thus assuming that the average response in a study group is representative of each individual's response to training (Mann, Lamberts and Lambert 2014; Timmons 2011; Bacon *et al.* 2013). While exercise interventions regularly display clear alterations in group mean parameters, these changes often have large between-athlete variability in response between participants which is reflected by a large sample standard deviation (Astorino and Schubert 2014; Sisson, Katzmarzyk, Earnest, *et al.* 2009; Scharhag-Rosenberger *et al.* 2010; Scharhag-Rosenberger *et al.* 2012a; Bouchard, An, Rice, Skinner, *et al.* 1999; Kohrt, Malley, Coggan, *et al.* 1991; Kuehnbaum, Gillen, Kormendi, *et al.* 2015; Lortie *et al.* 1984; Vollaard *et al.* 2009). The presence of between-athlete variability has led to the notion of '*responders*' and '*non-responders*' to training interventions as an explanation for large levels of between-athlete variability commonly reported by training studies (Mann, Lamberts and Lambert 2014). However, it is indeed possible to have large levels of between-athlete variability and no non-responders, as well as low levels of between-athlete variability and no responders, respectively. It has been

shown that an individual's genetics can influence baseline phenotype; for example, 50 % of the variance in maximal oxygen uptake ($\dot{V}O_{2max}$) could be explained by hereditary factors (Bouchard *et al.* 1998; Bouchard, An, Rice, Skinner, *et al.* 1999; Bouchard and Rankinen 2001). Training response can also be influenced by many other factors, such as epigenetic environmental stressors (Bouchard, Sarzynski, *et al.* 2011; Rankinen *et al.* 2012), and therefore an individual's hereditary influence may contribute more towards pre-training phenotype, and not necessarily the training response itself (Bouchard *et al.* 1998; Gaskill, Rice, *et al.* 2001; An *et al.* 2002). This pre-training phenotype is altered with training, which disrupts homeostasis results in a cascade of effective molecular signal processes and gene expression (Coffey and Hawley 2007). An individual's baseline phenotype can also influence training response in different parameters to varying degrees; for example, baseline measurements explain only 1 % of the changes in $\dot{V}O_{2max}$ following training, whereas 40 % of the changes in observed heart rate (HR) at 50 W can be explained by baseline phenotype (Bouchard and Rankinen 2001). Commonly acknowledged sources of error that influence levels of between-athlete and within-athlete variability are random measurement error (Hopkins *et al.* 2009; Hopkins 2000; Scharhag-Rosenberger *et al.* 2009), lifestyle factors (Ehlert, Simon and Moser 2013), and training program characteristics such as the basis for prescription of exercise intensity (Mann, Lamberts and Lambert 2014).

The presence of between-athlete and within-athlete variability in exercise stimulus leads to difficulty in applying the outcomes of exercise training studies to result in desired physiological adaptations on an individually optimised basis (Hopker and Passfield 2014). The extent to which between-athlete and within-athlete variability in the methods used to prescribe exercise training influences the outcomes of training interventions has not been fully explored (Mann, Lamberts and Lambert 2013). Indications that training intensity prescriptions may result in large degrees of between-athlete variability are apparent when examining differences in time to exhaustion between individuals when exercise is standardised to a set percentage of $\dot{V}O_{2max}$ (%$\dot{V}O_{2max}$; Coyle *et al.* 1988; Bouchard, An, Rice, Skinner, *et al.* 1999; Vollaard *et al.* 2009; Scharhag-Rosenberger *et al.* 2010). While it has been well established that there is a relationship between both submaximal and maximal laboratory measures and endurance performance (Joyner and Coyle 2008), such a relationship does not guarantee their efficacy as a means of prescribing training intensity. When exercising at 88 % $\dot{V}O_{2max}$, observed time to exhaustion (TTE) in cyclists can vary from 12 min to 75 min (Coyle *et al.* 1988), and large between-athlete variability in blood

lactate (BLa^{-1}) response (Coyle *et al.* 1988; Scharhag-Rosenberger *et al.* 2010), showing that differing levels of cellular and metabolic stress are present between individuals even when using a standardised method for prescribing exercise intensity. This raises serious questions about the suitability of prescribing training based on standardised percentages of maximal values (Hopker and Passfield 2014).

Effort-based training prescriptions have been used by coaches in the prescription of athletic training (Seiler and Hetlelid 2005; Seiler and Sjursen 2004; Stepto *et al.* 1999; Seiler *et al.* 2013; Sandbakk *et al.* 2013) and are implemented by instructing athletes to self-pace their exercise intensity according to a target level of perceived effort. This method has been utilised in training research, commonly being incorporated into high-intensity interval training by using a "*maximal session effort*" prescription, which involves participants regulating their exercise intensity so that when the exercise session is completed, maximal effort is achieved by the end (Seiler and Hetlelid 2005; Seiler *et al.* 2013; Seiler and Sjursen 2004). Previous research indicates that the between-athlete variability of performance and response in self-paced work bouts may vary in an intensity-dependent manner (Nicolò, Bazzucchi, Haxhi, *et al.* 2014; Seiler and Sylta 2017). Effort-based intensity prescriptions requires athletes to self-regulate their work rate to match a target perception of effort, allowing the athlete to adjust for the many factors that may influence their exercise performance on a given day; such as sleep, stress, wellness, or anxiety (Azevedo *et al.* 2021; Abbiss and Laursen 2008; Ungureanu *et al.* 2020; Azevedo *et al.* 2019; Millet 2011; Yoon *et al.* 2009). Gaining a further understanding of both the between-athlete and within-athlete variability surrounding the use of effort-based exercise intensity prescriptions will further facilitate its use in applied and research settings (Hopker and Passfield 2014).

Chapter 2 - Literature review

This review of the literature will explore the physiological adaptations and processes that occur following endurance training. The review will provide an overview of training structure and organisation, introducing high intensity interval training. Following this, the issue of individual variability in training response will then be explored, along with the proposed factors involved, examining specifically the inadequacy of existing standardised exercise intensity prescription methods. The use of effort-based intensity prescriptions will be reviewed as a potential method to address the individual variability in training response.

2.1 Physiological adaptations following endurance training

The training process involves the imposition of an activity that stresses homeostasis, followed by a period of recovery (Morton 1997). The initial impact of this stimulus will result in temporary fatigue if the stimulus is of sufficient duration, intensity, and frequency (Morton 1997; Budgett 1998). Following an exercise stimulus, a cascade of metabolic, respiratory, cardiovascular, endocrinal, and neuromuscular adaptations take place (Jones and Carter 2000). Adaptations such as these are crucial for the improvements observed in exercise performance and affect key parameters of endurance performance, such as exercise economy, $\dot{V}O_{2max}$ and the lactate threshold (Midgley, McNaughton and Jones 2007) and these are reviewed in the following section

Cardiorespiratory fitness is commonly assessed by the measurement of $\dot{V}O_{2max}$, which in turn is widely accepted as being representative of overall health and fitness, as it reflects the capacity of the pulmonary, cardiovascular, and neuromuscular systems during exercise (Blair 1996; Williams 2001; Jones and Poole 2005). $\dot{V}O_{2max}$ is the upper limit of maximal oxygen uptake achieved during high-intensity exercise (Joyner and Coyle 2008). $\dot{V}O_{2max}$ is one of the factors determining the maximal rate at which adenosine triphosphate (ATP) is resynthesised by primarily aerobic processes during exercise that lasts longer than a few seconds (Joyner 1991; Joyner 1993; Bassett and Howley 2000; Coyle 1995). Early research reported finding a linear relationship between exercise work rate and $\dot{V}O_2$, up until a work rate at which $\dot{V}O_2$ was observed to plateau and not increase any further (Hill and Lupton 1923). Following this discovery there were many investigations into the possible determinants of $\dot{V}O_{2max}$ (Hill and Lupton 1923; Bassett and Howley 2000; di Prampero 1985; di Prampero and Ferretti 1990; Wagner 1992; Wagner 1993), with the major factors being identified to be differences in maximal cardiac output and stroke

volume (Ekblom and Hermansen 1968), in addition to other factors such as blood oxygenation, muscular oxygen extraction, red blood cell mass and muscular blood flow (Bassett and Howley 2000; Kanstrup and Ekblom 1984; Rowell 1986; Dempsey 1986; Saltin and Strange 1992; Mitchell and Sproule 1958). With the adaptations leading to an increased $\dot{V}O_{2max}$ being well documented (Midgley, McNaughton and Jones 2007), $\dot{V}O_{2max}$ is commonly used as a reference point for prescribing exercise intensity (Bouchard, An, Rice, Skinner, *et al.* 1999; Midgley, McNaughton and Wilkinson 2006; Vollaard *et al.* 2009; Bacon *et al.* 2013; Gormley *et al.* 2008; Burgomaster *et al.* 2008; Howley, Bassett and Welch 1995; Ingham, Fudge and Pringle 2012).

It has been shown that the ability to maintain a high $\dot{V}O_2$ during exercise determines success in endurance competition (Joyner and Coyle 2008), and elite endurance athletes can record $\dot{V}O_{2max}$ values in the range of 70-85 $ml.kg^{-1}.min^{-1}$ for males, and 60-75 $ml.kg^{-1}.min^{-1}$ for females (Coyle *et al.* 1991; Lucia, Hoyos and Chicharro 2001; Lundby and Robach 2015; Lucía *et al.* 1998; Jones 2006). Interestingly, despite $\dot{V}O_{2max}$ displaying a strong correlation with performance levels in individuals across a range of fitness levels (Coyle *et al.* 1988; Vollaard *et al.* 2009), in highly trained athletes the relationship between performance and $\dot{V}O_{2max}$ is not as strong (Lucía *et al.* 1998; Lucia, Hoyos and Chicharro 2001; Jones 2006). Furthermore, it has been observed that in highly trained athletes there can be no relationship between changes in $\dot{V}O_{2max}$ and changes in performance (R^2 = .05; Vollaard *et al.* 2009; Costill *et al.* 1976). This indicates that there are other factors that determine endurance performance capability beyond $\dot{V}O_{2max}$ alone, such as the lactate threshold (LT) and exercise economy (Jones and Carter 2000).

In an acute bout of incremental exercise, the concentration of BLa^{-1} can be used to establish changes in metabolic substrate use (Midgley, McNaughton and Jones 2007). The extensive study of the BLa^{-1} response to exercise and the distinctive breakpoints that occur in the relationship between exercise intensity and BLa^{-1}, has led to numerous terms being used to describe them; LT (Ivy *et al.* 1980), anaerobic threshold (Heck *et al.* 1985), individual anaerobic threshold (Stegmann, Kindermann and Schnabel 1981), the onset of BLa^{-1} accumulation (Sjödin and Jacobs 1981), maximal lactate steady state (MLSS; Freund *et al*. 1986; Pringle and Jones 2002) and the lactate turn point (Davis *et al.* 1983). The first breakpoint that occurs in the relationship between BLa^{-1} and exercise intensity has been commonly referred to as the LT (Midgley, McNaughton and Jones 2007) and is associated

with the increase of BLa^{-1} from a steady baseline during incremental exercise. The most common criteria used to determine LT is a 1 $mmol.L^{-1}$ rise above baseline BLa^{-1} level, representing a marginal change in the accumulation of BLa^{-1} due to the increase in exercise intensity (Yoshida *et al.* 1987; Coyle *et al.* 1988; Jones and Carter 2000; Coyle *et al.* 1983). The secondary breakpoint, commonly referred to as the lactate turn point (LTP; Beneke 2003) is associated with a dramatic rise in BLa^{-1} (Hoefelmann *et al.* 2014), however, the main criteria used to establish LTP is greatly debated (Hoefelmann *et al.* 2014; Faude, Kindermann and Meyer 2009). Criteria have been presented for establishing LTP, such as a fixed concentration of 4 $mmol.L^{-1}$ (Sjödin and Jacobs 1981), 3.5 $mmol.L^{-1}$ (Heck *et al.* 1985), adding 1.5 $mmol.L^{-1}$ to the lowest ratio of lactate:workload (Coyle *et al.* 1983), or the "D_{max}" method involving the curvilinear relationship between BLa^{-1} and workload (Cheng *et al.* 1992). Despite this, the importance of the relationship between BLa^{-1} and exercise intensity on endurance performance is widely acknowledged. When exercising at 88 % $\dot{V}O_{2max}$ until exhaustion, athletes whose LT occurred at lower % of $\dot{V}O_{2max}$ (~66 % $\dot{V}O_{2max}$) reached exhaustion in almost half the time of those whose LT occurred higher relative to $\dot{V}O_{2max}$ (~82 % $\dot{V}O_{2max}$; ~ 29 min vs ~ 60 min; Coyle *et al.* 1988). This highlights the importance of the ability to sustain a high percentage of $\dot{V}O_{2max}$ at LT, and also indicates that this is a potential source of training-induced exercise performance improvements (Coyle *et al.* 1988).

Following a successful period of endurance training, a noticeable rightward shift of the lactate threshold can be observed, meaning higher intensities of exercise can be sustained and correspond with an improved LT and LTP. BLa^{-1} accumulation is dependent on the balance between the rate of lactate buffering from the muscle to the bloodstream and subsequent clearance from the blood (MacRae *et al.* 1992; Phillips *et al.* 1995). Exercise training results in reductions in BLa^{-1} accumulation at submaximal exercise intensities and also delays the occurrence of LT to higher absolute exercise intensity (Favier *et al.* 1986). An increased rate of lactate clearance from the muscle has been reported following endurance training (Donovan and Brooks 1982). The mechanisms behind these adaptations are thought to be mainly due to mitochondrial enzyme activity (Ivy *et al.* 1980; Holloszy and Coyle 1984; Coyle 1999; Coyle *et al.* 1985) and the increase in the levels of these enzymes along with the increase in size and number of mitochondria (Holloszy and Coyle 1984). Further improvements are likely due to the increase in the protein monocarboxylate transporter (MCT), as both lactate clearance capacity and the expression of MCTs in the muscle are improved after endurance training (Juel 2001). Within type I muscle fibres

MCT1 is the main MCT present, importing lactate into the muscle for oxidisation (McCullagh *et al.* 1996). This process is possible to be reversed, with MCT1 transporting lactate into the blood from the muscle, with this process is influenced by the concentration gradient of lactate across the sarcolemma (Bonen *et al.* 2000). Another MCT responsible for the handling of lactate is MCT4, predominantly located within fast-twitch muscle fibres (Wilson *et al.* 1998). Following endurance training, it has been shown that MCT1 concentration increases to a greater extent in comparison to MCT4 (Pilegaard, Terzis, *et al.* 1999; Pilegaard, Domino, *et al.* 1999). The observed increase in LT is likely to be due to the increased capacity to transport lactate out of the working muscle, in addition to the ability to uptake lactate, complementing lowered lactate production levels during exercise. To improve LT further following training, it is likely that a muscle fibre type transportation is required. In comparison to skeletal muscle containing high proportions of type I fibres, skeletal muscle containing high proportions of type II fibres displays approximately half the lactate transportation ability (Hawley and Stepto 2001). Following endurance training, hypertrophy of type I muscle fibres can occur, in addition to the transition of type IIb into type IIa fibres (Spina *et al.* 1996; Andersen and Henriksson 1977), and type IIa into type I in some cases (Simoneau *et al.* 1985; Sale *et al.* 1990).

A key adaptation to endurance exercise is the mitochondrial biogenesis that occurs (Irrcher *et al.* 2003; Wu *et al.* 2002), increasing mitochondrial content in the muscle, and enhancing endurance performance (Irrcher *et al.* 2003; Adhihetty *et al.* 2003). This increased mitochondrial content improves fatigue resistance and results in greater oxidative capacity (Hood *et al.* 2000), as displayed by an increased $\dot{V}O_{2max}$, LT, and exercise economy (Holloszy and Booth 1976; Sjödin, Jacobs and Svedenhag 1982; Hoppeler *et al.* 1985). Following endurance training, mitochondrial biogenesis is thought to change substrate use (Holloszy and Coyle 1984), decreasing carbohydrate utilisation rates and increasing fat utilisation at the same relative exercise intensity (Coggan *et al.* 1995). The sparing of muscle glycogen, occurring as a direct result of the alteration in substrate usage, has been shown to result in improved exercise performance (Hermansen, Hultman and Saltin 1967). Lowered reliance on carbohydrate oxidation and anaerobic metabolism would lead to lowered lactate production and consequentially an increase in LT, resulting in further improvements in performance (Holloszy and Coyle 1984). Alongside the alterations in substrate usage, higher levels of lactate oxidation as a result of increased levels of MCT1 may be a further result of mitochondrial biogenesis (Dubouchaud *et al.* 2000).

In addition to $\dot{V}O_{2max}$ and LT, the ability of an individual to effectively convert energy into work, also known as gross efficiency (GE) is a further key determinant of performance (Joyner and Coyle 2008; Gaesser and Brooks 1975). Generally, GE is commonly observed within a range of 18 – 23 % (Coyle *et al.* 1992; Hopker, Coleman and Wiles 2007; Moseley and Jeukendrup 2001; Moseley *et al.* 2004; Hopker *et al.* 2012; Nickleberry and Brooks 1996), and can be increased through training, although displaying an inverse relationship with $\dot{V}O_{2max}$ (Hopker *et al.* 2012; Hopker, Coleman and Passfield 2009; Hopker *et al.* 2010; Coyle 2005; Santalla, Naranjo and Terrados 2009). The exact determinants of GE remain to be fully elucidated, but muscle fibre type distribution, training status, and genetic factors may play key interacting roles (Jones 2006; Hopker *et al.* 2013; Holloszy *et al.* 1977). The observed positive relationship between GE and endurance training status (Hopker *et al.* 2013) may be likely due to muscle fibre type transformation from predominantly Type II to Type I, which has been shown to be associated with increased exercise economy (Dubouchaud *et al.* 2000). The alteration in fibre type is thought to result in lowered energy cost of contraction required to meet the necessary force requirement of exercise (Crow and Kushmerick 1982). This is further supported by the observation that Type I fibres display higher blood flow capacity and have a lower oxygen consumption than Type II fibres, and that Type I fibres are more abundant in the musculature of endurance-trained athletes (Joyner and Coyle 2008). However, despite the indication that muscle fibre type may be important, it has been observed that athlete training status influences GE to a greater extent than muscle fibre type (Hopker *et al.* 2013).

Professional cyclists are required to have high GE in order to sustain the high power outputs associated with successful performance in competition (Faria, Parker and Faria 2005b; Faria, Parker and Faria 2005a). It has been shown that GE is related to endurance performance in short time trials (5 min; $r = .48$) and longer performance trials (40 km and 1 h; $r = .58$; Jobson *et al.* 2012). Furthermore, a decrease in GE can be achieved following moderate-intensity exercise and is associated with reduced performance (Passfield and Doust 2000), which is thought to be due to progressively increasing levels of muscle oxygen consumption (O'Grady, Pageaux and Hopker 2014; Hopker, O'Grady and Pageaux 2017). It has been shown that training can improve GE, especially the completion of high-intensity interval training (HIIT; Hopker *et al.* 2010).

2.2 The training process

There are numerous ways in which training can be structured in order to improve performance involving the manipulation of frequency, intensity, and duration of training (Busso 2003; Esteve-Lanao *et al.* 2005; Hawley 2008). The goal of training is to elicit specific physiological stress in order to induce adaptations that are associated with improved performance (Borresen and Lambert 2009; Hawley and Burke 1998). However, the implementation of training is a complex process with many variations in training volume and intensity distribution possible (Seiler and Kjerland 2006) that in turn affect the resultant adaptive responses (Coffey and Hawley 2007). Training intensity has been shown as a key driver of improvement in $\dot{V}O_{2max}$ (Midgley, McNaughton and Wilkinson 2006; Bacon *et al.* 2013; Wenger and Bell 1986), leading to a wealth of investigations into the efficacy of varying distributions of training intensity for improving $\dot{V}O_{2max}$ (Tabata *et al.* 1996; Helgerud *et al.* 2007; Wisløff *et al.* 2007; Gormley *et al.* 2008; Neal, Hunter and Galloway 2011; Rodas *et al.* 2000). The following section will explore the interaction between training volume and training intensity, specifically low-intensity training, threshold training, HIIT, and the differences in associated training adaptations.

The traditional methods of improving endurance performance involve large volumes of exercise at a low intensity, typically equating to ~ 65 – 75 % of $\dot{V}O_{2max}$, < 2 mmol.L^{-1} BLa^{-1} or < 80 % maximal HR (HR_{max}; Laursen and Jenkins 2002; Seiler and Kjerland 2006). Over a period as short as 3 days, various metabolic and haemodynamic adaptations can be triggered following low-intensity training (Coyle 1999; Green *et al.* 1987; Green, Jones and Painter 1990). It is typical that further improvements in exercise performance can be obtained by increasing training volumes when using low-intensity training, as can be observed when completing 3 – 5 training sessions per week over a period of 3 – 5 weeks (Laursen and Jenkins 2002).

Following low-intensity training, the main physiological adaptations that occur are thought to be due to increased calcium-calmodulin kinase (CaMK) signalling pathway activity, due to increased peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) activation (Laursen and Jenkins 2002; Coffey and Hawley 2007). A cascade of cellular processes involved in mitochondrial biogenesis occurs following low-intensity training, beginning with increased cytosolic calcium concentrations resulting from increased

excitation-contraction coupling (Hood 2001). Elevated calcium concentration stimulates a number of kinases, including CaMK and phosphates such as calcineurin, triggering alterations in gene transcription (Hood 2001). In addition to these pathways relating to calcium concentrations, additional adaptations can also result from elevated ATP consumption rates, reduced ATP synthesis rates, as well as the associated adenosine monophosphate (AMP):ATP ratio increase. The AMP:ATP ratio has been shown to stimulate adenosine monophosphate activated protein kinase (AMPK; Hardie and Sakamoto 2006). Both calcium-related pathways and AMPK regulate the expression of PGC-1α.

The initiation of mitochondrial biogenesis is dependent on the metabolic fluctuations that occur during exercise, leading to a cascade of signalling responses, importantly CaMK and AMPK phosphorylation, leading to an upregulation of PGC-1α (Combes *et al.* 2015; Coffey and Hawley 2007; Jäger *et al.* 2007; Puigserver and Spiegelman 2003; Ojuka 2004). PGC-1α is widely recognised as holding a vital role of master regulator of mitochondrial biogenesis (Baar 2004; Puigserver and Spiegelman 2003), and it has been established that PGC-1α mRNA and proteins are increased following endurance training (Taylor *et al.* 2005). PGC-1α targets adaptations as a result of its interactions with several DNA binding transcription factors and the coordination of numerous biochemical events, such as recruiting of chromatin modifying enzymes (p300/CBP and SRC-1; Puigserver *et al.* 1999), triggering of basal transcription mechanisms (Wallberg *et al.* 2003), and connecting transcriptional signals to RNA splicing (Monsalve *et al.* 2000). Despite these involvements, it appears that in PGC-1α knockout mice other factors can exert training-induced adaptations (Leick *et al.* 2008). However, a blunted expression of genes and protein expression involved in oxidative metabolism is observed in PGC-1α knockout mice (Arany *et al.* 2005; Handschin and Spiegelman 2008; Leick *et al.* 2008), indicating that PGC-1α is still vital for both the adaptive response following exercise and responses that occur during endurance exercise.

Low-intensity exercise training has been shown to result in important adaptations, such as increases stroke volume and blood plasma, as well as mitochondrial biogenesis and capillarisation (Midgley, McNaughton and Wilkinson 2006; Romijn *et al.* 1993). Higher rates of glucose and fat utilisation have been reported in response to high volumes of low-intensity training, increasing exercise performance in endurance events (Romijn *et al.*

1993). Greater utilisation of fat and glucose would result in greater aerobic energy generation capacity and would likely reduce required recovery time following anaerobic energy production by improving oxidative flux (Stoggl and Sperlich 2014). One of the potential triggers of mitochondrial biogenesis is thought to be related to the higher relative fat oxidation rates that occur when training at low exercise intensities (Holloszy 2008). However, if an athlete's training consists of solely low-intensity training without progression, it is unlikely that $\dot{V}O_{2max}$ and LT would continue to improve over a longitudinal period, leading to a plateau in training response. This observed plateau in progression has been observed in well-trained athletes if no training intensities above the LT are completed (Costill *et al.* 1988; Laursen and Jenkins 2002; Stoggl and Sperlich 2014). Therefore, it is important that training prescription involves a mix of low and high-intensity training into athletes' regimes.

Initially designed as a method of completing targeted exercise training at or near intensities corresponding to an athlete's LT, threshold training (Seiler and Kjerland 2006) is a robust method for increasing exercise performance (Denis, Dormois and Lacour 1984; Gaskill, Walker, *et al.* 2001; Kindermann, Simon and Keul 1979; Londeree 1997). Threshold training has sometimes been the training modality that is favoured in several world-class cross-country skiers from Norway specialise in shorter duration sprint events (Sandbakk *et al.* 2011). It has also been shown that in elite cross-country skiers, running speed at LT and performance in a timed run was improved to a greater extent following training that results in BLa^{-1} concentrations of 3 – 4 $mmol.L^{-1}$ in comparison with low-intensity training at < 3 - 4 $mmol.L^{-1}$ (Evertsen, Medbø and Bonen 2001). However, there is some notion that threshold training alone for well-trained athletes could be ineffective or possibly even counter-productive to endurance exercise performance (Esteve-Lanao *et al.* 2005; Guellich and Seiler 2010). However, threshold training remains a valuable addition when included in small doses in conjunction with other training modalities (Seiler and Kjerland 2006).

In order to increase endurance performance to greater levels than those achieved when using solely low-intensity training, HIIT can be conducted; with the potential to observe improvements in both untrained and trained individuals (Laursen and Jenkins 2002; Wenger and Bell 1986; Bacon *et al.* 2013). The ability produce high levels of physiological stress over a short period of time demonstrates the potency of HIIT as a training intervention (Burgomaster *et al.* 2008; Gibala *et al.* 2006). Following HIIT, it has been

observed that endurance performance increases due to improvements in $\dot{V}O_{2max}$ (Burgomaster *et al.* 2008; Daussin *et al.* 2007; Daussin *et al.* 2008; Gibala *et al.* 2006; Laursen and Jenkins 2002; Midgley, McNaughton and Wilkinson 2006; Rønnestad, Hansen, *et al.* 2014) lactate and ventilatory thresholds (Acevedo and Goldfarb 1989; Edge *et al.* 2005), and both time trial (TT), and TTE performance (Lindsay and Hawley 1996). HIIT serves to trigger adaptations responsible for improving performance primarily due to the activation of AMPK and the resultant expression of PGC-1α (Laursen 2010). These adaptations are predominantly involved in allowing greater availability and extraction of oxygen, alongside increases in $\dot{V}O_{2max}$ (Daussin *et al.* 2007; Helgerud *et al.* 2007). Further adaptations are centred around improvements in aerobic and anaerobic metabolism (MacDougall *et al.* 1998) by means of increasing oxidative capacity and mitochondrial biogenesis (Burgomaster *et al.* 2008; Daussin *et al.* 2007; Daussin *et al.* 2008; Gibala *et al.* 2006), as well as improvements in stroke volume (Helgerud *et al.* 2007; Wisløff *et al.* 2007), blood volume (Shepley *et al.* 1992), and oxygen extraction (Daussin *et al.* 2007).

HIIT commonly incorporates intermittent bouts of longer (~2 – 6 min) or shorter (~ 30 – 60 sec) exercise bouts, completed at intensities at, or near $\dot{V}O_{2max}$ (e.g. > 90 % of $\dot{V}O_{2max}$; Gibala *et al.* 2006), and interspersed with periods of active or passive recovery (Burgomaster *et al.* 2005; Tabata *et al.* 1996). The potency of HIIT can result in significant increases in $\dot{V}O_{2max}$ following interventions ranging from 2 to 6 weeks in duration (Poole and Gaesser 1985; Tabata *et al.* 1996; Rodas *et al.* 2000; Edge *et al.* 2005; Daussin *et al.* 2007; Cunningham, McCrimmon and Vlach 1979; Driller *et al.* 2009). In addition, HIIT is effective at improving exercise performance in both untrained (Holloszy *et al.* 1977; Rodas *et al.* 2000; Gormley *et al.* 2008) and trained individuals (Laursen and Jenkins 2002). The duration of the work interval during HIIT is a key factor when programming training. Following an investigation into the differences between HIIT using 2 min and 30 sec periods, it was found that both groups increased their performance and physiological variables by a similar extent (~ 3 – 8 %; Laursen *et al.* 2002; Laursen *et al.* 2005). The interest in the impact of interval duration led to extensive discussion around the training protocols, which would result in the greatest accumulated time exercising above 90 % $\dot{V}O_{2max}$ (Buchheit and Laursen 2013; Laursen 2010; Laursen and Jenkins 2002; Midgley, McNaughton and Wilkinson 2006). It has been shown that shorter maximal intervals, such as ~ 30 sec, can result in superior training adaptations when compared with longer intervals, such as ~ 5 min which are also performed to the limit of tolerance (Rønnestad, Hansen, *et al.* 2014; Rønnestad *et al.* 2021). Furthermore, a key interaction is present

between HIIT interval duration and intensity with the accumulated workloads differing between prescriptions, but with varying outcomes observed. It has been shown that accumulating a duration of ~10 – 15 min at ~ 95 % HR_{max} is more effective at improving $\dot{V}O_{2max}$ than ~25 min at ~ 85 % HR_{max} (Helgerud *et al.* 2007). However, in contrast to this, it has also been shown that accumulating ~ 30 – 45 min at ~ 90 % HR_{max} is more effective at improving $\dot{V}O_{2max}$ than ~15 – 20 min at ~ 95 % HR_{max} (Seiler *et al.* 2013; Sandbakk *et al.* 2013).

A key factor in HIIT training relates to the ratio between work and recovery periods, with differences in this ratio resulting in varying neuromuscular and metabolic responses. Primarily, the intermittent nature of HIIT allows greater time to be spent at high levels of $\dot{V}O_{2max}$ when compared to continuous exercise (Billat 2001; Seiler *et al.* 2013). Furthermore, the transitions from work to rest result in a greater occurrence of $\dot{V}O_2$ fluctuations, thought to be one of the most important mechanisms of the improvements which occur following HIIT training (Cochran *et al.* 2014; Combes *et al.* 2017; Edge *et al.* 2013; Tucker *et al.* 2015). A comparison between HIIT programs using a ratio of 40:20 sec, 30:30 sec, and 20:40 sec demonstrated that 40:20 sec resulted in higher HR, minute ventilation (V_E), $\dot{V}O_2$, and BLa^{-1} than the other two protocols (Ballor and Volovsek 1992). It has also been demonstrated that compared to a work:rest ratio of 30:30 sec, a ratio of 40:20 sec results in longer periods of elevated BLa^{-1}, $\dot{V}O_2$, and other ventilatory parameters when completed at the same absolute work intensity (Nicolò, Bazzucchi, Lenti, *et al.* 2014). Interestingly, (Ballor and Volovsek 1992) utilised fixed absolute work intensities and overall durations, meaning that the 40:20 sec protocol was more demanding than the 30:30 and 20:40 sec protocols. Similarly, (Nicolò, Bazzucchi, Lenti, *et al.* 2014) utilised fixed absolute work intensities but prolonged the duration of the exercise protocol to exhaustion, resulting in considerably longer exercise duration in the 30:30 sec protocol compared to 40:20 sec. When both effort and exercise duration is controlled using an effort-based intensity prescription, similar physiological responses are observed between protocols using work:rest ratios of 40:20 sec, 30:30 sec, and 20:40 sec. Furthermore, it has been recently demonstrated that passive recovery allows participants to complete higher work bout power outputs with a lower overall session effort (Fennell and Hopker 2021). The above indicates that overall effort is a key parameter to control when comparing HIIT interventions, along with the total duration of work.

Summary

The organisational structure of training, involving the distribution of accumulated training time across various exercise intensities clearly can initiate a vast array of adaptive responses to improve endurance performance. The molecular response to training can involve the activation of both AMPK as a result of HIIT training, as well as CaMK from large volumes of low-intensity training (Laursen 2010). The adaptations that occur in an intensity-dependent manner influence a range of performance-related measures, such as increased numbers of type I muscle fibres, mitochondrial biogenesis, improved fat oxidation ability, and increases in glucose transportability (Coffey and Hawley 2007). Mixing exercise intensities has been shown to induce varying levels of cellular stress, increasing expression of signalling molecules which have similar downstream adaptations (Baar 2006), and therefore observed performance improvement is likely to be a product of the synergy of both intensity and duration (Hawley and Stepto 2001). However, there are often inconsistencies in the findings of exercise training studies, with some studies demonstrating HIIT results in greater improvements versus moderate-intensity training (Helgerud *et al.* 2007; Wisløff *et al.* 2007; Gormley *et al.* 2008), whilst others show similar levels of effectiveness between modalities (Burgomaster *et al.* 2008; Gibala *et al.* 2006).

2.3 Individual response to training

The most common reporting method throughout the literature on training studies is for group training effects to be reported as the mean response and standard deviation (SD). Interestingly, within normally distributed data sets, 32 % of training response measurements are greater than 1 SD away from the mean response (Cohen and Holliday 1979). This could indicate the presence of a large amount of between-athlete variability observed within traditional training studies if the observed SD is large. From a clinical perspective, between-athlete variability in exercise training response leads to a level of uncertainty following rehabilitative programs and reducing the occurrence of adverse or non-response to training (Bouchard, An, Rice, Skinner, *et al.* 1999; Bouchard, Blair, *et al.* 2012; Boule, Weisnagel, *et al.* 2005; Scharhag-Rosenberger *et al.* 2012; Vollaard *et al.* 2009; Timmons *et al.* 2005).

Between-athlete variability is a phenomenon that has been observed in various parameters, such as $\dot{V}O_{2max}$ (Hamel *et al.* 1986; Bouchard and Rankinen 2001; Scharhag-Rosenberger

et al. 2012; Kohrt, Malley, Coggan, *et al.* 1991; Hautala *et al.* 2003; Hautala *et al.* 2006; Sisson, Katzmarzyk, Earnest, *et al.* 2009; Vollaard *et al.* 2009; McPhee *et al.* 2010; Karavirta *et al.* 2011; Hautala *et al.* 2012), aerobic threshold (Prud'homme, Bouchard and Leblanc 1984), anaerobic threshold (Scharhag-Rosenberger *et al.* 2012; Prud'homme, Bouchard and Leblanc 1984), TT performance (Vollaard *et al.* 2009), resting HR (Scharhag-Rosenberger *et al.* 2012), exercise HR (Bouchard and Rankinen 2001; Scharhag-Rosenberger *et al.* 2012), resting muscle glycogen (Vollaard *et al.* 2009), muscle enzyme activity (Hamel *et al.* 1986; Vollaard *et al.* 2009; McPhee *et al.* 2011), and systolic blood pressure during exercise (Bouchard and Rankinen 2001). As many potential factors influence individual variation in response to exercise training, isolating exact training responses in individuals becomes more of a challenge (Hecksteden *et al.* 2015). Variation in these parameters complicates our understanding of the physiological response to training in that a high-responding individual in one parameter may display a low response in another parameter (Vollaard *et al.* 2009; Scharhag-Rosenberger *et al.* 2012). This has led to the adoption of the notion of individual responsiveness to training (Bouchard and Rankinen 2001; King *et al.* 2008; Mann, Lamberts and Lambert 2014).

Research into the sources of variability in training response was highlighted by the findings of a research consortium between five laboratories, with studies involving involved over 40 African American and 90 Caucasian families, termed the HERITAGE Family study (Bouchard, Leon, *et al.* 1995). The investigation required participants to follow an endurance training program over 20 weeks using a standardised intensity prescription of a progressive build in $\%\dot{V}O_{2max}$ within sessions from 55 % to 75 % and examined the resultant metabolic and cardiovascular responses. With entire families being recruited, the influence of genetics on training adaptation was explored by looking at familial aggregation (Bouchard, An, Rice, Skinner, *et al.* 1999; Perusse *et al.* 2001; Rice *et al.* 2001; Rice *et al.* 2002; Rico-Sanz *et al.* 2003), major gene effects (An *et al.* 2003; An *et al.* 2005), heritability (Gaskill, Rice, *et al.* 2001; Bouchard, An, Rice, Skinner, *et al.* 1999; Perusse *et al.* 2001; Rice *et al.* 2001; Rice *et al.* 2002), and specific polymorphisms (Bouchard, Sarzynski, *et al.* 2011; Rankinen *et al.* 2012). Heritability was found to explain up to 50 % of the observed variance in $\dot{V}O_{2max}$ improvements following training (Bouchard *et al.* 1998), up to 59 % of the observed variance in HR when exercising at 50 W prior to a training intervention (An *et al.* 2002), and up to 58 % of the variance in $\dot{V}O_2$ at VT_1 (Gaskill, Rice, *et al.* 2001). Familial aggregation was found to be present in the maximal enzyme activity in the untrained state of the oxidative, glycolytic, and phosphagen

pathways (Rico-Sanz *et al.* 2003). It was also observed that variance in 21 single nucleotide polymorphisms could explain approximately 50 % of the observed variance in $\dot{V}O_{2max}$ improvements following training (Bouchard, Sarzynski, *et al.* 2011), and a further 9 single nucleotide polymorphisms could explain approximately 36 % of the observed variance in HR when exercising at 50 W (Rankinen *et al.* 2012). Following the findings of the HERITAGE Family study, it was concluded that hereditary influence contributes greater towards the phenotype of the individual before training, rather than the magnitude of the training response itself (Bouchard *et al.* 1998; Gaskill, Rice, *et al.* 2001; An *et al.* 2002). As well as the apparent genetic basis, there are many other factors present which could influence an individual's training response, such as lifestyle factors, nutrition, and training program characteristics (Mann, Lamberts and Lambert 2014). It is well known that training-related improvements are greatly dependent on the relative stress that is exerted on the individual during training (Coyle *et al.* 1988; Hecksteden *et al.* 2015). Expanding our knowledge surrounding both within-athlete variability and between-athlete variability in exercise response can help researchers and practitioners prescribe more effective training interventions (Chrzanowski-Smith *et al.* 2020).

The training-related interaction factors such as exercise intensity, duration, and interval format exert a specific level of cellular stress, presenting a stimulus for adaptive processes to initiate. The manner in which exercise intensity is prescribed during research into training has predominantly involved standardised percentages of HR reserve (HRR), maximal minute power (MMP), HR_{max}, or $\dot{V}O_{2max}$. While these methods are easy to replicate, they open the possibility of introducing between-athlete variability as a result of differing levels of cellular stress incurred due to their reliance on a measure of maximal work rate or capacity (McPhee *et al.* 2010). It has been shown that individuals can adapt to training at low or moderate intensities, and non-response can be minimised by increasing the exercise dose (Montero and Lundby 2017). Intensity prescriptions which are relative to maximal capacity or work rates have also received criticism around the observed variation in time to exhaustion (Scharhag-Rosenberger *et al.* 2010) and BLa^{-1} response (Dwyer and Bybee 1983; Meyer, Gabriel and Kindermann 1999; Scharhag-Rosenberger *et al.* 2010) when exercising at fixed percentages of maximal parameters. A clear example of varying metabolic stress between individuals at the same standardised percentage of maximal capacity is the observed variability in time to exhaustion between 12 – 75 min when exercising at 88 % $\dot{V}O_{2max}$ (Coyle *et al.* 1988). This would be a clear indication that the standardised relative intensities result in different individuals exercising above or below

their individual LT. Similar findings were also observed when exercising until exhaustion at the intensity corresponding to critical power (CP), with time to exhaustion varying from between 20 – 40 min (Brickley, Doust and Williams 2002). These observations clearly suggest that the level of metabolic stress differs between individuals, rendering the prescription method ineffective at prescribing training intensity on an individual basis (Dwyer and Bybee 1983; Meyer, Gabriel and Kindermann 1999; Scharhag-Rosenberger *et al.* 2010; Vollaard *et al.* 2009; Katch *et al.* 1978). Varying levels of cellular stress at standardised percentages of maximal parameters would indicate that the position of training intensity relative to an individuals' threshold(s) could affect the signalling and adaptive processes that occur, thus presenting a potential source of exercise training variability (Gaskill, Walker, *et al.* 2001).

While it is appropriate to conclude that a proportion of variation in training adaptive response can be attributed to genetic factors, it is important to acknowledge that the prescription of relative exercise intensity is also influential. It is apparent that the between-athlete variation in cellular stress incurred by standardised training intensities could account for a significant proportion of between-athlete variability in the adaptive training response (Mann, Lamberts and Lambert 2014). In order to attenuate this variation due to training at standardised percentages of maximal parameters, it is necessary to develop a more individualised approach to training intensity prescription, which results in consistent training stress between individuals.

Summary

High levels of between-athlete variability have been a factor associated with response to standardised training prescriptions, which are frequently utilised for research purposes. While some of this variability can be explained by factors such as pre-training phenotype and hereditary factors, a significant proportion of the variability is likely to arise as a result of inconsistent levels of cellular stress between individuals during training. To address this, prescribed exercise intensities should transition from previous standardised approaches to more individualised methods of prescription.

2.4 Individualised training intensity prescription

Methods of individualising training intensity seek to elicit equivalent levels of training stress across individuals who display varying levels of physiological and functional capacity (Mann, Lamberts and Lambert 2013; Jamnick *et al.* 2020). The previous section outlined traditional approaches to prescribing exercise training, the utilisation of percentages of $\dot{V}O_{2max}$, HR_{max}, or MMP, all of which remain common methodologies within contemporary research (Sedlock *et al.* 2010; Killgore *et al.* 2010; Ferguson-Stegall *et al.* 2011; Van Proeyen *et al.* 2011; McPhee *et al.* 2011; Nordsborg *et al.* 2010), regardless of acknowledged variation (Meyer, Gabriel and Kindermann 1999; Scharhag-Rosenberger *et al.* 2010). Prescribing individualised exercise intensities can be achieved through several different methodologies, each with varying levels of efficacy and distinct challenges. The section below will describe some common methods of prescribing exercise intensity in a more individualised manner versus standardised percentages of maximal capacity, such as prescription relative to threshold, prescription using the individual's power-duration relationship, and effort-based prescriptions.

2.4.1 Prescription relative to thresholds

It follows a logical argument that if heterogeneous training outcomes are observed when utilising fixed percentages of maximal values, then prescribing relative to each individuals threshold may improve the homogeneity of response (Mann, Lamberts and Lambert 2013). However, there is a paucity of such prescriptions in the research literature that specifically consider individual variability in training response despite the above rationale.

An early study that addressed the variability in training response sought to identify the variability which occurs following training completed with intensity prescriptions based on the aerobic threshold compared to prescriptions at standardised percentages of $\dot{V}O_{2max}$ (McLellan and Skinner 1981). Whilst the findings of this study showed significantly greater improvements in $\dot{V}O_{2max}$ when training was prescribed using percentages of threshold versus $\%\dot{V}O_{2max}$ (38.3 to 47.2, and 36.9 to 43.6, respectively), no differences in between-athlete variability in training response were found between the two prescription methods. It is possible that the absence of differences in between-athlete variability could be due to small group sizes ($\%\dot{V}O_{2max}$; n = 6, % aerobic threshold; n = 8), or the attempt to match training intensity resulting in inadvertent manipulation in training loads across both

groups. However, despite utilising intensities prescribed relative to individual thresholds, there was no reduction in training response variability (McLellan and Skinner 1981). Further studies have sought to prescribe exercise intensities in an individual manner relative to thresholds, with the aim of reducing the level of variability in $\dot{V}O_{2max}$ change following training (Karavirta *et al.* 2011). Participants completed a 21-week combined strength and endurance training program using, where for the first 4 weeks they exercised at HR levels below aerobic threshold, from weeks 5 – 7 participants began to incorporate HR levels above aerobic threshold, weeks 8 – 14 continued to incorporate HR levels above the aerobic threshold in addition to HR levels above the anaerobic threshold. Finally, in weeks 15 – 21 training progressed by accumulating time at HR levels above the aerobic and anaerobic thresholds (Karavirta *et al.* 2011). Despite the individualised intensities relative to threshold, there was still a large between-athlete variability with the change in $\dot{V}O_{2max}$ ($\Delta\dot{V}O_{2max}$) ranging from -8 % to 42 %. This finding is of interest, as prescribing exercise intensity relative to each individual's thresholds should homogenise the level of metabolic and cellular stress during training bouts (Faude, Kindermann and Meyer 2009; Mann, Lamberts and Lambert 2013). However, a potential limitation relates to the wording of the prescription methods utilised for prescribing intensity, specifically: "*above*", "*below*", and "*between*" aerobic and anaerobic thresholds. With these criteria, it may be that the prescription was not precise enough to adequately standardise the level of stress across individuals, as the difference in metabolic stress would differ greatly between aerobic and anaerobic threshold, despite all being within the same prescription level in the study (Karavirta *et al.* 2011). In addition, the prescription method is based on HR, which often results in variability of physiological response between individuals (Meyer, Gabriel and Kindermann 1999; Hofmann *et al.* 2001).

The ability of threshold-based training prescriptions to reduce between-athlete variability in training response has been further explored, with individualised prescription being based around each individual's first and second ventilatory threshold (VT_1, VT_2, respectively), and standardised prescriptions based around set %HRR (Wolpern *et al.* 2015). The findings of this study showed increases in $\dot{V}O_{2max}$ in both %HRR and individualised prescription method groups (34.9 to 36.6, and 34.3 to 38.3, respectively; Wolpern *et al.* 2015). However, despite both groups increasing $\dot{V}O_{2max}$ based on group averages, it was observed that only 41.7 % of individuals displayed favourable increases in $\dot{V}O_{2max}$ (defined as $\Delta\dot{V}O_{2max} > 5.9\%$) when using %HRR, compared to 100 % of individuals when training was prescribed relative to VT_1 and VT_2. From presented data on the adherence to training

prescriptions, it was shown that both groups adhered to their relative exercise prescriptions, but the HR values recorded during exercise within the training group using prescriptions relative to VT_1 and VT_2 were noticeably higher than the %HRR group (139 bpm [76 % HR_{max}], versus 118 bpm [65 % HR_{max}], respectively; Wolpern *et al.* 2015). A marginally smaller standard deviation was observed in actual HR values between the training group using prescriptions relative to VT_1 and VT_2 and the %HRR group (± 15 bpm, versus ± 12 bpm, respectively), potentially indicating a more homogenous exercise intensity prescription. The above findings indicate that it may be possible to individualise exercise intensity, resulting in more homogenous levels of exercise-induced cellular and metabolic stress and the resulting training adaptations (Buford, Roberts and Church 2014; Mann, Lamberts and Lambert 2014; Mann, Lamberts and Lambert 2013).

2.4.2 Mathematically modelled training intensities

Over a century ago, (Kennelly 1906) performed an analysis of human and animal exercise performance records and outlined a distinct relationship between exercise intensity and time. This work was further extended when (Hill 1925) expanded on the relationship between exercise intensity and time using running world records. The above studies identified that as intensity increases, sustainable duration decreases, and vice versa, adopting a curvilinear relationship between exercise intensity and time. The resultant interest in the physiological underpinnings of the relationship between exercise intensity and duration led to models being produced using an athlete's best performances, with the aim of predicting performance for varying durations. A mathematical framework was developed from the observation of the function of various muscle groups and individual muscles, which sought to describe the relationship surrounding how muscle groups fatigue during exercise at varying intensities, which became termed the Critical Power (CP) model (Monod and Scherrer 1965; Pringle and Jones 2002; Morton 2006; Jones *et al.* 2010; Moritani *et al.* 1981). The application of the CP model assumes that the relationship between exercise intensity and time to exhaustion is hyperbolic and represents the upper limit of a sustainable workload (Poole and Gaesser 1985). The CP model breaks down the relationship between intensity and duration and calculates estimates of aerobic and anaerobic parameters of performance, named CP and W', respectively (Monod and Scherrer 1965; Pringle and Jones 2002; Morton 2006; Jones *et al.* 2010; Moritani *et al.* 1981). In order to generate the CP model, a series of time to exhaustion trials within the severe exercise intensity domain are completed (Hill 1993; Moritani *et al.* 1981; Morton

2006; Mattioni Maturana *et al.* 2018), which can be performed on separate days (Gaesser and Wilson 1988; Poole, Ward and Whipp 1990), or with at least 30 min rest separating each trial (Housh, Housh and Bauge 1989; Galbraith *et al.* 2014; Karsten *et al.* 2015). A 3 min all-out exercise test can be used to accurately establish CP but underestimates the W' and is unable to produce the hyperbolic relationship between exercise intensity and duration (Vanhatalo, Doust and Burnley 2007).

The CP model can be useful for identifying training prescriptions as the CP represents the boundary between the exercise intensity domains classified as "*heavy*" and "*severe*" (Vanhatalo, Jones and Burnley 2011). This allows athletes to not only prescribe exercise training intensities based on the different physiological responses in each exercise intensity domain (Black *et al.* 2017), but also establish the exercise intensities at which exercise becomes "*non-steady-state*" which results in fatigue occurring at a more rapid pace (Vanhatalo, Jones and Burnley 2011). Despite the advantages of the application of the CP model, there are some notable limitations. The CP model relies on only two components of human energy supply during exercise; the aerobic domain, which is assumed to be unlimited in capacity and limited in energy contribution rate, and the anaerobic domain, which is limited in capacity but not rate-limited, possibly indicating a reductionist approach of explaining exercise performance and fatigue (Morton 2006). Furthermore, the CP model has been found to be limited in its ability to describe and predict performance of durations between 2 and 20 min (Hill 1993; Dekerle, Vanhatalo and Burnley 2008). In addition to this, it is also assumed based on the hyperbolic relationship between exercise intensity and duration that exercise can be maintained indefinitely at or below CP, as well as the ability to produce an infinite amount of power as time approaches zero (Hill 1993; Jones *et al.* 2010).

Despite extensive research being conducted exploring CP's physiological significance, it remains unclear how CP relates to other established thresholds such as the heavy and severe exercise domain boundary (Bull *et al.* 2008), AT (Moritani *et al.* 1981; Poole *et al.* 1988), LT (Housh, Devries, *et al.* 1991b), and $\dot{V}O_{2max}$ (Housh, Johnson, *et al.* 1991a; Hopkins *et al.* 1989; Pepper, Housh and Johnson 1992). This debate has given light to the notion of interpreting the relationship between exercise intensity and time purely as a mathematical model of performance with no physiological assumptions being made (García-Manso *et al.* 2012). The power law (PL) relationship, or log-log model, was first

observed in the velocity/distance relationship for various athletic and horse racing events, showing that performance capacity progressively declines as duration or intensity increases (Kennelly 1906; Grubb 1998). The most high-profile use of a PL model has been to estimate and predict performances at world record pace in athletics (Kennelly 1906; Katz and Katz 1999; Savaglio and Carbone 2000), with swimming performances also being predicted accurately within a narrow range of durations (Hinckson and Hopkins 2005; Osiecki *et al.* 2014). CP and PL models have been used in attempts to individualise exercise training prescriptions (García-Manso *et al.* 2012). The PL model has been successfully applied to intermittent exercise in cycling (Chidnok *et al.* 2012), however, the model was unable to account for the curvilinear response of W' reconstitution during recovery periods (Ferguson *et al.* 2010). In response to this, a model of W' reconstitution during intermittent exercise was developed by (Skiba *et al.* 2012), which was also further developed based on different work and recovery durations (Skiba *et al.* 2014). While it can be observed that CP and PL models can provide reliable and non-invasive methods of prescribing individualised training intensities (García-Manso *et al.* 2012), the time consuming and intensive testing required to generate the models limits the effectiveness of incorporation into training prescription (Karsten *et al.* 2017). The CP and PL method could also be limited in the range of exercise intensity prescription wider than the boundary between the heavy and severe exercise domains. Furthermore, training that is prescribed using CP and PL models remain potentially susceptible to day-by-day alterations in an individuals' exercise capacity.

2.4.3 Effort-matched training intensities

The psycho-physical occurrence of perception of effort has been defined as the intensity of subjective effort, stress, discomfort, and fatigue which is felt during exercise or physical activity (Noble and Robertson 1996; Eston 2012), and has been extensively studied in an exercise training setting (Borg and Linderholm 2009; Borg 1982a; Ekblom and Golobarg 1971; Mihevic 1981; Borg 1970; Myles and Maclean 1986; Eston and Williams 1988; Dunbar *et al.* 1994; Stephen Seiler and Hetlelid 2005; Green *et al.* 2009; Halperin and Emanuel 2019). Measuring perception of effort commonly involves the use of the rating of perceived exertion (RPE) scale, with perceived effort being defined as "*the feeling of how heavy and strenuous a physical task is*" (p. 8; Borg 1998). The RPE scale involves an individual rating their perceived effort on a visual analogue 6 – 20 scale with 6 representing "*no exertion at all*", and 20 representing "*maximal exertion*" along with the

instructions that "*perception of exertion depends mainly on the strain and fatigue in your muscles and on your feeling of breathlessness or aches in the chest*" (p. 47; Borg 1998). Varying perspectives on perception of effort have been presented over the years, such as the central governor model (St Clair Gibson 2004), and the psychobiological model (Marcora 2008; Marcora, Bosio and de Morree 2008), with the latter model defining perception of effort as the "*conscious sensation of how heavy, and strenuous a physical task is*" (Marcora 2008). Furthermore, it is important to differentiate effort and exertion, which are commonly utilised interchangeably. Effort has been defined as "*the amount of mental or physical energy being given to a task*", with a clear focus on the psychological investment into a task, differentiating itself from Borg's definition of RPE, which remains focused on the physical aspects of the task (Abbiss *et al.* 2015). Despite this, there remains confusion within the research surrounding effort and exertion, with recent calls for fewer definitions, terms, and instructions to be used (Halperin and Emanuel 2019).

The most common use of the RPE scale is as a tool to measure a subjective response to a given workload, but it can also be used as a tool to prescribe exercise intensity in '*production'* mode (Borg 1998) as a method to regulate exercise (Helms *et al.* 2018; Parfitt, Evans and Eston 2012; Graham and Cleather 2019; Myles and Maclean 1986). The production mode uses the RPE scale as a framework with which exercising individuals can use to regulate their exercise work rate or resistance (Borg 1998; American College of Sports Medicine 2014; Noble and Robertson 1996), in order to manage the development of fatigue (Azevedo *et al.* 2021). As the production mode prescription method requires only the presence of an RPE scale, it has been studied in a wide range of populations, including clinical, active, untrained (Eston *et al.* 2006; Eston and Williams 1988; Coquart *et al.* 2016; Noble and Robertson 1996; Dunbar *et al.* 1998; Noble 1982; Robertson 2001; Hartshorn and Lamb 2004), and trained athletes (Perrey *et al.* 2003; Schallig *et al.* 2017; Garcin, Danel and Billat 2008; Groslambert *et al.* 2004). In addition to using perceptually regulated intensity prescription for individual bouts of exercise, effort-based training prescriptions have been used by coaches in the prescription of whole session intensity by instructing athletes to self-pace their exercise intensity throughout a training session according to the desired level of perceived effort (Seiler and Hetlelid 2005; Seiler and Sjursen 2004; Stepto *et al.* 1999; Seiler *et al.* 2013; Sandbakk *et al.* 2013). Commonly, this method is incorporated into HIIT training by using a '*maximal session effort*' prescription, which involves participants regulating their exercise intensity in order to complete exercise session with maximal effort being achieved by the end of the exercise (Seiler and Hetlelid

2005; Seiler *et al.* 2013; Seiler and Sjursen 2004). Participants can also evaluate the subjective load of an entire training session using Foster's session-RPE (sRPE; Foster *et al.* 2001). The widely used sRPE method incorporates both the intensity and duration of the exercise session and represents the subjective 'mean' exertion felt by the participant and has been shown to be both reliable and valid (Haddad *et al.* 2017).

Insights into effort-based training may indicate that the variability of performance and response vary in an intensity-dependent manner, with higher individual variability in power output observed as intensity increases (Nicolò, Bazzucchi, Haxhi, *et al.* 2014; Seiler and Sylta 2017). Despite power output variability increasing as work phase intensity increases during short intermittent exercise, BLa^{-1} response variability was observed to decrease (Nicolò, Bazzucchi, Haxhi, *et al.* 2014; Nicolò, Bazzucchi, Lenti, *et al.* 2014), highlighting that high variability in one measure may result in lower variability in another measure. As effort-based prescriptions require that athletes self-pace their exercise in accordance with session goals, this may help to ensure consistency in between-protocol exercises intensity to manage the associated physiological perturbations (Seiler and Sjursen 2004a). The influence of various life and environmental stressors affect the body and brain via the autonomic, neuroendocrine, and immune systems (McEwen 2007; Ganzel, Morris and Wethington 2010). When performing self-paced exercise, the athletes may be more aware of the alterations in factors that influence pre-training fatigue, such as stress, sleep, and environmental factors (Gallo *et al.* 2016). In support of this, it has been shown that following two weeks of high-intensity interval training using peak power output as a fixed intensity target, athletes with positive training responses display consistent levels of life stressors, whereas athletes who did not adapt positively to the training intervention reported higher levels of life stressors (Capostagno, Lambert and Lamberts 2021). In a recent study, four self-paced interval training sessions were investigated for time spent ≥ 90 % $\dot{V}O_{2max}$ when using a maximal session effort prescription; finding that both 4-min and 8-min work periods can result in between 15.9 % and 24.2 % of total session duration at ≥ 90 % $\dot{V}O_{2max}$, the higher ranges being achieved using a work:recovery ratio of 2:1 (e.g., 4-min work, 2-min recovery; Dall' Agnol, Turnes and De Lucas 2020). It is possible that had the training been prescribed using maximal effort-based intensity targets, the athletes who reported higher levels of life stress might have adjusted their exercise workload adequately in order to achieve the highest tolerable session effort and aid in reducing between-athlete variability (Seiler and Sylta 2017).

Summary

Several prescription methods are present which can help to identify specific exercise intensities at which training may be completed to produce a consistent level of cellular and metabolic stress. However, many of these methods have been demonstrated to result in variability when prescribed in a standardised manner. Using an effort-based training intensity prescription may provide a method that may allow athletes to self-regulate their exercise workload based on perceived exertion during exercise. However, the specific use of using effort-based exercise intensity prescriptions to reduce individual variability in exercise response has yet to be examined.

2.5 Metabolomics

With the advancements in the field of systems biology, it is now possible to study the complex interactions that occur as a result of the imposition of a stressor on a biological system (Dunn, Broadhurst, *et al.* 2011a; Bruggeman and Westerhoff 2007; Kell 2006a; van der Greef, Hankemeier and McBurney 2006). This presents an opportunity to investigate the metabolic effect of exercise across numerous metabolites involved in many different metabolic pathways (Zhao *et al.* 2020; Renata Garbellini Duft *et al.* 2017; Schranner *et al.* 2020). Metabolites can be described as low molecular-weight (typically < 1,500 Da) molecules of either organic or inorganic origin (Griffiths 2007; Harrigan and Goodacre 2003; Lindon, Nicholson and Holmes 2006; Bundy, Davey and Viant 2009). Metabolites are linked throughout various processes within a biological system. Metabolites are the building blocks for many other biological components (e.g., proteins, ribonucleic acid, deoxyribonucleic acid, and cell walls), they are central in intermediary metabolism, they provide many necessities for life (e.g., ATP for energy release) and they have an active role in the regulation of homeostasis and cellular signalling. The term metabolome is used to describe the collection of these compounds in a biological system, first used by (Oliver 1998) and accordingly, metabolomics is the measurement of the metabolome using a variety of biospecimens (Goodacre *et al.* 2004; Fiehn 2002; Nicholson, Lindon and Holmes 1999). When describing the analysis of the metabolome, the terms metabolomics and metabonomics are often used interchangeably in the literature but reflect slight differences. Nicholson et al. introduced the term ‘metabonomics’ in 1999 to describe; *“the quantitative measurement of the dynamic multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modification”*, which indicates a specific use of metabolite analysis in response to specific stimuli in a biological system (Nicholson,

Lindon and Holmes 1999). Fiehn introduced the definition of 'metabolomics' in 2002 as being; *"A comprehensive and quantitative analysis of all metabolites",* thus, representing a wide-ranging description of the method of global metabolite analysis (Fiehn 2002). The fields of metabolomics and metabonomics involve the analysis of a global profile of metabolites present in a biological system (Dunn, Broadhurst, *et al.* 2011; Fiehn 2002; Goodacre *et al.* 2004; Griffin 2006; Nicholson, Lindon and Holmes 1999), and the complete human metabolome remains unfinished, but the total number of metabolites is estimated to be between 2,000 – 20,000, with only a fraction of these being identified and described within metabolomics databases e.g. the human metabolome database (HMDB; Bernini *et al.* 2011; Wishart *et al.* 2007; Wishart *et al.* 2009).

Within any biofluid, there are thousands of metabolites with diverse physical and chemical properties, physiological concentrations, and molecular structures (Dunn *et al.* 2008). Some biofluids present a 'regional' metabolome to the area or network of the body, such as saliva (Sugimoto *et al.* 2010; Wei *et al.* 2011), semen (Li *et al.* 2007; Hung *et al.* 2009), amniotic fluid (Romero *et al.* 2010), synovial fluid (Zhai *et al.* 2010; Giera *et al.* 2012), cerebrospinal fluid (Dunne *et al.* 2005; Huang *et al.* 2006), as well as homogenated tissue samples (Huang *et al.* 2010; Römisch-Margl *et al.* 2012). In contrast to the regional nature of the above biofluids, both blood and urine reflect the global state of an organism (Álvarez-Sánchez, Priego-Capote and Luque de Castro 2010; Maher *et al.* 2007). Blood plasma presents a near-instantaneous representation of the current metabolic state at the time of collection, whereas the urinary metabolome represents an averaged reflection of recent metabolic processes (Álvarez-Sánchez, Priego-Capote and Luque de Castro 2010). Human urine is generated by the kidneys as an end process of their extraction of soluble wastes, excess water, sugars, and numerous other compounds from the bloodstream (Bouatra *et al.* 2013). Urine contains high concentrations of urea (an end product of amino acid metabolism), inorganic salts (such as chloride, potassium, and sodium), ammonia, creatinine, organic acids, water-soluble toxins, and end products of the breakdown of haemoglobin. (Bouatra *et al.* 2013). As a result, analysing the metabolomic profile of urine could provide a reflection of the state of the human system at a given sample time-point (Yin, Lehmann and Xu 2015; Nicholson, Lindon and Holmes 1999). The use of urine as a biofluid for metabolomics analysis has gained popularity in recent years, with demonstrations that it yields stable and non-invasive results (Bernini *et al.* 2011; Gika *et al.* 2007; Gika *et al.* 2008; Sun *et al.* 2008).

2.5.1 Analytical platforms and techniques

Metabolomics analyses are conducted on a wide range of platforms, however, the primary platforms of use are nuclear magnetic resonance (NMR) and mass spectrometry (MS) coupled to liquid (LC) or gas (GC) chromatography (Dunn and Ellis 2005; Nicholson *et al.* 2002) as a separation technique. With diverse chemical structures present in the metabolome, it can be common to combine multiple platforms to provide complementary information and complete coverage of metabolites (Dettmer, Aronov and Hammock 2007). NMR is one of the key analytical techniques for metabolomics due to its high level of reproducibility and relative ease of sample preparation to provide quantitative metabolite analysis, whereas MS shows greater sensitivity, resolution, and a higher number of metabolite classes that can be profiled (Want, Cravatt and Siuzdak 2005). Metabolomics research is conducted in either a '*targeted*' or an '*untargeted*' manner (Dunn, Broadhurst, *et al.* 2011b) and can be performed on a wide range of systems and analytes. Untargeted metabolic profiling has gained popularity as an effective tool for hypothesis generation to investigate system-wide biological variations in settings such as disease (Jansson *et al.* 2009; Chen *et al.* 2010; Fan, Bai and Shen 2005; Vallejo *et al.* 2009; Wei *et al.* 2011), xenobiotics (Loo *et al.* 2012; Ji *et al.* 2011; Pujos-Guillot *et al.* 2013), and nutrition (Llorach *et al.* 2010; Pujos-Guillot *et al.* 2013; Krupp *et al.* 2012; Nieman *et al.* 2012). Untargeted metabolomics is a comprehensive analysis of measurable metabolites in the specimens including previously unassigned metabolites, whilst targeted approaches are conducted by measuring specific metabolites of interest, and analytical optimisation is performed to provide quantitative measurements of these specific metabolites (Lu, Bennett and Rabinowitz 2008; Roberts *et al.* 2012), but potentially missing previously unknown metabolites of interest. Untargeted protocols utilise wide scopes of mass-to-charge (*m/z*), typically 50 – 1000, and therefore has the potential to capture thousands of metabolites within the sample of interest (Kell 2006b; Dunn *et al.* 2013; Quintás *et al.* 2018). This is advantageous as no prior knowledge of relevant metabolites is required and can detect previously unpredicted metabolites of interest (Kell and Oliver 2004). Optimisation of the metabolomics platform can assist in increasing data accuracy and can be tuned for each experiment to ensure optimal data acquisition (Wang and Griffiths 2008). Key elements of the performance of a metabolomics platform are; mass accuracy (detecting a *m/z* as close as possible to the theoretical *m/z* based on a compounds molecular structure), mass range (the range of *m/z* that can be detected), scan speed (the time taken by the analyser to scan over a specific mass range), transmission (the ratio of ions that enter the mass spectrometer

versus the ions that reach the detector), and resolution (differentiating between two ions with the same mass, but different *m/z*).

Chromatography

It is possible to inject raw biofluids into an MS system, however, this may result in ion enhancement or suppression, fragmentation, and adduct formation due to the number and complexity of molecules within the biological sample (Lenz and Wilson 2007; Remane *et al.* 2010; Drexler, Reily and Shipkova 2011). LC and GC platforms serve to separate sample molecules prior to analysis based on polarity by using an analytical column (Dettmer, Aronov and Hammock 2007), which reduces ion suppression and allows for more accurate metabolite detection and quantification (Want *et al.* 2007). GC and LC present differences in the ability to separate molecules based on their polarity, with GC being advantageous for the detection of low-polar or volatile compounds, whereas LC is ideal for highly polar and non-volatile compounds. The major advantage of LC over GC lies in the simplicity of sample preparation, with urine, plasma, and serum requiring minimal sample preparation steps (Álvarez-Sánchez, Priego-Capote and Luque de Castro 2010; Want *et al.* 2010; Yin, Lehmann and Xu 2015). LC is performed using an analytical column with derivatised silica, called the stationary phase, and pressurised organic and aqueous solvents, called the mobile phase (Wilson *et al.* 2005) running through the column. The sample of interest is injected into the column, and separation of the sample is achieved by creating a gradient of the mobile phase composition, which results in selective elution of compounds depending on their chemical properties. Once samples have eluted through chromatography, they are detected by the MS system. Over the years, LC has been developed by reducing column size, increasing temperature, and increasing flow rates (Cheng, Lu and Neue 2001; Tiller, Romanyshyn and Neue 2003; Neue and Mazzeo 2001), with high-performance liquid chromatography (HPLC) being developed, and further refined into ultra-performance liquid chromatography (UPLC; Waters Corp., MA, USA). HPLC is achieved by decreasing the particle size within the analytical column to 10μm and increasing the flow pressure up to 6,000 psi (Horvath and Lipsky 1969) and was commonly used throughout the early application of metabolomics (Gavaghan *et al.* 2000; Fan, Bai and Shen 2005; Yang *et al.* 2004; Lenz *et al.* 2004; Williams *et al.* 2005). UPLC advanced upon HPLC techniques by using smaller particle sizes within columns (< 2 μM) and higher pressures (10,000 – 15,000 psi) thus offering enhanced resolution with lower acquisition times.

Mass spectrometry

Once analytes have been separated by chromatography, they pass through to the MS system, where the molecular mass of a specific molecule is determined by detecting the *m/z* ratio of charged ions in the compound (Maher, Jjunju and Taylor 2015). An MS unit is conducted of an ion source, mass analyser, detector, and an accompanying data system. Through the unit, compounds are ionised from the liquid phase to a gas phase, separated by their *m/z* ratio, and then detected by the mass analyser (Maher, Jjunju and Taylor 2015). The ionisation of the sample is commonly performed using electrospray ionisation (ESI), comprising of a charged capillary needle (1 – 3 kV applied) and an opposing counter electrode which is oppositely charged, creating an electric field gradient (Kebarle and Verkerk 2009). The sample is pumped through the capillary needle, creating charged liquid droplets within the 'desolvation zone'. Here, the sample droplets shrink in size, with the aid of drying agents such as nitrogen, resulting in Coulombic fissions when the surface tension of the droplet surface is exceeded by the charge repulsion. The shrinking droplets finally enter the gas phase via either the 'charged residue theory' (Dole *et al.* 1968) or the 'ion evaporation' theory (Iribarne 1976; Iribarne, Dziedzic and Thomson 1983). Once in the gas phase, the ions are able to enter a vacuum chamber in the mass analyser for detection (Wang and Griffiths 2008). Mass analysers separate ions from the sample according to their *m/z* ratio using different methods but commonly using quadrupole or time-of-flight (TOF) MS analysers. Mass analysers, such as quadrupole-TOF (qTOF) or triple-quadrupole setups (Yates 2000), can be used alone or in tandems. TOF-MS calculates the transit time of ions as they pass through the analyser, based on the notion that ions having identical kinetic energy applied to them, but different masses, will require different durations to travel a fixed distance (Maher, Jjunju and Taylor 2015). Quadrupole-MS (qMS) uses four parallel electrodes to generate an electric field that can selectively allow ions of specific *m/z* ratios to pass through the detector (Maher, Jjunju and Taylor 2015; Kicman, Parkin and Iles 2007). TOF-MS results in greater resolution and mass accuracy allowing differentiation between compounds that are close or identical in mass, whereas qMS results in greater sensitivity allowing detection of analytes at low concentrations within the sample (Wang and Griffiths 2008). The final process of the MS analysis system is for the conversion of the information collected by the mass analyser into a form where it can be transferred to a computational system to generate mass spectra (Figure 3.1). This is commonly performed by quantifying the abundance of ions colliding with the detectors and then emitting secondary particles such as electrons and photons. During each *m/z* scan, the abundance of ions is recorded, and after numerous *m/z* scans are

completed, information containing the *m/z* ratio, ion abundance, and retention time (RT) is delivered to the data system, where mass spectra and chromatograms are produced.

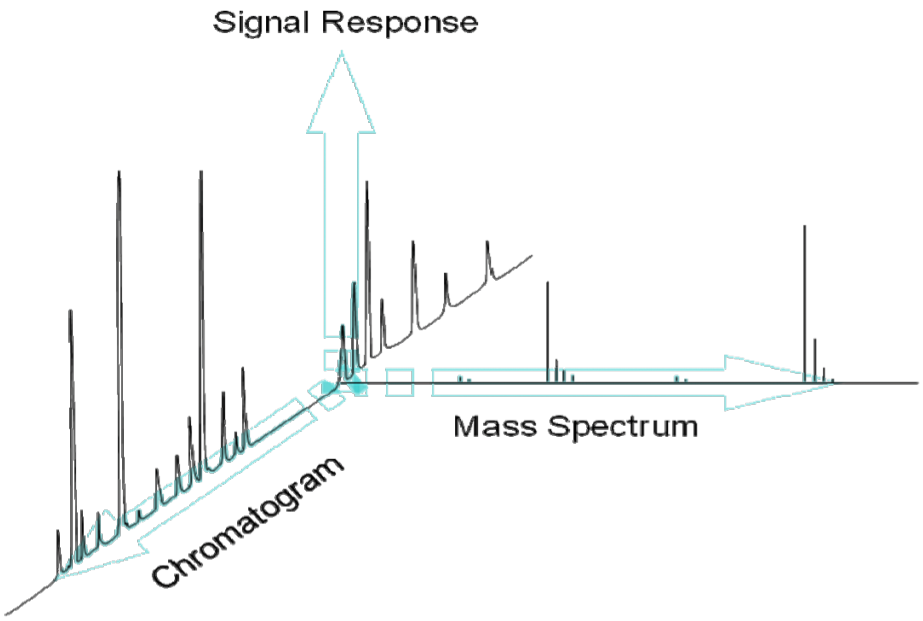


Figure 2.1 – Graphical representation of the chromatogram and mass spectrum data output (Reproduced from Sargent 2013).

An example of a typical chromatogram acquired using LC-MS global profiling is presented in Figure 3.2. The total intensities for all ions detected in each scan are displayed as a total ion chromatogram (TIC; Figure 3.2A). Reduced noise and higher-resolution peaks can be seen in the base peak intensity (BPI; Figure 3.2B) chromatograms, where only the most intense peak within each scan is displayed. Desired *m/z* ratios of interest can be searched, and an extracted ion chromatogram (EIC, Figure 3.2C) can be produced; in this example, for hippuric acid (mass: 180.0661). Investigating the retention time peak in Figure 3.2C, a mass spectrum can be produced, which shows the mass and intensity of the main peak along with any other compounds present (Figure 3.2D).

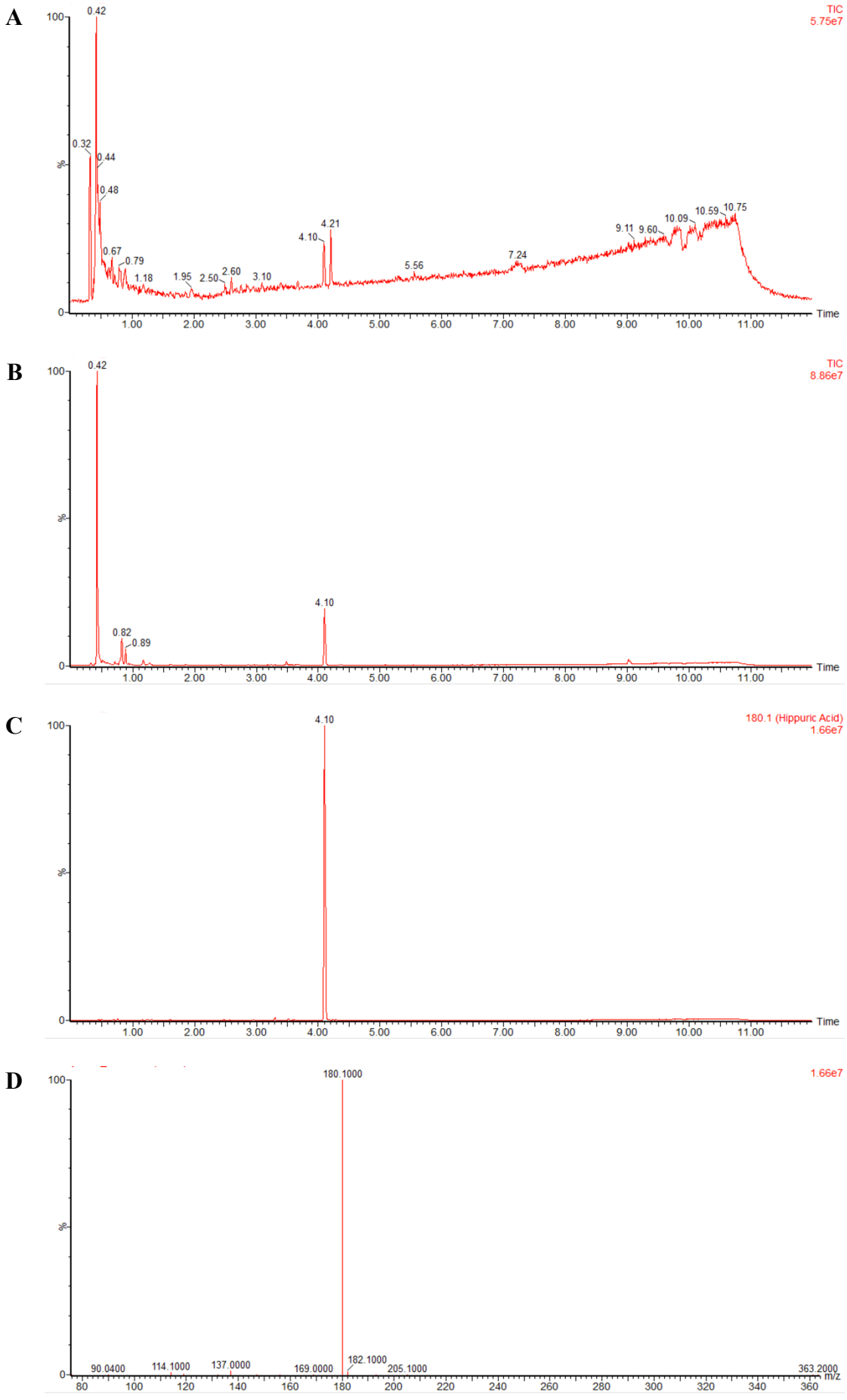


Figure 2.2 - Raw MS data presented in chromatogram forms. A) total ion chromatogram, B) base peak intensity, C) extracted ion chromatogram, D) mass spectrum.

Quality control and system conditioning

The stability of the analytical platform is known to change over time, and distinct differences in the retention time and sensitivity of metabolite peaks are often observed in the early stages of analysing samples, especially when new columns are used (Want *et al.* 2010; Gika *et al.* 2007; Michopoulos *et al.* 2009; Zelena *et al.* 2009). Column ageing, temperature and pressure changes, mobile phase composition, contaminant build-up and communication issues are all common sources of retention time and sensitivity variability.

In order to account for this initial variance when analysing samples, quality control (QC) samples were used throughout Chapters 7 and 8 to evaluate the stability and precision of the data from analysis (Begou *et al.* 2018; Dunn *et al.* 2012; Bijlsma *et al.* 2006; Want *et al.* 2010; Gika *et al.* 2007). Identical QC samples are interspersed throughout analytical runs in order to check for any changes in retention time, intensity, random errors or fluctuation (Begou *et al.* 2018; Dunn *et al.* 2012; Bijlsma *et al.* 2006; Want *et al.* 2010; Gika *et al.* 2007). Conditioning QC (cQC) samples are also repeatedly injected at the beginning of an analytical run to provide a level of 'dirtiness' in the system, resulting in a stable platform for samples to be analysed. QC samples are prepared by mixing small and equal aliquots of each experimental sample into a pooled sample which contains the mean concentration of all the metabolites present within the experimental group (Begou *et al.* 2018).

Metabolomics data pre-processing

Following the analysis of the UPLC-MS system stability, any metabolites that failed to reach threshold values were removed, and the dataset proceeded onto the data pre-processing stage. Data pre-processing involves converting the raw spectral data (chromatograms) into a data matrix that can be interpreted, quantified, and on which statistical analyses can be performed. The following sections outline the main steps involving pre-processing of LC-MS data;

- *Metabolite feature detection*

Feature detection can also be called "*peak picking*" and involves the extraction of detected peaks within each chromatogram to remove baseline noise. There are several methods that can be used to perform feature detection, and within this thesis, the "*centWave*" method

was used. The *centWave* method identifies peaks as regions of interest based on analysis of the density of the chromatogram in the *m/z* domain, and then a continuous wave transformation is applied in order to resolve chromatographic peaks (Tautenhahn, Böttcher and Neumann 2008). Following this, metabolites with a mass accuracy of ≥ 30 ppm are removed, consistent with most metabolomics studies (Wang and Griffiths 2008), in addition, an intensity filter can be used to eliminate any peaks with low amplitude if necessary. Further unnecessary data can be removed from the dataset using a minimum signal-to-noise ratio filter of 3:1. The above thresholds ensure that the data taken forward through further analysis are highly reproducible and are not confounded by data errors.

- *Retention time alignment*

Many factors can alter the retention time measured during UPLC-MS, such as column degradation, sample degradation, mobile phase composition, pressure and temperature fluctuations, and contaminant build-up (Rasmussen *et al.* 2011). Using maximum and minimum retention time drifts which were identified using raw chromatogram outputs, peak alignment can be performed to re-aligning the dataset so that matching peaks are unified to a common retention time within the data matrix. Incorrect retention time alignment could lead to peak-splitting, where the signal for one metabolite of interest is separated into two different peaks.

- *Peak filling and integration*

To account for samples which have zero values for specific peaks of interest due to their values being below noise or mass accuracy thresholds, peak filling can be performed, allowing for further comparisons to be made.

- *Data filtering*

To ensure that the metabolite of interest is present within the majority of the complete sample set, filtering can be applied to remove peaks that are only apparent in a minority of samples.

- *Data normalisation*

Systemic variation such as sample dilution and instrument drift can still be present at this stage and can be removed in a normalisation step in the data pre-processing. There are several methods that can be used to perform normalisation, and within this thesis, median fold change (MFC) normalisation methods are used. MFC identifies the mean target profile

for each metabolite of interest and matches the remainder of the samples within the dataset to this profile (Veselkov *et al.* 2011).

- *CV Filtering*

Further analytical variation within the dataset can be removed by CV filtering, which involves removing peaks that are inconsistent over time following normalisation. The CV values for all features observed within the QC samples are calculated, and features with CV values > 30 % are removed from the dataset.

- *Data scaling*

To account for the large variance observed between variables detected in UPLC-MS analyses, scaling is performed. There are many methods that can be performed, but unit variance (UV) and pareto scaling are the two most common methods in metabolomics investigations. UV scaling is performed by mean centring the data and then dividing by the SD of the variability, thus ensuring that variables with low concentration are not outweighed by highly concentrated variables, but this may increase the likelihood that noise and data artefacts are amplified. On the other hand, pareto scaling is performed by dividing the data by the square root of the SD, and therefore reduces the relative importance of high intensity peaks. Since different forms of data pre-treatment can greatly affect the output of the modelling, both UV and pareto scaling are evaluated throughout this thesis as identified.

Metabolomics data analysis

Metabolomic analytical runs generally result in large datasets with information that does not typically suit univariate statistical analyses due to a likelihood of high rates of false positives (Worley and Powers 2013; Eriksson *et al.* 2004). Multivariate statistical analysis is more suited to dealing with metabolomic data and is useful for not only the visualisation of the datasets, as well as assessing variance between and within groups of samples. Additionally, discriminant analysis can be performed which allows for classification of groups within the datasets and determine metabolomic features that are responsible for the difference between sample groups (Worley and Powers 2013). Two main forms of multivariate analysis are conducted in this thesis: principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA). All multivariate tests were performed in SIMCA-P (Version 12, Umetrics, Sweden).

- *Principal Component Analysis (PCA)*

PCA is used to reduce the dimensionality of complex datasets with high dimensions down to a smaller number of dimensions. This is done by transforming variables into uncorrelated principal components (PC). These PCs are used to construct individual scatter plots, which present the relationship between the dataset observations (scores) and individual variables (loadings). The first PC (PC1) represents the largest variation in a directional plane, and then additional PCs can be added to PCA (PC2, PC3…), with each subsequent PC containing less variation (Worley and Powers 2013; Wold, Esbensen and Geladi 1987) but orthogonal to the previous PC. The application of PCA is typically used to provide an overview of the acquired spectroscopic (e.g. LC-MS and NMR) data by observing the QC samples to be tightly grouped together within the scores plot (Eriksson *et al.* 2013; Trygg, Holmes and Lundstedt 2007). The scores plot of each PCA can also be used to visualise the distribution of the samples within the dataset and can be used to identify clusters of sample groups, outliers, and trends within the dataset (Figure 3.3; Wiklund 2008). Samples that fall outside the Hotelling's T_2 95 % confidence ellipsis represent significant outliers (Trygg, Holmes and Lundstedt 2007). Outliers were assessed and either included if their presence shows biological reasoning or excluded if the outlier is due to a data analysis or analytical artefact. The loadings plot of a PCA presents the variables which are responsible for the patterns observed within the scores plot. Displacement of loadings from the mean centre point of the plot influences score placement on the scores plot, and distance from the centre indicates the observation strength.

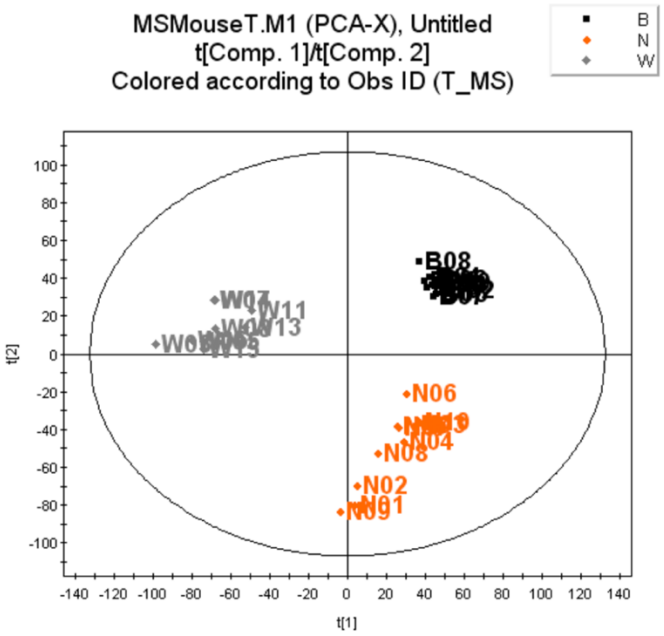


Figure 2.3 – PCA scores plot, showing variance within the dataset on the first and second principal components. In this example, three groups have been identified. Taken from (Wiklund 2008).

- *Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) Analysis*

Standard Partial Least Squares (PLS) analyses are used to determine relationships between X- and Y- matrices by maximising covariance by linear regression. In metabolomics analyses, the X-matrix represents the processed chromatographic data consisting of the RT, *m/z,* and intensity, and the Y-matrix would contain information such as categorical or continuous data for discriminant analysis or continuous variables. The components loadings of the PLS methods are comprised of class data, the direction of the component does not effectively distinguish between classes. For this, Orthogonal PLS (OPLS) can be performed, where the variation in the X-matrix that is uncorrelated to the Y-matrix is removed in the orthogonal components (Trygg and Wold 2002; Wold *et al.* 1998) from the predictive component and therefore making the interpretation of the dataset easier (Figure 2.4). Similar to PLS analysis, the Y-matrix can be categorical or continuous in nature, allowing for regression analysis in OPLS or OPLS-DA. The predictive ability of the model can be explained by three factors: the percentage variance of the X-matrix (R^2X) and the Y-matrix (R^2Y), and the robustness of the model (Q^2Y) to provide an estimate of the predictive strength of the model based on 7-fold cross-validation. The closer the Q^2Y value is to 1, the better the predictive ability of the model.

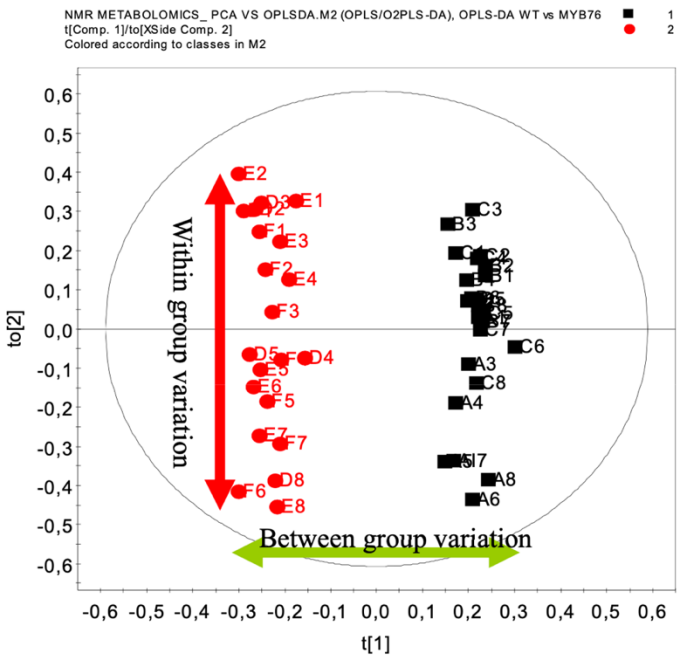


Figure 2.4 - OPLS-DA scores plot, showing the predictive scores (t1) that differentiate between the two groups, and the variation within the group that does not differentiate between the two groups is captured in the orthogonal component (to). Taken from (Wiklund 2008).

Following assessment of the robustness and predictive strength of the OPLS-DA models, an S-plot can be used to identify potential biomarkers of interest from the OPLS-DA loadings by presenting the covariance from the model with the correlation in a scatter plot. The resulting plot normally forms an S when there is variation in peak intensities within the dataset (Figure 2.5; Wiklund 2008). The X-axis of the S-plot displays the magnitude of the variable, and the Y-axis displays the variable reliability. Variables with large magnitude (in either positive or negative direction along the x-axis) and large reliability (also in either positive or negative direction along the y-axis) present as reliable candidate biomarkers for investigation. The data points that fall on the negative side of the X and Y axes in the S-plot indicates *m/z* values that are higher in samples from the sample class that is represented on the left-hand side of the scores plot, and the opposite for data points on the positive side of the X and Y axes.

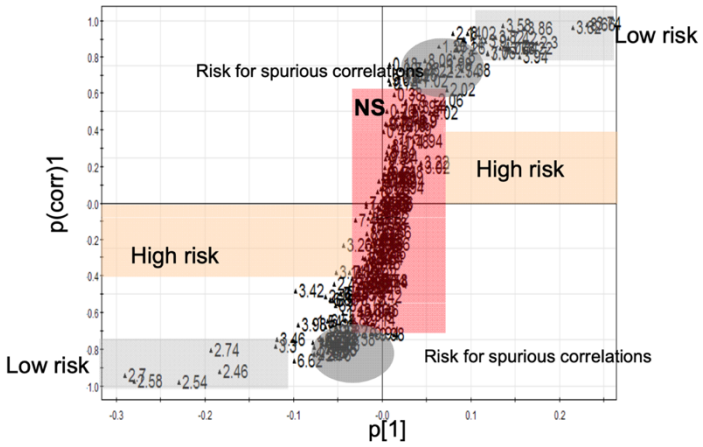


Figure 2.5 - Example S-Plot, showing sectors of interest, risk, and significance. Taken from (Wiklund 2008).

2.5.2 Metabolomics in exercise

The acute response to stressors placed on the human body is primarily metabolically focused in most cases, which are then followed by transcriptional and translational changes (Dunn, Broadhurst, *et al.* 2011a). The aim of investigating the metabolic profile of a biological system is to identify either single or patterns of metabolites that are possible to link to a specific physiological state and gain a more in-depth insight into the processes at work (Chorell *et al.* 2009). The acute response to exercise involves a cascade of hormonal and cell-signalling events, which are related to the degree of homeostatic perturbation and resultant intracellular and systemic metabolic response (Chorell *et al.* 2009). These responses to exercise stress likely influence gene and protein expression, which in turn result in phenotypic adaptation aimed at reducing the stressful impact of subsequent exercise bouts (Coffey and Hawley 2007). Metabolomics provides an insight into the whole-body systemic metabolic perturbations, as well as recovery and adaptation, are now able to provide new insights into exercise science research (Bundy, Davey and Viant 2009; Primrose *et al.* 2011; Wishart *et al.* 2007). There has been steady incorporation of metabolomics in exercise research to date, investigating both the acute response to single exercise bouts and the chronic changes in human metabolome in response to exercise training (Enea *et al.* 2010; Yan *et al.* 2009; San-Millán *et al.* 2020; Bally *et al.* 2017; Berton *et al.* 2017; Chorell *et al.* 2009; Danaher, Gerber, Wellard, *et al.* 2016; Heaney, Deighton and Suzuki 2017; Kuehnbaum, Gillen, Kormendi, *et al.* 2015; Huang *et al.* 2010; Lee *et al.* 2010; Mach *et al.* 2017; Mukherjee *et al.* 2014; Schranner *et al.* 2020), indicating that the technique has been providing interesting insights compared to existing methods (Chorell *et al.* 2009; Siopi and Mougios 2018).

Due to the large energy demand of exercise, the metabolites that are most altered following exercise are those related to energy metabolism (Hawley *et al.* 2014; Calbet *et al.* 2020; Schranner *et al.* 2020). A comparison of three acute exercise training session protocols; continuous, long HIIT (3 min work:3 min recovery), short HIIT (30 sec work:30 sec recovery), utilising an isoeffort intensity prescription identified no difference in the metabolic perturbations between formats, but identified metabolic changes before and after the training sessions (Zafeiridis *et al.* 2016). These differences were related mainly to glycolysis and the tricarboxylic acid (TCA) cycle, emphasising the advantage of metabolomics use in exercise research (Zafeiridis *et al.* 2016). Differences between acute exercise training session formats were observed in a comparison between two short HIIT protocols; 20 sec sprints at 150 %$\dot{V}O_{2max}$ with 40 sec recovery, against 10 sec sprints at 300 %$\dot{V}O_{2max}$ with 50 sec recovery; with the 10 sec sprint protocol resulting in significantly greater disturbances in metabolites related to glycolysis, as well as lipid and fatty acid metabolism (Danaher, Gerber, Wellard, *et al.* 2016). In a comparison of continuous exercise at 75 %$\dot{V}O_{2max}$ until exhaustion and 30 sec maximal sprints, no difference was found in the metabolites that were altered, but the magnitude of the metabolic response increased in an intensity-dependent manner (Enea *et al.* 2010). A further comparison of continuous exercise at 65 %$\dot{V}O_{2max}$, and a HIIT protocol involving 10 repeats of 4 min at 80 %$\dot{V}O_{2max}$ matched to the continuous exercise for volume; the HIIT group displayed altered metabolomic profiles relating to the metabolism of lipids, proteins, and the TCA cycle (Peake *et al.* 2014). The human metabolome has also been shown to be altered in response to chronic exercise training (Zhao *et al.* 2020; Renata Garbellini Duft *et al.* 2017; Schranner *et al.* 2020). In response to a 6-week HIIT intervention utilising 60 sec sprints at 90 %$\dot{V}O_{2max}$, the expression of o-acetylcarnitine was increased, a metabolite indicative of improved muscle oxidative capacity (Kuehnbaum, Gillen, Kormendi, *et al.* 2015; Kuehnbaum, Gillen, Gibala, *et al.* 2015). However, the population involved in this study were overweight-obese and sedentary young women, so the study may have limited applicability to highly trained athlete populations (Kuehnbaum, Gillen, Kormendi, *et al.* 2015; Kuehnbaum, Gillen, Gibala, *et al.* 2015). Research assessing both the acute and chronic exercise response using repeated three 80 metre running sprints with either 10 sec or 1 min recovery between sprints identified that both inosine and hypoxanthine are altered with HIIT exercise (Pechlivanis *et al.* 2010; Pechlivanis *et al.* 2013; Pechlivanis *et al.* 2015). Hypoxanthine and inosine are two metabolites related to purine metabolism and are indicative of high ATP turnover and resynthesis (Lewis *et al.* 2010).

Existing literature on the application of metabolomics in exercise shows that the change in metabolic profiles are associated with different exercise formats and time points surrounding exercise bouts (Al-Khelaifi *et al.* 2018; Yan *et al.* 2009; Wagner *et al.* 2007). The metabolites commonly found within exercise-related investigations are associated with energy metabolism, such as lactate (Goodwin *et al.* 2007; Berton *et al.* 2017), products of adenine breakdown (Dudzinska *et al.* 2010), and amino acid alterations (Berton *et al.* 2017; Leibowitz *et al.* 2012). However, the specific application of metabolomics as a tool to provide further depths to the evaluation of individual variability in response to exercise training has yet to be performed.

2.6 Summary

Based on the findings of this literature review, the aim of this thesis was to investigate the use of effort-based methods as a standardised method of exercise intensity prescription. Individual variability observed in both exercise performance and the physiological response will be investigated when using these targets, with the goal of achieving low levels of individual variability in positive exercise training response and adaptations. A key part of this investigation will be an exploration of the interaction of duration and intensity when using effort-based intensity targets on physiological and psychological responses to exercise. The exercise formats used will include single bouts, whole sessions, and chronic response to exercise training. Metabolomics will be explored as a tool to investigate the global metabolic response to effort-based exercise and establish potential differences between exercise formats and associations with varying levels of individual variability.

Chapter 3 - General methods

3.1 Introduction

In this chapter, the general methods utilised throughout the investigations included in this thesis are outlined. Within each individual study chapter, further specific methods are outlined.

3.2 Participants

All participants were recruited using word-of-mouth, advertisements (physical and online), and prospective contact. In Chapters 4 and 6, both male and female cyclists were recruited to participate, and in Chapter 5, only male cyclists were recruited. To obtain as homogenous participant groups as possible, participants completed a Cycling Experience Questionnaire and were required to be regularly competing in local and national level cycling or triathlon competitions and have been training regularly. Focusing on cyclists who have competitive experience had the rationale that these cyclists would regularly be exposed to perceptually regulated exercise within a competition setting. The performance level criteria (Table 3.1) set out by de Pauw *et al.* (2013) were used to classify participants within each experimental chapter, allowing for effective comparison between participants' performance level within this thesis, as well as for future research.

Table 3.1 – de Pauw *et al.* (2013) classification criteria for cyclists' performance level.

	PL1	**PL2**	**PL3**	**PL4**	**PL5**
	Untrained	Recreationally trained	Trained	Well trained	Professional
Absolute MMP (W)	< 280	280 - 319	320 - 379	380 - 440	> 350
Relative MMP ($W.kg^{-1}$)	< 4.0	3.6 - 4.5	4.6 - 5.5	4.9 - 6.4	> 5.5
Relative $\dot{V}O_{2max}$ ($ml.kg.min^{-1}$)	< 45	45 - 54.9	55 - 64.9	65 - 71	> 71
Absolute $\dot{V}O_{2max}$ ($L.min^{-1}$)	< 3.7	3.4 - 4.2	4.2 - 4.9	4.5 - 5.3	> 5.0
Cycling training ($hrs.week^{-1}$)	< 2-3	4	≥ 5	≥ 10	≥ 10
Cycling experience (years)	-	-	-	≥ 3	≥ 5

3.3 Informed consent and ethical approval

Ethical approval for all studies was obtained from the University of Kent local research ethics committee. Upon recruitment, participants were provided with approved information sheets that detailed the rationale of each study, as well as the procedures, benefits, and potential risks involved. All participants provided informed consent for the studies they were involved with and were aware of their right to withdraw their participation at any time. A standardised medical health questionnaire was completed by all participants prior to the completion of any data collection and were excluded from participation if any contraindications to exercise were identified. In the case where participants became injured or unwell during their participation, they were encouraged to withdraw from the specific study and re-start their participation when appropriate.

3.4 Experimental conditions and locations

All testing procedures were conducted within University of Kent laboratories at Medway Park, Gillingham, or on the Medway Campus, Gillingham. Both locations are maintained with similar environmental conditions of and ambient temperatures of approximately 19 °C. Each laboratory was equipped with air conditioning units, adequate airflow, and cooling fans.

3.5 Standardised control measures

Participants were provided with detailed instructions of the pre-testing control procedures and considerations required for each study prior to each laboratory visit with the aim of achieving homogenous physiological and psychological states during participation. These procedures will be detailed within each experimental chapter.

3.6 Anthropometric measurements

Height and body mass data of each participant were measured (SECA Beam scale and stadiometer, Birmingham, UK) during the first visit of each study, and body mass was recorded during subsequent visits to ensure accurate reporting of body-mass relative values. Participants were instructed to stand barefoot wearing only the clothing they would complete the testing in, with their feet touching the measurement backboard and their

gluteus muscles and upper backs against the stadiometer. Using the Frankfort plane, participants adopted a level head position and were instructed to take a deep breath before the stadiometer sliding measurement level being lowered to the top of their head. Measurements were made to the nearest 0.1 cm and 0.1 kg.

3.7 Maximal incremental test

All studies included in this thesis incorporated a continuous incremental ramp test to maximal volitional exhaustion on a cycling ergometer. The purpose of this test was to identify MMP, $\dot{V}O_{2max}$, and HR_{max}, but it also served to familiarise participants with the laboratory and collect information on physiological and psychological response to incremental exercise.

Participants completed the maximal incremental test on a bicycle ergometer (Cyclus2, RBM Electronics, Leipzig, Germany). After riding at 100 W for a period of 10 min, the external load was increased by 20 W every 60 sec until volitional exhaustion, defined as the point where self-selected cadence dropped below 60 rpm despite strong verbal encouragement. MMP was calculated as the highest power output averaged over a period of 60 sec, $\dot{V}O_{2max}$ was calculated as the highest $\dot{V}O_2$ achieved over a period of 30 sec, and HR_{max} was identified as the highest HR value reached in the incremental test. After a period of 30 min (10 min cool-down at 100 W, 10 min seated rest, and 10 min warm-up at 100 W), participants were instructed to exercise at MMP until volitional exhaustion to identify TTE at an intensity corresponding to $\dot{V}O_{2max}$ and also to confirm $\dot{V}O_{2max}$ values recorded during the incremental test.

3.8 General experimental equipment and procedures

3.8.1 Cyclus2 ergometer

The testing ergometer which was used for the duration of the research studies included in this thesis was a Cyclus2 ergometer (Cyclus2 ergometer, RBM Electronics, Leipzig, Germany). This ergometer has been used previously in cycling research and is widely considered as having good accuracy and reproducibility (Rodger *et al.* 2016; Reiser *et al.* 2000). Participants were able to use their own bicycles whilst using this ergometer, which reduces the need for familiarisation and improves test comfort. In addition to the use of their own bicycles, the axles of the Cyclus2 ergometer are constructed elastically, allowing

for a more ‘natural’ cycling movement versus a static machine. During the maximal incremental testing procedures, the resistance provided by the Cyclus2 was controlled using the internal programming included with the unit in accordance with the test protocol and allowing participants to freely choose their pedalling cadence. During self-paced exercise bouts, participants were able to control the resistance by using the gear shifting capabilities on their own bicycles to adjust the chosen gearing on the Cyclus2 fixed cassette. Where participants were unable to attend the laboratories with their own bicycle, an appropriately sized bicycle was fitted from a stock of laboratory bicycles. As changes in bike setup has been shown to affect efficiency (Price and Donne 1997), subject's setup was kept identical between all tests.

3.8.2 CompuTrainer cycling trainer

During the training intervention involved in Chapter 6, participants completed training sessions using a CompuTrainer Pro unit (RacerMate, Seattle, USA). These units are electromagnetically braked cycling ergometers upon which participants were able to mount their own bicycles, and resistance was applied on the rear wheel. When using the CompuTrainer device there are specific calibration procedures that must be followed, including the standardisation of rear tyre pressure. A total of nine trainer units were set up in the laboratory to allow for group training participation, and adequate spacing between units was given. The CompuTrainer has been regularly used in cycling training research (Mauger, Jones and Williams 2010; Micklewright *et al.* 2010) and display good reliability (coefficient of variation [CV]: 1.2 – 1.9 %) of test-retest power output across a wide range of intensities (Stone *et al.* 2011; Noreen, Yamamoto and Clair 2010; Davison, Corbett and Ansley 2009; Zavorsky *et al.* 2007). The units allow participants to regulate the intensity of cycling by altering their cadence and gear selection throughout exercise, which closely mimics the experience of cycling outdoors.

3.9 Physiological measurements

3.9.1 Heart rate

During Chapters 4, 5, and 6, the Cyclus2 ergometer collected ANT+ heart rate signals measured from a heart rate chest strap worn by each participant (Cyclus2 heart rate strap,

RBM Electronics, Leipzig, Germany). During training sessions completed by participants in Chapter 6, heart rate data was collected by the CompuTrainer units.

3.9.2 Expired gas

During Chapters 4, 5, and 6, gas exchange data were measured using an online breath-by-breath metabolic system (Cortex Metalyser 3B, Leipzig, Germany) and analysed using compatible software (Metasoft III, Cortex, Leipzig, Germany). The unit measures ventilatory volume using a digital turbine transducer, which uses a photocell sensor to detect the rotation of a turbine in the gas sampling unit attached to a face mask. Inspired and expired gas was sampled in a continuous manner from the sampling unit to measure factors such as $\dot{V}O_2$ and $\dot{V}CO_2$ through a specialised sampling line. Immediately prior to all testing bouts, the system was calibrated for volume using a 3 L syringe (Hans Rudolph Inc., Kansas City, USA) and for oxygen (O_2) and carbon dioxide (CO_2) using ambient gas and certified calibration gas mixtures (14.98 % O_2 and 4.97 % CO_2). CV of the ambient and calibration gas over multiple days was measured < 0.02 %, and CV of volume calibration was measured over multiple days at three different flow rates; 0.5 L/s (inspiration; -1.9 %, expiration; -0.7 %), 1 L/s (inspiration; -2.6 %, expiration; -0.9 %), and 3 L/s (inspiration; -1.3 %, expiration -1.3 %).

3.9.3 Near-infrared spectroscopy

Muscle oxygenation status was measured using near-infrared spectroscopy (NIRS) in Chapters 4 and 5, which is based on the difference in light absorbency between oxygenated haemoglobin (O_2Hb) and deoxygenated haemoglobin (HHb). Working skeletal muscle has been shown to deoxygenate during exercise, and the degree of deoxygenation is heavily influenced by training status and the intensity of exercise (Nioka *et al.* 1998; Hansen *et al.* 1996). The oxygenation status of the muscle, therefore, provides an insight into the level of oxidative stress present within the muscle during exercise, particularly as trained endurance athletes have display increased mitochondrial function and oxidative capacity (Hawley 2002; Holloszy and Coyle 1984; Davies, Packer and Brooks 1981). The NIRS probe (Portamon, Artinis Medical Systems BV, Netherlands) transmits near-infrared light through muscle tissue and is then measured by a receiver. The NIRS probe was placed on the right leg, at the distal end of the vastus lateralis muscle, covered with blackout cloth, and held securely in place with an elasticated bandage. The placement was determined by

asking subjects to extend their leg to locate the distal end of the muscle body, and the NIRS probe was placed in a manner that ensured the sensors were covering muscle tissue. Care was taken when securing the NIRS probe to ensure that enough emitted light was able to be detected by the NIRS receiver. The Portamon probe was set up with 35 mm distance between probe and receiver (allowing the signal to penetrate approximately 17.5 mm into the muscle tissue; Malagoni *et al.* 2010).

As near-infrared light sent by the NIRS probe travels through muscle tissue, part of the signal is absorbed whilst the remainder is scattered by the tissue, which can then be measured by the NIRS probe detector. The changes in tissue O_2Hb and HHb across time was calculated using a modified form of the Beer-Lambert Law using the received optical densities from continuous wavelengths of near-infrared light (762 nm and 848 nm), corresponding to the wavelengths of absorption of O_2Hb and HHb. Changes in total haemoglobin (tHb) were calculated by the sum of O_2Hb and HHb and used as an index of change in regional blood volume within the illuminated area (Van Beekvelt *et al.* 2001). The dynamic balance between O_2 supply and consumption is represented by the tissue saturation index (TSI, expressed in %) and was calculated as $O_2Hb/HHb + tHb \times 100$. The NIRS data were collected at 10 Hz, and then for the purposes of further analysis, a 10-point moving average was applied when transferring data to a personal computer from the Portamon probe. The data were normalised to a baseline period (0 μM) to express the magnitude of change (Δ; Smith and Billaut 2010; Subudhi, Dimmen and Roach 2007; Subudhi *et al.* 2009; Shibuya *et al.* 2004a; Shibuya *et al.* 2004b). This baseline period lasted 120 sec and occurred immediately prior to the initiation of the warm-up prior to exercise, where the subject remained in a seated position and instructed to remain still and relax their leg.

3.10 Psychological measurements

Prior to the measurement of any psychological ratings, full instructions were delivered to participants to ensure correct comprehension of the related scales. The majority of study participants were also regular participants in the laboratory and thus have had previous experience with the scales used.

3.10.1 Perceived Exertion and Session Perceived Exertion

Subjective ratings of perception of effort were recorded during various exercise procedures using Borg's 6 – 20 scale (Borg 1970; Borg 1982a), which provides descriptive categories such as; '*no exertion at all*', '*very light*', and '*maximal exertion*' with corresponding numerical values. RPE provides a score of the subjective evaluation of a participants' total physical strain experienced at a specific moment during exercise and contains both physical and psychological components. To assess the overall subjective workload within an exercise testing session, participants were asked to evaluate the session using Foster's sRPE (Foster *et al.* 2001). The sRPE has been shown to be a reliable and valid method that incorporates both the intensity and duration of the exercise session and represents the subjective 'mean' exertion felt by the participant (Haddad *et al.* 2017).

3.10.2 Life Demands and Stress

To assess participant's total load of stress with regards to exercise and general life stress, the Daily Analyses of Life Demands for Athletes (DALDA; Rushall 1990) was used prior to testing and exercise sessions. The DALDA is formed of an inventory of ratings that measure sources and symptoms of stress and is valid for competitive athletes over the age of eleven years. A total of 9 sources of stress and 25 symptoms of stress are examined, and three ratings can be given by the participant: (a) "*worse than normal*", (b) "*normal*", or (c) "*better than normal*". For the purposes of this thesis, the number of "*worse than normal*" responses for each session was used to assess the influence of outside-of-sport stressors that may have interfered with training.

3.11 Metabolomics analysis

3.11.1 Urine sample preparation

Details of urine sample collection processes and timings are presented within experimental Chapters 7 and 8. Following collection, urine samples were centrifuged at 1500 x g for 10 – 15 min to remove any debris (including any cellular material). The acellular supernatant was then aliquoted for storage/freezing in a locked freezer and thawed prior to analyses. Cellular material was disposed of in a clinical waste bin following standard clinical SOPs. Sample preparation was carried out following the guidelines of Want *et al.* (2010). Neat urine samples were thawed at room temperature and were vortexed for 15 sec and

centrifuged at 10,000 g for 10 min. Using an individually wrapped disposable syringe (1 ml Terumo Syringe, Terumo, Tokyo, Japan), 1 ml of urine was then drawn and passed through a molecular filter (Minisart Syringe Filter, Sartorius, Goettingen, Germany) to remove any further cellular debris still contained in the sample. Filtered urine was subsequently diluted using UPLC-grade water with a 1:1 dilution ratio, vortexed for 15 sec and centrifuged at 10,000 x g for a further 5 min before being transferred into glass vials (2 ml Fisherbrand™ Glass Vials, Fischer Scientific, UK) for UPLC-MS analysis (Danaher, Gerber, Wellard, *et al.* 2016; Enea *et al.* 2010; Lehmann *et al.* 2010).

3.11.2 UPLC-MS analysis

All urine samples for Chapters 7 and 8 were prepared and analysed using UPLC-MS in both positive (ESI^+) and negative (ESI^-) ionisation modes. Analyses were performed using Waters ACQUITY equipment (Waters Corp., Milford, MA), which was comprised of a Quaternary Solvent Manager, an H-Class Sample Manager, Acquity QDa quadrupole mass analyser with an ESI probe (Jagadabi *et al.* 2019; Mistry, Lee and Wood 2019; Gay *et al.* 2014). Instrument control and data acquisition were performed using MassLynx v4.1 software (Waters Corp., Milford, MA). Chromatographic separation was performed using a Waters Acquity UPLC HSS T3 100Å 1.8 µm column (2.1 mm ɵ x 100 mm length; Waters Corp., Milford, MA), with a mobile phase comprising of eluent A (de-ionised H_2O + 0.1 % formic acid), and eluent B (acetonitrile + 0.1 % formic acid). The gradient flow rate was held at a constant 0.5 $ml.min^{-1}$ over a gradient lasting 12 min (Table 3.2). A regulated autosampler held samples at 4 °C during analyses, with an injection volume of 5 µL. MS ESI conditions were optimised from previous experiments using urine samples and are presented in Table 3.3. An *m/z* range of 50 – 800 was used in resolution mode for data acquisition, with a 0.5 sec scan time. Ten QC injections were used to condition (cQC) the UPLC-MS system prior to experimental samples being run and again at the end of the sample run, as well as repeated every 10 samples throughout the entire analytical run. UPLC-Grade H_2O samples were used as 'blanks' to assess the spectroscopic effects of the solvent system, and these were incorporated at the start and end of each analytical run. Test mixes were created containing known compounds from chemical standards, known to be responsive to exercise, dissolved in UPLC-Grade H_2O (dilution 1:5) to enable putative identification of metabolites of interest in the biological samples, and are detailed within Chapters 7 and 8.

3.11.3 Quality control and system conditioning

As QC samples should reflect analytes similar to the samples of interest, all QC samples were prepared for each exercise condition by pooling 100 μL of urine taken from each urine sample prior to dilution but following filtering and centrifugation. Prior to any experimental samples being analysed 10 cQC samples were run, and further QC samples were repeated at least every 10 experimental sample injections.

Table 3.2 - Gradient used for urine sample analysis; A, UPLC grade water & 0.1 % formic acid; B, Acetonitrile & 0.1 % formic acid.

Time (min)	A%	B%
0	99	1
1	99	1
3	85	15
6	50	50
9	5	95
10	5	95
10.1	99	1
12	99	1

Table 3.3 - Electrospray Ionisation conditions utilised for the untargeted metabolic profiling of urine in both positive and negative modes.

	ESI^+	ESI^-
Source temperature (°C)	150	150
Desolvation temperature (°C)	500	500
Cone gas flow ($L.h^{-1}$)	100	100
Desolvation gas flow ($L.h^{-1}$)	1200	1200
Capillary voltage (kV)	2	2.4
Cone voltage (kV)	20	32

3.11.4 Data preparation

Following samples being injected through the UPLC-MS system and analysed, data was analysed in chromatogram form using MassLynx v4.1 software (Waters Corp., Milford, MA). The DataBridge tool within MassLynx4.1 was then used to convert MS data output files (.raw) to netCDF format. The netCDF data files were then processed using the XCMS package for R software (Smith *et al.* 2006).

3.11.5 Univariate analysis of raw data

Visual comparison of overlaid QC BPI chromatograms followed by assessment of retention time drift, mass accuracy, and alterations to the peak intensities of the most abundant point of the putatively identified metabolites included in the test mixes in both ESI^+ and ESI^- modes were used to assess the analytical reproducibility of the UPLC-MS system. Following confirmation that the analytical chromatograms contain no errors or indicate any experimental failures, further analysis of the UPLC-MS system stability was performed using putatively identified metabolites. Test mixes were prepared using selected compounds, which are commonly found in exercise-related urinary analyses and are presented within both Chapters 7 and 8. These compounds contained within the test mixes were used to putatively identify metabolites within the experimental samples based on their accurate mass, RT, and ion fragment patterns as well as cross-referenced in HMDB (Wishart 2008; Kind and Fiehn 2007). For retention time drift, mass accuracy, and alterations to the peak intensities, measures were taken from the most abundant point of the selected metabolite peak of interest. Acceptance criteria thresholds were; retention time drift ≤ 6 sec, mass accuracy error to ≤ 30 ppm, and sensitivity to ≤ 0.3 C, where the CV was calculated using the following equation:

$$Coefficient\ of\ Variance = \frac{Standard\ deviation}{Mean}$$

3.11.6 Pre-processing parameters

Metabolomics data pre-processing within Chapters 7 and 8 were performed using the XCMS package in R, and the parameters were optimised based on inspection of chromatograms from all datasets and were as follows; minimum chromatographic peak width = 1 sec, maximum chromatographic peak width = 20 sec, ppm threshold for peak detection = 30, signal to noise ratio = 3, mass tolerance for peak grouping = 0.5 Da, retention time error = 2 sec, and scan range = 20 – 540 sec. Thresholds for data extraction, normalisation, and CV filtering were; minimum fraction filtering to 50 %, retention time drift to ≤ 6 sec, mass accuracy error to ≤ 30 ppm, and sensitivity to ≤ 0.3 CV.

3.11.7 Multivariate analysis of metabolomics data

Within Chapters 7 and 8, multivariate analysis of metabolomics data was performed using OPLS-DA models within SIMCA-P (Version 12, Umetrics, Sweden). PCA was performed within Chapters 7 and 8 using pareto scaling, and the reproducibility of the datasets were assessed by observing clustering of QC samples within the scores plot. The sample variability for each analysis group was also assessed on the PCA scores plot. OPLS-DA analysis groups are outlined within Chapter 7 and 8. OPLS-DA models were subjected to permutation tests (n = 100) to assess the validity of the model, and Q^2Y was used to assess the goodness of the prediction parameters of the models. OPLS-DA models were deemed valid if the Q^2Y from the original model remains significantly higher (at $P < .05$) than the Q^2Y values generated from the permutation models. From valid OPLS-DA models, S-Plots were generated to identify the metabolites responsible for the separation. Metabolites were considered discriminatory metabolites if their VIP ≥ 1, p[1] ≥ 0.03, and p(corr) ≥ 0.4, where VIP scores provide an overview of the influence of an individual variable on the OPLS-DA model (Farrés *et al.* 2015; Akarachantachote, Chadcham and Saithanu 2014), p[1] represents the magnitude of each variable, and p(corr) represents the reliability of each variable.

3.11.8 Materials and suppliers

Materials that were used throughout Chapters 7 and 8 are listed and detailed in Table 3.4.

Table 3.4 – General metabolomics materials used throughout this thesis.

Material	Supplier	Cat No.
Acquity UPLC HSS T3 Column 100Å (1.2 mm x 100 mm)	Waters Corp. Milford, MA	186003539
UPLC Grade water	Sigma Aldrich, UK	34877
UPLC Grade acetonitrile	Sigma Aldrich, UK	34998
UPLC Methanol	Sigma Aldrich, UK	34860
Formic acid	Fischer Scientific, UK	56302
Urine collection containers	Fischer Scientific, UK	11592842
2 ml Fisherbrand™ glass vials	Fischer Scientific, UK	13-622-186
1 ml Terumo syringe	Fischer Scientific, UK	13197664

Chapter 4 - Variability of acute physiological response to submaximal self-paced exercise bouts of different intensity and duration

4.1 Abstract

Introduction: The use of rating of perceived exertion (RPE) as a training intensity prescription has long been used by competitive athletes and coaches. However, the individual variability in the physiological response to exercise prescribed in this manner has not been investigated. **Methods:** Twenty well-trained competitive cyclists (18 = male, 2 = female, $\dot{V}O_{2max}$: 55.07 ± 11.06 $mL.kg^{-1}.min^{-1}$) completed 3 exercise trials, each consisting of nine self-paced exercise bouts in a randomised order consisting of either short (1 min), medium (4 min), or long (8 min) bouts at self-selected workloads to elicit at low (RPE 9), medium (RPE 13), or high (RPE 17) intensities. Power output and physiological responses were monitored to identify within- (WAV), between-athlete (BAV) variability using CV, and Total variability (TV), which was calculated as the ratio of WAV and BAV. **Results:** Power output, HR, work done, volume of consumed oxygen ($\dot{V}O_2$), volume of output carbon dioxide ($\dot{V}CO_2$), minute ventilation ($\dot{V}_E$), and ΔHHb all increased significantly at higher intensities ($P < .001$). ΔTSI% and ΔO_2Hb decreased as intensity increased ($P < .001$). During RPE 9 bouts, shorter durations resulted in lower $\dot{V}O_2$ ($P < .05$), whereas ΔTSI% decreased and ΔHHb increased as duration increased ($P < .05$). During RPE 13 bouts, shorter durations resulted in lower $\dot{V}O_2$, $\dot{V}_E$, and $\%\dot{V}O_{2max}$ ($P < .001$), higher power output, HR, ΔHHb ($P < .001$) and ΔTSI% ($P < .05$). During RPE 17 efforts, power output ($P < .001$) and ΔTSI% ($P < .05$) decreased as duration increased. As intensity and duration increased, variability in power output, work done, heart rate, $\dot{V}O_2$, $\dot{V}CO_2$, and $\dot{V}_E$ decreased, and variability in muscle oxygenation data increased. **Conclusion:** Self-paced exercise intensity prescriptions at higher effort levels and longer durations result in the lowest WAV and BAV, suggesting that as the required effort level increases, athletes' power output and physiological responses become more homogenous. Future investigations should investigate maximal session effort prescriptions to provide greater consistency of training stimulus.

4.2 Introduction

Perception of effort is defined as the intensity of subjective effort, stress, discomfort, and fatigue which is felt during exercise or physical activity (Noble and Robertson 1996; Eston 2012). The common method of measuring perception of effort is the RPE scale (Borg 1998) which is believed to be influenced by factors such as fatigue, effort, strain, discomfort, and/or pain (St Clair Gibson *et al.* 2006). It has been demonstrated that increased RPE can be the result of increases in oxygen consumption, metabolic acidosis,

ventilation, and heart rates (Borg 1998; Ekblom and Golobarg 1971; Robertson *et al.* 1986). The RPE scale can be used during exercise to record the RPE experienced whilst an individual is exercising at an exercise workload or resistance (Myles and Maclean 1986). The RPE scale can also be used as a tool to prescribe exercise intensity in *production mode* (Borg 1998) as a method to regulate exercise (Helms *et al.* 2018; Parfitt, Evans and Eston 2012; Graham and Cleather 2019). The RPE production mode provides a perceived exercise intensity continuum that an exercising individual can use to regulate their exercise work rate or resistance (Borg 1998; American College of Sports Medicine 2014; Noble and Robertson 1996). The ease of use of the RPE scale in production mode during self-paced exercise has been studied in a wide range of populations, including clinical, active, untrained (Eston *et al.* 2006; Eston and Williams 1988; Coquart *et al.* 2016; Noble and Robertson 1996; Dunbar *et al.* 1998; Noble 1982; Robertson 2001; Hartshorn and Lamb 2004), and trained athletes (Perrey *et al.* 2003; Schallig *et al.* 2017; Garcin, Danel and Billat 2008; Groslambert *et al.* 2004).

When using RPE in production mode, it is unclear whether both the intensity of the RPE anchor and the duration of the work bout would influence the accuracy and reliability of the exercising individual to adjust their work rate or resistance to maintain a specified RPE level or anchor. The reproducibility of this approach to exercise prescription has been investigated involving blind (Buckley 2000), child (Eston *et al.* 2000; Thompson and Lamb 2001), and healthy participants (Hartshorn and Lamb 2004), with varying results. Thompson and Lamb (2001) concluded that there was no difference in reliability in children when exercise intensity was prescribed using RPE in production mode with, or without, an anchoring protocol during both low and high levels of exertion. Increased reliability using RPE in production mode after a series of trials has been demonstrated in blind men and women (Buckley 2000) and children aged 7 – 10 years old (Eston *et al.* 2000) which may indicate a learning effect of using the scale in this manner. Although, conversely, a large study of 2,560 Caucasian men and women indicates that healthy individuals are able to use RPE in production mode to accurately achieve specified exercise intensities (Scherr *et al.*, 2013). As duration and intensity are both known to impact an individual's perception of effort, it is, therefore, likely to impact upon reliability of the exercise intensity that is selected in response to a specific RPE anchor (Seiler and Sylta 2017). Indeed, the impact of intensity on perceptually regulated exercise has been studied by Hartshorn and Lamb (2004), who demonstrated increased reliability with greater exercise intensity. For example, within-subject variability in power output as a CV

decreased from 58.3 % at RPE 9 to 39.7 % at RPE 15. Only Eston *et al.* (2006) have explored the impact of duration on perceptually regulated exercise. Their data suggest that shorter duration exercise (e.g., 2 min) has greater repeatability and reliability when compared to longer duration (e.g., 4 min). Both duration and intensity of perceptually regulated exercise have not yet been considered within the same study, and therefore the interaction is unknown. It is possible that both the intensity of the RPE anchor and the duration of work bout itself could affect an individual's ability to regulate their exercise intensity or work rate accurately and reliably to the desired target.

Therefore, the aim of this study was to assess the individual variability in repeated self-paced submaximal exercise of different intensities in trained competitive cyclists using long, medium, and short workload periods. Specifically, this study explores how exercise intensity and duration influence variability of performance and physiological responses, as well as the interaction between the two.

Hypotheses

$H1_1$ – Longer work bouts result in lower total variability in both performance and physiological response to self-paced exercise.

$H1_0$ – There is no difference observed in variability between the different workload durations.

$H2_1$ – Higher intensity work bouts result in lower total variability in both performance and physiological response to self-paced exercise.

$H2_0$ – There is no difference observed in variability between different intensity exercise bouts.

4.3 Methodology

4.3.1 Participants

Twenty trained/well-trained cyclists (18 males, 2 females; mean ± SD (95 % confidence interval; [CI]): age 38 ± 11 years (CI: ± 5), height 176.6 ± 9.7 cm (CI: ± 4.6), mass 72.4 ± 9.2 kg (CI: ± 4.4), $\dot{V}O_{2max}$ 55.07 ± 11.06 mL.kg^{-1}.min^{-1} (CI: ± 5.26), maximum minute power (MMP) 337 ± 54 W (CI: ± 26), HR_{max} 180 ± 9 bpm (CI: ± 4)), with at least 3 years of cycling training and racing experience (corresponding to Performance Level 3 – 4; de Pauw *et al.* 2013; Decroix *et al.* 2016), provided written informed consent to voluntarily participate in the study which held full ethical approval from the local institutional ethics committee according to the Declaration of Helsinki.

4.3.2 Study design and experimental procedures

Participants visited the exercise testing laboratory on four separate occasions over a period of 5 ± 2 weeks, with visits separated by at least 72 h to ensure full recovery between each. Participants were requested to arrive in a rested and euhydrated state to each visit. In Visit 1, participants completed an incremental exercise test to identify $\dot{V}O_{2max}$ and performance parameters, followed by a $\dot{V}O_{2max}$ confirmation effort (see *Maximal incremental test* for more details). During Visit 1, participants were also familiarised with the laboratory equipment used during the subsequent exercise testing visits. Visits 2 to 4 comprised of 3 supervised exercise sessions, each consisting of 3 separate self-paced exercise bouts; 3 RPE-anchored exercise intensities (RPE 9, 13, 17) lasting either 1 (SHORT), 4 (MED), or 8 (LONG) min completed in a randomised order during each visit (see: *Exercise testing sessions*). All visits for each participant were completed within the same 3-h period of the day, and participants were asked to maintain a consistent diet and lifestyle and to avoid alcohol and strenuous exercise the day before the sessions. To allow for adequate familiarisation prior to data collection, participants were asked to attempt to incorporate effort-based training bouts in their own training before commencing the study. A cooling fan was present, and plain water was available for participants to drink ad libitum.

4.3.3 Maximal incremental test

Participants completed a maximal incremental test on a bicycle ergometer (Cyclus2, RBM Electronics, Leipzig, Germany) to identify MMP, $\dot{V}O_{2max}$, and HR_{max}. After riding at 100

W for a period of 10 min, the external load was increased by 20 W every 60 sec until volitional exhaustion, defined as the point where self-selected cadence dropped below 60rpm despite strong verbal encouragement. MMP was calculated as the highest power output averaged over a period of 60 sec, $\dot{V}O_{2max}$ was calculated as the highest $\dot{V}O_2$ achieved over a period of 30 sec, and HR_{max} was identified as the highest HR value reached in the incremental test. After a period of 30 min (10 min cool-down at 100 W, 10 min seated rest, and 10 min warm-up at 100 W), participants were instructed to exercise at MMP until volitional exhaustion in order to confirm $\dot{V}O_{2max}$ values recorded during the incremental test.

4.3.4 Exercise testing sessions

After a warm-up period of 10 min easy cycling, participants completed work bouts of either 1, 4, or 8 min (SHORT, MED, LONG) clamped at RPEs of either 9, 13, 17 (6 – 20 scale: Borg 1998) in a randomised order, with 5 min easy cycling between each. A cool-down period of 10 min was completed following the final bout. Participants were able to self-select their cycling power output to achieve desired RPE anchor by using their gearing system on their bicycle. Elapsed time was available for participants during all bouts, but they were blind to all other data and information, and no encouragement was given during exercise to minimise the effects of external factors (Currell and Jeukendrup 2008).

4.3.4.1 Physiological measurements

During each session, power output was measured, and heart rate was transmitted using a compatible heart rate strap (Cyclus2 heart rate, RBM Electronics, Leipzig, Germany). Data were continuously measured and was subsequently segmented into the 9 sections corresponding to the 9 exercise bouts for analysis.

Respiratory gas exchange data were assessed continuously throughout all sessions using an online gas analyser (Metalyzer 3B, CORTEX Biophysik GmbH, Leipzig, Germany) and an appropriately sized facemask covering the nose and mouth. A 10-sec rolling average was used when analysing respiratory gas exchange data. Expired gas data were analysed to quantify $\dot{V}O_2$, $\dot{V}CO_2$, $\dot{V}_E$, and breathing frequency (B_f).

Muscle oxygenation was measured using spatially resolved dual-wavelength near-infrared spectroscopy (NIRS; Portamon, Artinis Medical Systems, BV, Netherlands), with the optode positioned 10 cm superior to the lateral epicondyle of the femur at the distal end of the vastus lateralis muscle and secured with muscle tape and bandage. NIRS data were analysed relative to a 2-min resting baseline measurement completed prior to each testing session to provide relative change (Δ) in TSI%, O_2Hb, and HHb.

4.3.4.2 Psychological measurements

The DALDA (Coutts, Slattery and Wallace 2007) questionnaire was administered immediately prior to each exercise session. Immediately following each exercise session, the subjective workload was assessed using the National Aeronautics and Space Administration Task Load Index (NASA-TLX; Hart and Staveland 1988; Hart 2006) in order to check for consistency in the overall perceived session load.

4.3.5 Data processing and statistical analysis

Data were processed according to the combination of exercise duration (SHORT, MED, and LONG), intensity (RPE 9, 13, 17), and session repeat (3 x SHORT, MED, and LONG). Prior to statistical analysis, all data were checked for normality of distribution. Sphericity of the data was investigated using the Mauchly test, and the Greenhouse-Geisser adjustment was made when data was deemed non-spherical. Data are reported as mean ± SD, and CVs are presented as a percentage unless specified otherwise. When assessing variability, low CVs indicating a consistent response and high CVs displaying variable response.

Repeated measures analysis of variance (ANOVA) was used to analyse power output and physiological response data between exercise session visits, and two-way repeated-measures ANOVA (duration x intensity) was used to analyse performance and physiological parameters. When significant differences were found, post-hoc testing with Bonferroni correction was used to determine where differences occurred. Effect sizes were calculated using partial eta squared (η_p^2) and were defined as small, medium, or large based upon 0.10, 0.25, and above 0.40, respectively (Cohen 1988).

Quantification of individual variation observed was completed by calculating CVs for the WAV, BAV, and TV of each parameter by expressing the standard deviation relative to the mean for each parameter. Linear mixed modelling was applied to analyse the variability in power output, work done, HR, %MMP, $\%HR_{max}$, $\dot{V}O_2$, $\dot{V}CO_2$, $\dot{V}_E$, $\%\dot{V}O_{2max}$, $\%B_{fmax}$, TSI%, O_2Hb, and HHb for each combination of duration and intensity. Furthermore, linear mixed modelling was used to assess the relative contribution of both WAV and BAV to the total variability observed (Bliss 1967; Hansen *et al.* 1997; Bagger, Petersen and Pedersen 2003).

Critical difference levels were used to indicate whether the difference observed between two work bout measurements was significant (Fraser, Hyltoft and Lytken 1990; Fraser and Harris 1989; Harris and Yasaka 1983; Bagger, Petersen and Pedersen 2003). The following equation is utilised to calculate critical difference;

$$first\ measurement \times Z \times \sqrt{2} \times WAV$$

where the first measurement is the measurement of interest, Z =1.65, which results in establishing a two-tailed probability of 10 %, and WAV is the observed within-subjects CV for the parameter involved.

Linear mixed models, ANOVA's, and post-hoc tests were conducted using the Statistical Package for the Social Sciences, version 26 for Mac OS X (SPSS, IBM®, Armonk, New York, USA), and an alpha level was set at $P < .05$ for the criteria for detection of significance in all cases. CV and critical differences were calculated in Microsoft Excel (Excel v16.3 Microsoft, Redmond, Washington, USA).

4.4 Results

4.4.1 Psychological response comparisons

Monitoring questionnaires were used to assess similarity in participant's perceived 'readiness to train' and perceived demand of each session. No differences were observed for perceived levels of stress prior to sessions ($P = .765$, $\eta_p^2 = .008$) and load attributed to mental ($P = .338$, $\eta_p^2 = .048$), physical ($P = .576$, $\eta_p^2 = .025$), temporal ($P = .257$, η_p^2 .06),

performance (P = .748, η_p^2 = .013), effort (P = .569, η_p^2 .025), and frustration (P = .860, η_p^2 = .007) sources following each testing session (Figure 4.1).

4.4.2 Session Order Differences

To assess whether there was any learning effect or influence of session order, the differences in the repeated exercise bouts were investigated. All data for repeated sessions were not significantly different for RPE 9 ($P \geq .098$, $\eta_p^2 \leq .115$), RPE 13 ($P \geq .109$, $\eta_p^2 \leq .11$), and RPE 17 ($P \geq .056$, $\eta_p^2 \leq .154$), except for both $\dot{V}CO_2$ (P = .045, η_p^2 = .18) and $\dot{V}_E$ (P = .026, η_p^2 = .168) which were higher in repeat 2 versus repeat 1 in SHORT_RPE17.

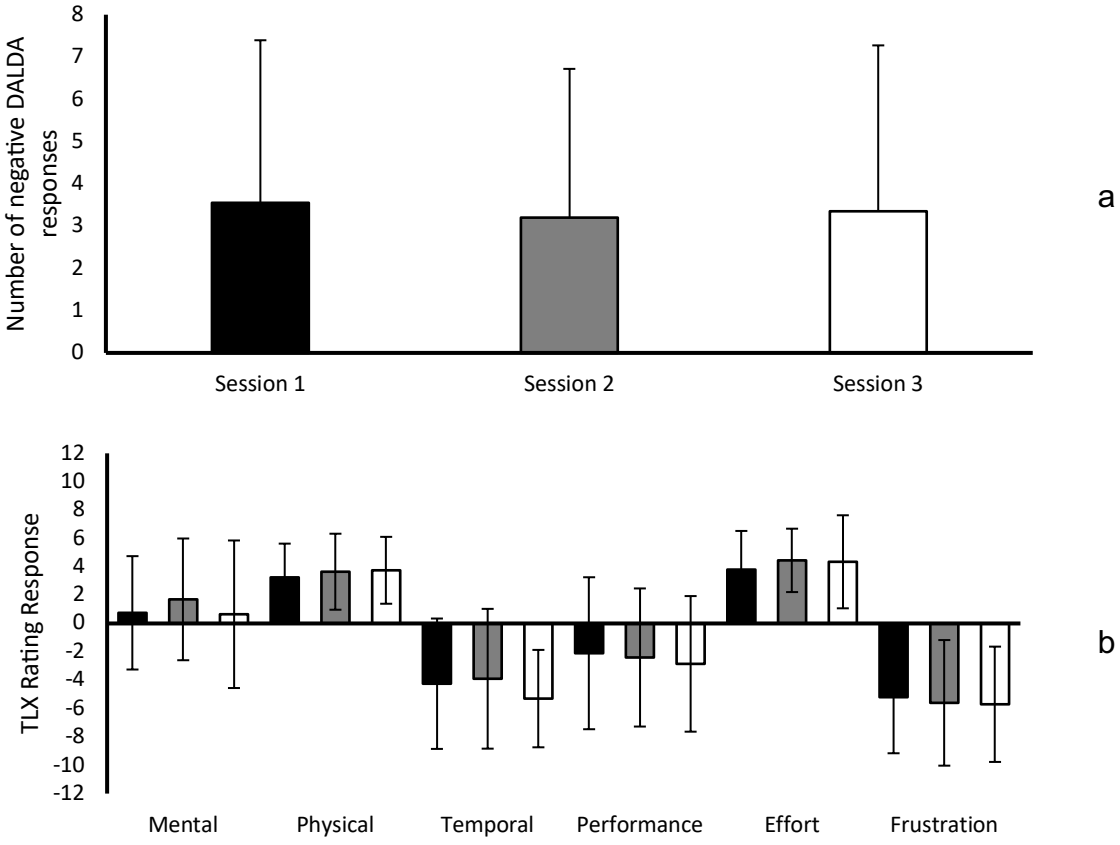


Figure 4.1 – DALDA (a) and NASA-TLX (b) responses across training session repeats (Black bar; session 1, grey bar; session 2, white bar; session 3).

4.5 Power output and cardiovascular response to exercise bouts

Power output, heart rate, and work done are reported in Table 4.1, and power as %MMP and HR as $\%HR_{max}$ are reported in Table 4.2. Significant changes in power output ($F_{(1.517, 89.53)}$ = 596.297; η_p^2 = 910), HR ($F_{(1.539, 90.829)}$ = 681.286; η_p^2 = .920), work done ($F_{(1.467,}$

$_{86.553)}$ = 633.586; η_p^2 = .915), %MMP ($F_{(1,59)}$ = 919.212; η_p^2 = .940), and %HR_{max} ($F_{(1.578,}$ $_{93.095)}$ = 709.357; η_p^2 = .923) were found as RPE anchor increased ($P < .001$). Significant changes in power output ($F_{(1.301, 76.771)}$ = 71.292; η_p^2 = .547), HR ($F_{(2, 118)}$ = 282.581; η_p^2 = 827), work done ($F_{(1.045, 61.678)}$ = 1309.505; η_p^2 = 957), %MMP ($F_{(1.414, 83.444)}$ = 22.101; η_p^2 = .273), and % HR_{max} ($F_{(2, 118)}$ = 270.719; η_p^2 = 821) were found as time increased ($P < .001$). There was an interaction effect observed for power output ($F_{(2.562, 151.172)}$ = 51.178; η_p^2 = .465), HR ($F_{(2.816, 166.160)}$ = 29.766; η_p^2 = .335), work done ($F_{(2.383, 140.613)}$ = 314.413; η_p^2 = .842), %MMP ($F_{(1.829, 107.922)}$ = 14.640; η_p^2 = .199), and HR as %HR_{max} ($F_{(2.773, 163.623)}$ = 29.634; η_p^2 = .334)($P < .001$). Locations of observed differences are highlighted in Table 4.1 and Table 4.2.

Variability Analysis

As shown in Table 4.1, power output and work done tended to decrease in terms of TV, BAV, and WAV as intensity and duration increased. Total CV in power output was lowest in LONG bouts of RPE 17 and highest in SHORT bouts of RPE 9. Heart rate displayed lower CVs in comparison to power output and work, with greater consistency being displayed as exercise intensity increased. Table 4.2 demonstrates that total, BAV, and WAV CV were all higher when reporting %MMP compared to %HR_{max}, with higher levels of consistency being found as intensity and duration increases.

Table 4.1 – Power output and cardiovascular response during RPE-clamped exercise bouts showing mean data, standard deviation, coefficients of variation, variance components, and critical difference levels.

						Coefficient of variation			Variance components			Critical difference
Variable			**Mean**	**SD**	**TV (%)**	**BAV (%)**	**WAV (%)**	**Var total**	**BAV (%)**	**WAV (%)**	**WAV (%)**
Power output (W)											
RPE 9	*a*	SHORT	95	41	43.1	43.5	13.1	1713.4	87.6	12.4	228.0
		MED	93	40	42.8	43.3	15.4	1633.4	83.4	16.6	271.4
		LONG	96	39	41.0	41.3	19.7	1581.2	72.6	27.4	375.7
RPE 13	*a*	SHORT *b*	228	69	30.3	30.5	15.2	4889.0	68.2	31.8	668.6
		MED	200	49	24.6	24.9	10.8	2512.6	79.5	20.5	403.2
		LONG	202	48	23.9	24.2	9.4	2402.0	78.1	21.9	356.1
RPE 17	*a*	SHORT *b*	349	97	27.7	8.9	10.6	9662.7	80.1	19.9	694.2
		MED *b*	275	61	22.1	22.4	8.6	3805.6	83.2	16.8	443.9
		LONG *b*	261	50	19.3	19.5	5.3	2596.5	91.6	8.4	259.0
Work done (kJ)											
RPE 9	*a*	SHORT *b*	6	2	44.0	44.4	11.8	6.3	86.9	13.1	12.3
		MED *b*	22	10	43.1	43.6	16.1	94.8	82.8	17.2	67.7
		LONG *b*	46	19	40.8	41.2	19.7	362.4	72.4	27.6	181.0
RPE 13	*a*	SHORT *b*	14	4	30.7	30.9	15.5	17.9	66.3	33.7	40.9
		MED *b*	48	12	24.7	25.0	10.7	145.6	79.7	20.3	95.9
		LONG *b*	97	23	23.9	24.3	9.3	553.6	78.2	21.8	170.1
RPE 17	*a*	SHORT *b*	21	6	27.4	8.6	11.1	34.3	79.5	20.5	43.9
		MED *b*	66	15	22.2	22.5	8.5	221.0	83.2	16.8	105.7
		LONG *b*	125	24	19.2	19.5	5.2	594.6	91.6	8.4	123.4
Heart rate (bpm)											
RPE 9	*a*	SHORT	109	12	11.3	11.5	5.3	155.6	76.6	23.4	107.7
		MED	111	15	13.8	14.0	7.5	242.0	69.6	30.4	159.1
		LONG	111	16	14.6	14.7	6.5	265.5	72.0	28.0	136.6
RPE 13	*a*	SHORT *b*	127	14	10.9	11.0	6.1	196.5	63.1	36.9	144.6
		MED *c*	138	13	9.7	9.9	6.6	186.8	44.0	56.0	169.9
		LONG	142	15	10.3	10.5	6.1	223.1	54.7	45.3	163.3
RPE 17	*a*	SHORT *b*	139	12	8.5	3.0	3.4	145.2	70.8	29.2	86.8
		MED *b*	154	12	8.0	8.1	4.2	158.1	69.5	30.5	120.9
		LONG *b*	160	11	6.7	6.8	3.0	117.8	71.4	28.6	88.1

a = Significant difference observed between all RPE's (P < .001). b = P < .001 vs. all other durations. c = P < .05 vs. LONG.

Table 4.2 – Relative power output and cardiovascular response during RPE-clamped exercise bouts showing mean data, standard deviation, coefficients of variation, variance components, and critical difference levels.

					Coefficient of variation			Variance components			Critical difference
Variable			**Mean**	**SD**	**TV (%)**	**BAV (%)**	**WAV (%)**	**Var total**	**BAV (%)**	**WAV (%)**	**WAV (%)**
Power as % MMP (%)											
RPE 9	*a*	SHORT	28	11	39.2	39.6	13.5	125.0	85.2	14.8	69.4
		MED	28	11	40.5	41.1	15.6	129.1	79.9	20.1	81.8
		LONG	29	11	39.5	39.9	19.6	129.8	68.5	31.5	111.2
RPE 13	*a*	SHORT	67	17	24.7	25.0	15.2	285.5	56.5	43.5	196.8
		MED	59	12	19.4	19.7	10.8	138.3	65.9	34.1	119.3
		LONG	60	11	17.6	17.9	9.4	115.1	62.7	37.3	106.1
RPE 17	*a*	SHORT *b*	103	20	19.8	6.5	10.6	432.1	58.0	42.0	206.0
		MED *b*	81	9	11.7	11.8	8.6	92.8	32.3	67.7	131.5
		LONG *b*	77	6	7.8	7.5	5.2	35.2	34.4	65.6	75.8
Heart rate as % HR_{max} (%)											
RPE 9	*a*	SHORT	60	6	10.6	10.8	5.5	41.9	72.2	27.8	61.8
		MED	62	8	13.6	13.8	7.6	72.1	66.8	33.2	89.4
		LONG	61	9	14.2	14.3	6.4	77.4	71.1	28.9	74.2
RPE 13	*a*	SHORT *b*	70	7	10.1	10.2	6.0	52.2	56.1	43.9	79.2
		MED *c*	77	7	9.6	9.7	6.4	56.1	44.8	55.2	92.0
		LONG	79	8	10.0	10.1	6.1	64.5	50.9	49.1	90.4
RPE 17	*a*	SHORT *b*	77	6	7.3	2.5	3.3	32.1	60.5	39.5	46.8
		MED *b*	85	6	6.7	6.8	4.2	33.8	56.4	43.6	67.5
		LONG *b*	89	4	5.0	5.0	2.9	20.0	52.2	47.8	48.4

a = Significant difference observed between all RPE's (P < .001). b = P < .001 vs. all other durations. c = P < .05 vs. LONG.

4.6 Expired gas responses during exercise bouts

$\dot{V}O_2$, $\dot{V}CO_2$, and $\dot{V}_E$ are reported in Table 4.3, and $\%\dot{V}O_{2max}$ and $\%B_{fmax}$ are reported in Table 4.4. Significant increases in $\dot{V}O_2$ ($F_{(1.473, 86.936)} = 529.082$; $\eta_p^2 = .90$), $\dot{V}CO_2$ ($F_{(1.485, 87.629)} = 494.818$; $\eta_p^2 = .893$), $\dot{V}_E$ ($F_{(1.507, 88.896)} = 371.169$; $\eta_p^2 = .863$), $\%\dot{V}O_{2max}$ ($F_{(1.676, 98.908)} = 684.862$; $\eta_p^2 = .921$), and $\%B_{fmax}$ ($F_{(1.667, 98.328)} = 346.81$; $\eta_p^2 = .855$) were found as RPE anchor increased ($P < .001$). Significant changes were found in $\dot{V}O_2$ ($F_{(1.728, 101.944)} = 228.521$; $\eta_p^2 = .795$), $\dot{V}CO_2$ ($F_{(1.723, 101.629)} = 203.813$; $\eta_p^2 = .776$), $\dot{V}_E$ ($F_{(1.796, 105.985)} = 158.104$; $\eta_p^2 = .728$), $\%\dot{V}O_{2max}$ ($F_{(1.738, 102.55)} = 194.221$; $\eta_p^2 = .767$), $\%B_{fmax}$ ($F_{(1.708, 100.794)} = 28.552$; $\eta_p^2 = .326$) as time increased ($P < .001$). There was an interaction effect observed for $\dot{V}O_2$ ($F_{(3.177, 187.454)} = 39.009$; $\eta_p^2 = .398$), $\dot{V}CO_2$ ($F_{(3.11, 183.511)} = 36.972$; $\eta_p^2 = .385$), $\dot{V}_E$ ($F_{(2.914, 171.899)} = 43.228$; $\eta_p^2 = .423$), $\%\dot{V}O_{2max}$ ($F_{(3.448, 203.438)} = 32.817$; $\eta_p^2 = .357$), $\%B_{fmax}$ ($F_{(3.18, 187.635)} = 16.789$; $\eta_p^2 = .222$)($P < .001$). Locations of observed differences are highlighted in Table 4.3, and Table 4.4.

Variability Analysis

As shown in Table 4.3 and Table 4.4 $\dot{V}O_2$, $\dot{V}CO_2$, and $\%\dot{V}O_{2max}$ tended to decrease in terms of total variability, BAC, and WAV, as intensity and duration increased. Variability in $\dot{V}_E$ and $\%B_{fmax}$ were similar across intensities and durations. Total CV in $\dot{V}O_2$ was lowest in LONG bouts of RPE 17, and highest in LONG bouts of RPE 9.

Table 4.3 – Expired gas response during RPE-clamped exercise bouts showing mean data, standard deviation, coefficients of variation, variance components, and critical difference levels.

						Coefficient of variation			Variance components			Critical difference
Variable				**Mean**	**SD**	**TV (%)**	**BAV (%)**	**WAV (%)**	**Var total**	**BAV (%)**	**WAV (%)**	**WAV (%)**
$\dot{V}O_2$ (L.min^{-1})												
RPE 9	*a*	SHORT	*c*	1.50	0.38	25.3	24.8	14.8	0.1	68.6	31.4	4.0
		MED		1.51	0.39	26.1	26.3	10.4	0.2	75.1	24.9	2.9
		LONG		1.53	0.43	27.9	28.3	11.2	0.2	78.9	21.1	3.2
RPE 13	*a*	SHORT	*b*	2.21	0.52	23.7	23.7	12.2	0.3	68.8	31.2	5.1
		MED	*c*	2.54	0.53	20.9	20.9	9.6	0.3	73.4	26.6	4.6
		LONG		2.69	0.54	20.0	20.2	8.5	0.3	77.3	22.7	4.2
RPE 17	*a*	SHORT	*b*	2.65	0.61	22.9	8.3	10.2	0.4	77.9	22.1	4.8
		MED	*b*	3.22	0.63	19.6	19.8	7.6	0.4	84.0	16.0	4.6
		LONG	*b*	3.36	0.57	16.9	17.1	4.3	0.3	92.5	7.5	2.7
$\dot{V}CO_2$ (L.min^{-1})												
RPE 9	*a*	SHORT		1.39	0.38	27.1	27.2	10.9	0.1	76.2	23.8	2.8
		MED		1.42	0.41	28.7	29.1	10.8	0.2	76.1	23.9	2.9
		LONG		1.48	0.44	29.9	30.3	15.0	0.2	65.6	34.4	4.3
RPE 13	*a*	SHORT	*b*	2.08	0.55	26.6	26.6	14.5	0.3	59.1	40.9	5.8
		MED	*c*	2.51	0.65	25.8	25.6	12.4	0.4	69.7	30.3	5.9
		LONG		2.73	0.61	22.5	22.5	11.0	0.4	69.2	30.8	5.6
RPE 17	*a*	SHORT	*b*	2.75	0.84	30.7	10.5	15.9	0.7	70.6	29.4	7.4
		MED		3.62	0.82	22.8	23.0	11.1	0.7	76.5	23.5	7.6
		LONG		3.67	0.67	18.2	18.4	5.9	0.5	88.8	11.2	4.1
$\dot{V}_E$ (L.min^{-1})												
RPE 9	*a*	SHORT		42.17	11.16	26.5	26.4	10.8	128.5	71.7	28.3	84.9
		MED		42.33	12.18	28.8	29.2	12.4	153.4	73.4	26.6	99.7
		LONG		42.94	10.99	25.6	25.8	13.3	124.5	67.2	32.8	109.0
RPE 13	*a*	SHORT	*b*	61.49	17.94	29.2	29.2	15.2	332.9	64.7	35.3	175.2
		MED	*c*	72.32	20.18	27.9	28.0	14.5	421.2	67.5	32.5	193.8
		LONG		77.53	20.68	26.7	27.0	11.6	440.8	72.8	27.2	167.6
RPE 17	*a*	SHORT	*b*	81.50	25.37	31.1	11.0	14.4	643.9	74.5	25.5	202.4
		MED	*b*	104.20	27.71	26.6	27.0	10.6	791.3	83.4	16.6	209.5
		LONG	*b*	111.77	24.20	21.7	22.0	7.0	605.2	87.4	12.6	146.9

a = Significant difference observed between all RPE's (P < .001). b = P < .001 vs. all other durations. c = P < .05 vs. LONG.

Table 4.4 – Relative expired gas response during RPE-clamped exercise bouts showing mean data, standard deviation, coefficients of variation, variance components, and critical difference levels.

Variable				Mean	SD	Coefficient of variation			Variance components			Critical difference
						TV (%)	BAV (%)	WAV (%)	Var total	BAV (%)	WAV (%)	WAV (%)
$\dot{V}O_2$ as % $\dot{V}O_{2max}$ (%)												
RPE 9	*a*	SHORT		38.6	8.3	21.5	21.7	10.0	70.1	70.5	29.5	71.9
		MED		39.4	9.9	25.2	25.6	10.7	101.4	73.2	26.8	79.7
		LONG		40.6	10.1	24.8	25.1	13.0	104.6	61.1	38.9	100.9
RPE 13	*a*	SHORT	*b*	56.7	10.5	18.5	18.6	12.0	112.5	47.5	52.5	129.0
		MED	*c*	65.2	11.5	17.6	17.3	9.7	134.3	54.1	45.9	117.2
		LONG		69.2	11.9	17.2	17.2	8.6	142.4	62.3	37.7	110.5
RPE 17	*a*	SHORT	*b*	67.7	11.0	16.2	5.0	10.3	117.3	49.7	50.3	123.2
		MED	*b*	82.2	10.8	13.1	13.2	7.6	118.3	53.8	46.2	117.2
		LONG	*b*	86.3	11.4	13.3	13.4	4.2	135.0	88.5	11.5	68.1
B_f as % $B_f$max (breaths.min^{-1})												
RPE 9	*a*	SHORT		46.0	8.3	18.0	18.3	7.1	70.8	78.8	21.2	59.9
		MED		44.1	9.4	21.3	21.4	7.9	90.3	70.3	29.7	65.9
		LONG		44.3	8.2	18.5	18.5	8.9	68.2	83.0	17.0	71.8
RPE 13	*a*	SHORT	*b*	52.9	10.9	20.6	20.7	9.0	122.1	76.7	23.3	86.9
		MED		55.1	11.4	20.7	20.2	8.9	132.5	71.9	28.1	88.9
		LONG		56.6	11.7	20.6	20.9	8.8	68.7	79.2	20.8	91.4
RPE 17	*a*	SHORT	*b*	62.7	11.2	17.8	5.9	6.6	126.8	79.7	20.3	74.3
		MED	*b*	66.8	13.1	19.5	19.8	5.7	176.4	86.6	13.4	70.9
		LONG	*b*	70.9	12.4	17.5	17.6	5.2	157.8	86.6	13.4	67.4

a = Significant difference observed between all RPE's (P < .001). b = P < .001 vs. all other durations. c = P < .05 vs. LONG.

4.7 Muscle oxygenation response during exercise bouts

ΔTSI%, ΔO_2Hb, and ΔHHb are reported in Table 4.5. Significant decreases in ΔTSI% ($F_{(1.245, 23.660)} = 65.598$; $\eta_p^2 = .775$), ΔO_2Hb ($F_{(1.147, 21.791)} = 61.594$; $\eta_p^2 = .764$), and increases in ΔHHb ($F_{(1.056, 20.073)} = 27.735$; $\eta_p^2 = .593$) were found as RPE anchor increased ($P < .001$). Significant decreases in ΔTSI% ($F_{(1.503, 28.561)} = 11.798$; $\eta_p^2 = .383$) and increases in ΔHHb ($F_{(1.223, 23.233)} = 13.385$; $\eta_p^2 = .413$) were found as time increased ($P < .001$). No significant decrease was observed in ΔO_2Hb ($F_{(1.468, 27.901)} = .918$; $\eta_p^2 = .046$, $P = 383$) as time increased. No significant interaction effect was observed for ΔTSI% ($F_{(4, 76)} = .695$; $\eta_p^2 = .035$, $P = 598$), ΔO_2Hb ($F_{(4, 76)} = .988$; $\eta_p^2 = .049$, $P = 420$), or ΔHHb ($F_{(2.538, 48.223)} = 1.115$; $\eta_p^2 = .055$, $P = 346$). Locations of observed differences are highlighted in Table 4.5.

Variability Analysis

As shown in Table 4.5, ΔTSI%, ΔO_2Hb, and ΔHHb displayed increasing levels of TV CV and BAV CV as effort level and duration increased. Total CV in ΔHHb was lowest in SHORT bouts of RPE 17 and highest in MED bouts of RPE 9.

Table 4.5 – Muscle oxygenation response during RPE-clamped exercise bouts showing mean data, standard deviation, coefficients of variation, variance components, and critical difference levels.

						Coefficient of variation			Variance components			Critical difference
Variable				**Mean**	**SD**	**TV (%)**	**BAV (%)**	**WAV (%)**	**Var total**	**BAV (%)**	**WAV (%)**	**WAV (%)**
ΔTSI%												
RPE 9	*a*	SHORT	*b*	-2.7	9.7	-355.6	-515.3	3.3	95.0	71.3	28.7	-1.7
		MED		-4.5	12.0	-264.5	-278.0	-10.3	149.4	64.6	35.4	10.1
		LONG		-4.5	12.0	-264.5	-278.0	-62.2	149.4	64.6	35.4	61.3
RPE 13	*a*	SHORT	*b*	-12.9	12.3	-96.0	-97.5	-83.8	156.3	79.5	20.5	201.2
		MED		-14.2	13.6	-95.7	-98.0	-81.2	187.7	87.7	12.3	218.9
		LONG		-14.8	13.2	-89.4	-90.9	-50.6	179.2	84.8	15.2	139.9
RPE 17	*a*	SHORT	*d*	-16.0	12.5	-78.0	-23.6	-45.0	160.1	86.5	13.5	129.7
		MED		-16.9	12.8	-75.7	-76.8	-34.1	167.5	83.9	16.1	106.4
		LONG		-17.1	13.9	-81.4	-83.0	-39.6	197.1	84.9	15.1	121.3
ΔO₂Hb												
RPE 9	*a*	SHORT		2.6	7.6	292.5	299.9	-41.9	64.0	69.7	30.3	-17.3
		MED		2.5	9.2	363.7	395.6	16.4	86.7	66.7	33.3	6.9
		LONG		2.6	7.6	292.5	299.9	25.1	60.0	60.6	39.4	10.4
RPE 13	*a*	SHORT		-8.4	10.9	-128.7	-128.8	-124.8	121.5	73.7	26.3	175.7
		MED		-8.4	10.3	-122.8	-124.8	112.3	109.1	82.2	17.8	-164.0
		LONG		-9.5	10.4	-110.0	-112.4	-231.1	110.8	78.0	22.0	395.8
RPE 17	*a*	SHORT		-11.5	9.8	-84.9	-34.8	-133.9	98.5	85.1	14.9	257.6
		MED		-12.1	10.8	-89.2	-90.7	0.1	120.1	84.5	15.5	-0.2
		LONG		-11.7	12.1	-103.8	-105.8	422.7	151.3	77.5	22.5	-837.7
ΔHHb												
RPE 9	*a*	SHORT	*b*	5.7	5.6	98.0	100.2	33.7	32.3	56.1	43.9	34.1
		MED		6.4	7.3	114.6	114.8	38.9	55.0	48.4	51.6	46.7
		LONG		6.4	6.1	95.4	97.4	44.4	37.7	58.7	41.3	53.4
RPE 13	*a*	SHORT	*b,c*	13.2	11.4	86.9	82.1	33.6	133.4	50.7	49.3	73.3
		MED		14.7	10.7	72.8	72.7	28.8	117.4	78.5	21.5	75.0
		LONG		15.5	11.4	73.1	74.0	20.9	133.8	85.6	14.4	61.2
RPE 17	*a*	SHORT		15.0	9.9	66.2	20.6	18.1	101.5	85.1	14.9	48.0
		MED		16.8	11.7	69.6	69.6	20.2	140.3	83.6	16.4	59.7
		LONG		17.4	13.0	74.8	75.5	22.9	174.7	84.1	15.9	70.5

a = Significant difference observed between all session formats (P < .001). b = P < .05 vs LONG. c = P < .001 vs MED. d = P < .05 vs MED

4.8 Discussion

The present study aimed to investigate both the physiological response and consistency of said response during self-paced submaximal exercise over different intensities and durations in trained competitive cyclists. The main findings of this study were that there were interactions between intensity and duration across all measured variables. Specifically, increases in intensity and duration resulted in greater consistency within measured parameters.

Unsurprisingly, as demonstrated in other research, increasing the RPE anchor resulted in higher cycling power outputs and greater physiological responses (Borg 1998; Hartshorn and Lamb 2004). Moreover, when duration increased, power output remained similar during RPE 9 bouts but decreased during RPE 13 and 17 bouts, suggesting that participants altered their power output in order to maintain the same perception of effort as the duration of the bout is extended (Seiler and Sylta 2017). The interaction between duration and intensity is also shown by changes in difference in the work done during each bout, as this is influenced by both duration and intensity.

As shown by Table 4.1, the current study found lower levels of variability during exercise at higher RPE anchors. When exercising at higher absolute exercise intensity, a small change in power output can result in large changes in physiological response and fatigue compared to lower absolute exercise intensities (Burnley and Jones 2018); thus participants are likely to control their exercise intensity within a closer bandwidth. This finding is supported by the work of Bagger, Petersen and Pedersen (2003), who demonstrate lower variability in measured physiological variables at higher exercise intensity, with the lowest variation during maximal conditions. It is likely that as the intensity of exercise increases, the cyclist will likely commit more conscious attention towards the required work rate and physiological responses, such as regionalised pain and pulmonary ventilation (Noble and Robertson 1996). Indeed, as HR, $\dot{V}O_2$, $\dot{V}CO_2$, and $\dot{V}_E$ increased as RPE anchor and duration increased in the current study, the WAV decreased, indicating greater homogeneity in the workloads produced by the athletes at a given RPE. The heightened perception of changes in the aforementioned physiological parameters likely results in a shift in the cyclist's attention towards internal-associative modes at the higher intensities and durations and away from external-dissociative mode experienced at lower intensities

(Noble and Robertson 1996). This is a possible explanation for the reduced variability in power output, and therefore physiological responses, as intensity and duration increased. However, in contrast to the findings of the current study, some research has suggested that when athletes are instructed to perform maximal effort time trials, reliability of performance is high but may decline as duration is increased (Schabort *et al.* 1998). The apparent reasons for these conflicting findings are unclear but could be related to fatigue over the longer duration efforts involved in the study of (Schabort *et al.* 1998), as well as methodological in nature as participants were instructed to complete the exercise "as fast as possible" and therefore may have resulted in differing pacing profiles to the present study.

Changes in skeletal muscle oxygenation follow expected patterns of decreasing ΔTSI% ΔO_2Hb and increasing ΔHHb with the increase of exercise intensity (Jones, Hesford and Cooper 2013; Niemeijer *et al.* 2017; Sperandio *et al.* 2009; Kemps *et al.* 2009). Duration could be seen to impact skeletal muscle oxygenation less than intensity, with differences only being found for ΔTSI% and ΔHHb during SHORT bouts, likely due to inadequate time for steady-state skeletal muscle oxygen consumption to be attained before the end of the exercise bout, compared to MED or LONG (Saltin *et al.* 1998; Nioka *et al.* 1998; Sperandio *et al.* 2009). Interestingly, ΔO_2Hb did not differ in this manner, displaying similar levels across all durations for each RPE anchor. NIRS data displayed extremely large levels of both WAV and BAV, particularly ΔTSI% and ΔO_2Hb, with ΔHHb presenting lower levels of variability in most cases. The levels of WAV observed in ΔTSI%, ΔO_2Hb, and ΔHHb were not affected by changes in intensity or duration, although BAV reduced with increased intensity (Table 4.5). These findings are somewhat in contrast to previous research, which has shown increased reliability of skeletal muscle oxygenation measurements at higher versus lower work rate (Gerz *et al.* 2013), suggesting that blood volume and blood flow may be more variable at lower intensities due to the reduced physiological demand on the working muscle.

Previous research has demonstrated a difference in perceptual response to exercise between trained and untrained individuals (Hassmén 1990), suggesting competitive athletes may possess a heightened ability to accurately and reliably utilise RPE as a method to regulate their exercise intensity. Indeed, Barroso *et al.* (2014) have previously suggested that perceptual responses (in this case, sRPE) are more accurate when the athlete has more experience. The notion that more experienced athletes are better equipped to perceive

effort accurately and reliably is supported by research suggesting that individuals can better identify intensity levels if they experience these levels frequently (Gearhart 2004). As the present study did not involve a comparison group of untrained individuals, it is not possible to support or refute this notion, however, future research may look to investigate the differences in the changes in reliability between trained and untrained individuals as intensity and duration are manipulated.

Maximal time trials have been observed to have higher reliability compared to any of the durations or intensities investigated in the current study. WAV observed from 4 min efforts in the current study display increasing reliability as intensity increases; 15.4 % (RPE 9), 10.8 % (RPE 13), and 8.6 % (RPE 17), which shows agreement with lower CVs displayed from maximal 4 min TT's; 2.2 % (MacInnis, Thomas and Phillips 2019) and 2.0 % (Driller *et al.* 2014). Longer maximal efforts similarly display higher levels of reliability compared to shorter efforts; such as 20 min TT 1.4 % (MacInnis, Thomas and Phillips 2019), 20 min TT 1.3 % (Driller 2012), 16.1 km TT 2.7 % (Sparks *et al.* 2016), and 20 km TT 2.7 % (Sporer and McKenzie 2007). Similarly, in the present study, increased levels of reliability were observed during 8 min efforts; 19.7 % (RPE 9), 9.4 % (RPE 13), and 5.3 % (RPE 17). The above suggests that the adoption of intensity prescriptions of a high or maximal self-paced intensity and longer duration intervals in a training session format could provide a novel opportunity to homogenise the exercise prescription. The higher the self-paced exercise intensity, the more consistent the power output distribution and physiological response on a single-bout basis. The intensity prescription of *maximal session effort*, which is the maintenance of high levels of physical exertion over a duration that would result in a maximal exertion for a given training session, has been utilised in research (Seiler and Sylta 2017; Abbiss *et al.* 2015), but not with the goal of assessing individual variability in exercise training response. However, based on the findings in the current study, the utilisation of effort-based prescriptions to elicit a reliable exercise stimulus may be limited to high or maximal session effort prescriptions, and therefore limit the application within lower intensity training. Nevertheless, this training methodology could hold potential for decreasing levels of individual variability in response to high-intensity training.

4.9 Conclusion

In conclusion, the present study demonstrates that using self-paced exercise intensity prescriptions at higher effort levels and longer durations result in the greatest consistency on both a within-athlete and between-athlete. This presents a direction to investigate the use of maximal effort prescriptions for whole training sessions in order to provide greater consistency of training stimulus and potentially greater consistency in long-term training response.

Chapter 5 - Variability of acute physiological response to maximal isoeffort training sessions

5.1 Abstract

Introduction: Prescribing exercise training intensity as %$\dot{V}O_{2max}$ has been shown to elicit variable responses between and within individuals. This study aimed to investigate individual variability associated with various formats of self-paced effort-based training. **Methods:** Seventeen well-trained male competitive cyclists ($\dot{V}O_{2max}$: 59.4 ± 8.2 ml·kg·min^{-1}) completed 9 exercise trials, each consisting of either short (SHORT) or long (LONG) interval, or continuous (CONT) effort based training formats, each repeated 3 times. Intensity prescription used a maximal session effort-based approach. Exercise performance and physiological responses were analysed to identify the within- (WAV) and between-athlete (BAV) variability using CV, and total variability (TV) which was calculated as the ratio of WAV to BAV. **Results:** Exercise power output was different across the 3 formats (SHORT; 382 ± 55 W, LONG 310 ± 45 W, CONT: 262 ± 40 W: $P <$ .001), with no difference in HR (SHORT: 164 ± 10, LONG: 163 ± 10, CONT: 164 ± 10 bpm; $P >$ .05). Average BLa^{-1} during SHORT and LONG was higher than in CONT (9.5 ± 3.0 and 9.1 ± 2.4 mmol.L^{-1} vs. 6.3 ± 2.1 mmol.L^{-1}; $P <$ .001). Average session RPE was highest in SHORT and LONG compared to CONT (18 ± 1 and 18 ± 1 vs. 17 ± 1, $P <$ .001), but final RPE was matched between all formats (SHORT: 19 ± 1, LONG: 19 ± 1, CONT: 19 ± 1; $P >$ .01). Average CV's for power output, cardiovascular, and perceptual response TV, BAV, and WAV were lowest in LONG (10.8 %, 10.9 %, 4.3 %) followed by SHORT (11.4 %, 11.5 %, 4.7 %) and highest in CONT (12.4 %, 12.5 %, 5.0 %). Expired gas TV and BAV were lowest in LONG (11.8 % and 11.9 %), followed by SHORT (12.5 % and 12.6 %), and CONT (13.3 % and 13.4 %). Expired gas WAV was lowest in LONG (4.3 %), followed by CONT (4.4 %), and SHORT (5.1 %). **Conclusion:** LONG format sessions display the greatest overall consistency in both performance and physiological response compared to SHORT and CONT sessions. When using an effort-based intensity prescription, LONG intervals result in a more homogenous training stimulus compared to shorter intervals or continuous exercise.

5.2 Introduction

The findings of Chapter 4 indicate that when self-paced exercise intensity is performed at higher effort levels and longer durations, within-athlete and between-athlete variability is lower compared to lower effort levels and shorter durations. This highlights the potential for the use of effort-based prescriptions to prescribe individualised exercise intensity (Seiler 2010) in order to generate a targeted stimulus for adaptation (Coffey and Hawley

2007; Booth *et al.* 1998). Extensive research has been completed to investigate the efficacy of various training prescriptions, leading to a better understanding of the physiological responses and adaptations that follow training (MacInnis and Gibala 2016; Booth *et al.* 1998; Petriz *et al.* 2017; Camera, Smiles and Hawley 2016; Stepto *et al.* 2009). Further insight has been gained from the analysis of the training regimens of elite performers (Smith 2003; Seiler and Tønnessen 2009). Despite this deep understanding, it could be argued that a solid scientific basis from which to derive effective training programs is still lacking (Borresen and Lambert 2009; Hopker and Passfield 2014). The direct applicability of scientific theory to the implementation of training becomes difficult due to the confounding factors present in training research, one of which is the presence of individual variability in the physiological response to an exercise training stimulus.

Individual variability in response to exercise training confounds research findings as it reduces effect sizes, increasing the required number of participants, and creates uncertainty regarding the efficacy of the training prescriptions involved. High levels of between-athlete variability are seen in response to standardised chronic training prescriptions not only in terms of physical performance but also most common physiological measurements (Mann, Lamberts and Lambert 2014; Bouchard and Rankinen 2001). Some of the variability in chronic training response has been linked to a genetic basis for so-called training "*responders*" and "*non-responders*" (Ehlert, Simon and Moser 2013; Bouchard and Rankinen 2001; Bouchard, Sarzynski, *et al.* 2011). Further, variability in the training response may be accounted for by random measurement error (Hopkins 2004; Hopkins 2000; Hopkins *et al.* 2009; Scharhag-Rosenberger *et al.* 2012). However, an issue yet to be fully explored as a potential source of variability relates to the way training is prescribed and standardised (Mann, Lamberts and Lambert 2014).

There are three distinct aspects of individual variability; within-athlete variability, between-athlete variability, and the between-athlete variability of within-athlete variability, also known as "*total individual variability"* (Nesselroade 1991). The total individual variability describes how an individual's response varies between repeated interventions and how different this variation is between individuals within an investigation. A first step in reducing the individual variability resulting from a training intervention is to prescribe training intensity in a manner that results in a homogenous and predictable response at both an individual and group level. Whilst the external training load, such as cycling power

output or running speed, is commonly the means of specifying the training prescription, it is probably the internal exercise-induced signalling cascade that dictates resulting in training adaptations, not the external load itself (Mann, Lamberts and Lambert 2013; Coffey and Hawley 2007). The distinct challenge in this regard appears to be identifying the way training can be prescribed easily and consistently across a group of individuals (Hopker and Passfield 2014). Traditionally, the prescription of exercise training intensities has been derived from standardised percentages of $\dot{V}O_{2max}$ (Mann, Lamberts and Lambert 2013; Mayes, Hardman and Williams 1987; Poole and Gaesser 1985; Hurley *et al.* 1984). However, the between-athlete variability in performance that occurs during exercise prescribed in this manner is large (Coyle *et al.* 1988; Scharhag-Rosenberger *et al.* 2010; Katch *et al.* 1978; Meyer, Gabriel and Kindermann 1999; Orok *et al.* 1989; Weltman *et al.* 1990; Weltman *et al.* 1989). For example, the time to exhaustion when exercising at 88 %$\dot{V}O_{2max}$ can range from 12 to 75 minutes (Coyle *et al.* 1988). In addition, at a fixed exercise intensity of 75%$\dot{V}O_{2max}$, BLa^{-1} responses ranged from 1.4 – 4.6 $mmol.L^{-1}$ and intensity relative to each individual's anaerobic threshold ranged from 86 % – 118 % (T Meyer, Gabriel and Kindermann 1999). Scharhag-Rosenberger *et al.* (2010) found that as prescribed intensity increases from 60 %$\dot{V}O_{2max}$ to 75 %$\dot{V}O_{2max}$, the variability in the metabolic response increases. These findings suggest that a consistent level of training stimulus between different individuals is difficult to achieve. Considering this, it is likely that the utilisation of standardised percentages of $\dot{V}O_{2max}$ contributes a source of variability to not only the acute training session stimulus but also the subsequent chronic training adaptation. A different method of prescribing training that may elicit a more homogenous response within- and between-individuals is the "*isoeffort*", or effort-based approach (Seiler *et al.* 2013; Seiler and Hetlelid 2005; Seiler and Sjursen 2004). The isoeffort approach is used by coaches to prescribe training (Seiler and Hetlelid 2005; Seiler and Sjursen 2004) and requires athletes to self-pace exercise intensity to produce a specified session effort (Seiler and Sylta 2017; Abbiss *et al.* 2015; Seiler *et al.* 2013). Currently, the individual variability in training response to effort-based training sessions and whether this can reduce the individual variability observed is unknown.

It has been established that HIIT is an effective method of improving performance in endurance athletes (Laursen 2010), and can be classified into two common formats; longer work intervals of 3 – 5 min, or shorter work intervals < 1 min, both types interspersed with recovery periods (Tschakert and Hofmann 2013). It has been shown that HIIT is capable of improving endurance performances in trained endurance athletes using both long (Tabata

et al. 1996; Iaia *et al.* 2008; Gunnarsson and Bangsbo 2012) and short (Westgarth-Taylor *et al.* 1997; Rønnestad, Ellefsen, *et al.* 2014; Lindsay *et al.* 1996) interval formats. Despite the potential for overall improvement in endurance performance in trained athletes, there are shortcomings of HIIT in improving $\dot{V}O_{2max}$, fractional utilisation of $\dot{V}O_{2max}$ (Seiler *et al.* 2013; Rønnestad, Hansen and Ellefsen 2014), and work economy (Rønnestad, Hansen and Ellefsen 2014; Sylta *et al.* 2016; Kohn, Essén-Gustavsson and Myburgh 2011). Furthermore, differences in performance improvements between HIIT interval formats have been observed, such as the cyclists being able to tolerate higher BLa^{-1} concentration during performance trials following short interval training versus long interval training (Rønnestad *et al.* 2015). A recent comparison of short and long interval HIIT using effort-based intensity prescriptions has demonstrated differences in chronic training response (Rønnestad *et al.* 2020), which further highlights the need to fully understand the acute physiological responses between effort-based training and the individual variability which is present.

Therefore, the aim of this study was to investigate how using either long or short interval formats or continuous formats affect the individual variability observed when session effort is controlled using an isoeffort prescription. It was hypothesised that the individual variability would differ based on session format, with shorter interval format sessions resulting in higher variability overall in comparison to longer intervals or continuous efforts.

Hypotheses

H_1 – Sessions utilising short interval formats result in higher total variability in both performance and physiological response to exercise compared to long interval or continuous training formats.

H_0 – There is no difference observed in variability between the short interval, long interval, or continuous session formats.

5.3 Methodology

5.3.1 Participants

Seventeen well-trained male cyclists (mean ± SD (95 % CI): age 35 ± 12 years (CI: ± 5.5), height 175.6 ± 8.8 cm (CI: ± 4.2), mass 71.4 ± 11.0 kg (CI: ± 5.3), $\dot{V}O_{2max}$ 59.4 ± 8.2 $mL \cdot kg \cdot min^{-1}$ (CI: ± 3.8), maximum minute power (MMP) 378 ± 45 W (CI: ± 22), HR_{max} 182 ± 9 bpm (CI: ± 4)), with at least 3 years of cycling training and racing experience (corresponding to Performance Level 3 – 4 ;de Pauw *et al.* 2013), provided written informed consent to voluntarily participate in the study which held full ethical approval from the local institutional ethics committee according to the Declaration of Helsinki.

5.3.2 Study design and experimental procedures

Participants visited the laboratory on 10 separate occasions to complete one ramp test to exhaustion and 9 exercise testing sessions over a period of 9 ± 3 weeks. In Visit 1, participants completed an incremental exercise test to identify $\dot{V}O_{2max}$ and performance parameters (see *Maximal incremental test* for details). During Visit 1, participants were also familiarised with the laboratory equipment used during the subsequent standardised exercise sessions. Visits 2 to 10 comprised of 9 supervised exercise sessions involving 3 exercise session formats, each repeated 3 times in a randomised manner. Exercise sessions consisted of short intervals comprising of 2 sets of 20 repeats of 30 sec work and 30 sec recovery (SHORT), long intervals comprising of 4 repeats of 5 min work and 5 min active recovery (LONG), or a continuous exercise bout for 40 min (CONT), see Table 5.1 and Figure 5.1. Sessions were completed using a maximal iso-effort and iso-time format of 40 min. (see *Exercise testing sessions* for details). All visits for each participant were completed within the same 3-h period of the day, and participants were asked to attend in a euhydrated state, to maintain a consistent diet and lifestyle, as well as to avoid alcohol and strenuous exercise the day before the sessions. Visits were separated by at least 72 h to ensure full recovery between exercise testing sessions. To allow for adequate familiarisation prior to data collection, participants were asked to attempt to incorporate effort-based training bouts in their own training before commencing the study. A cooling fan was present, and plain water was available for participants to drink ad libitum.

Table 5.1- Characteristics of the SHORT, LONG, and CONT exercise protocols.

	SHORT	LONG	CONT
Duration of work interval (s)	30	300	2,400
Duration of rest period (s)	30	300	0
Number of repeats per set	20	4	1
Number of sets	2	1	1
Recovery time between sets (s)	300	0	0
Total work duration per session (s)	1,200	1,200	2,400
Total rest duration per session (s)	1,200	1,200	0
Total 'training period' duration (s)	2,400	2,400	2,400
Total session time (s)	4,200	3,600	3,900

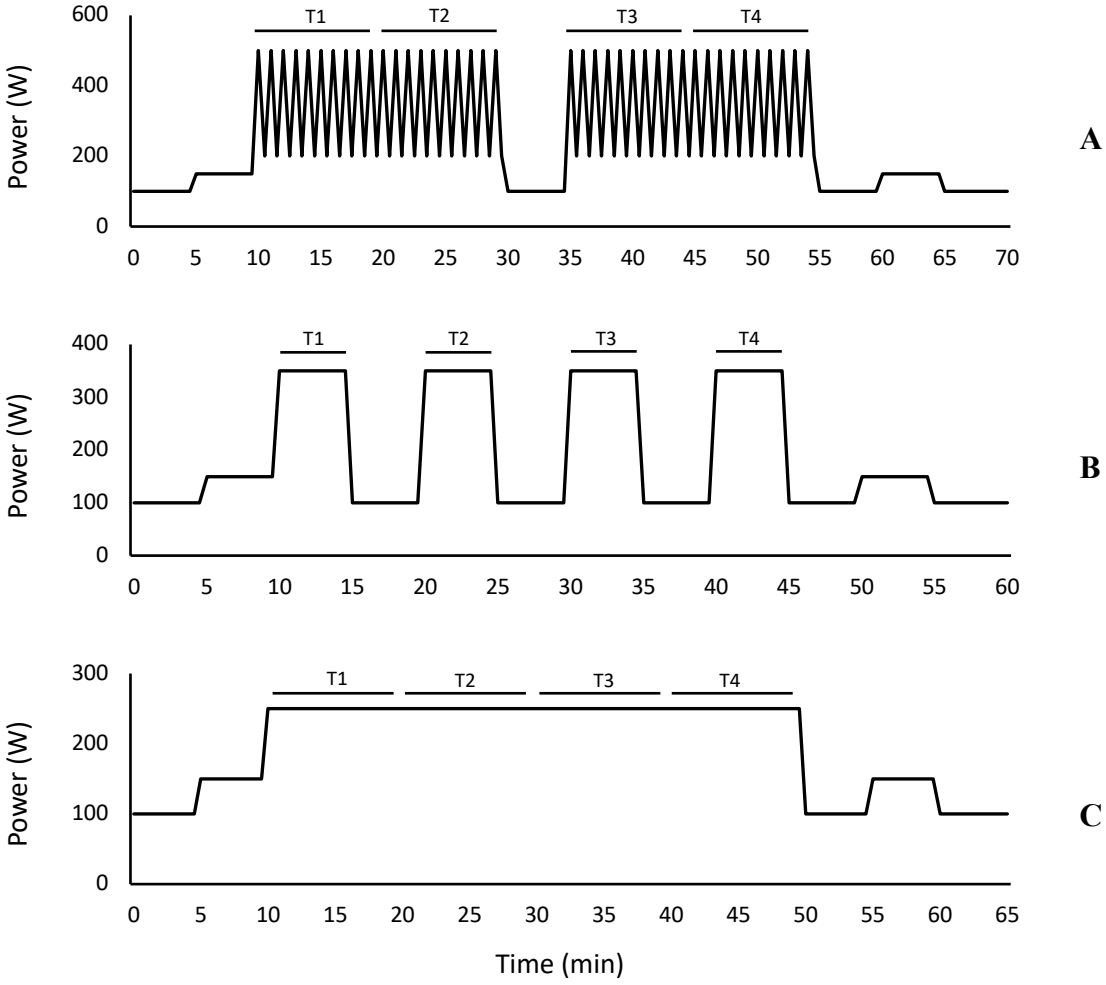


Figure 5.1 – Session format protocols and time segments for analysis. A = SHORT, B = LONG, C = CONT. T1, T2, T3, and T4 denote time segments used for analysis.

Maximal incremental test

Participants completed a maximal incremental test on a bicycle ergometer (Cyclus2, RBM Electronics, Leipzig, Germany) to identify MMP, $\dot{V}O_{2max}$, and HR_{max}. After warming up at 100 W for a period of 10 min, the external load was increased by 20 W every 60 sec until volitional exhaustion, defined as the point where self-selected cadence dropped below 60 rpm despite strong verbal encouragement. MMP was calculated as the highest power output averaged over a period of 60 sec, $\dot{V}O_{2max}$ was calculated as the highest $\dot{V}O_2$ averaged over a period of 30 sec, and HR_{max} was identified as the highest HR value reached in the incremental test.

Exercise testing sessions

Participants were provided with elapsed session time and their performance data during all training sessions, along with a graphical representation of the session format. Uniform and strong verbal encouragements were given from investigators across every session to ensure a maximal session effort was achieved.

5.3.2.1 Physiological measurements

During each session, power output was measured, and heart rate was transmitted using a compatible heart rate strap (Cyclus2 heart rate, RBM Electronics, Leipzig, Germany).

Respiratory gas exchange data were assessed continuously throughout all sessions using an online gas analyser (Metalyzer 3B, CORTEX Biophysik GmbH, Leipzig, Germany) to $\dot{V}O_2$, $\dot{V}CO_2$, $\dot{V}_E$, and ventilatory equivalents $\dot{V}_E$ /$\dot{V}O_2$ and $\dot{V}_E$ /$\dot{V}CO_2$.

Muscle oxygenation was measured using spatially resolved dual-wavelength NIRS (Portamon, Artinis Medical Systems, BV, Netherlands), with the optode positioned 10cm superior to the lateral epicondyle of the femur at the distal end of the vastus lateralis muscle and secured with muscle tape and bandage. Blood oxygenation was quantified by the change in TSI%, O_2Hb, HHb, and tHb. All NIRS data were corrected to a pre-session baseline measurement.

BLa^{-1} concentration was measured using a finger-prick capillary blood sample to serve as a method of establishing the degree of energy produced using anaerobic glycolysis (Gladden 2008). The thumb or index finger of the participant was cleaned using an alcohol wipe and allowed to dry. A disposable safety lancet (AccuCheck Safe-T-Pro Plus, Mannheim, Germany) was then used to puncture chosen sample site, and the first drop of blood was cleaned away. A 20 µL capillary sampling tube was then used to collect a fresh blood sample, and the wound covered using a sterile tissue if further sampling was required. The capillary tube was immediately placed in a 10 mL vial containing 2 mL heparinised phosphate-buffered solution and placed in an automated lactate analyser (Biosen C_Line, EKF Diagnostic, Barleben, Germany). The analyser automatically self-calibrated at 60-min intervals and was also calibrated prior to samples being run using a 12 $mmol.L^{-1}$ standard solution.

5.3.2.2 Psychological measurements

The DALDA (Coutts, Slattery and Wallace 2007) questionnaire was administered immediately prior to each exercise session. Subject RPE using the Borg 6 – 20 scale (Borg 1982b) was recorded at the end of every time segment. Immediately following each exercise session, the perceived session workload was assessed using the NASA-TLX (Hart and Staveland 1988; Hart 2006).

Data processing and statistical analysis

Gas data were analysed using a 10-sec rolling average and analysed for each time segment in the case of LONG and CONT, and the 30 sec of data in response to each effort were used for analysis in SHORT. Expired gas data were analysed relative to $\dot{V}O_{2max}$ and expressed as a percentage to quantify time spent exercising relative to $\dot{V}O_{2max}$.

NIRS data is presented for the average of the change in oxygenation metric for each time segment in a session (the difference between first and last 30 sec of NIRS data for each time segment; $\bar{X}$ Interval Δ), as well as the change between the first and the last time segment in a session (the difference between T1 – T4; Session Δ).

For the purposes of data analysis, sessions data from the three conditions (SHORT, LONG, and CONT) and three repeats of each condition (SHORT_R1/R2/R3, LONG_R1/R2/R3, and CONT_R1/R2/R3) were segmented into four separate time points; T1, T2, T3, T4 (see Figure 5.1) in order to provide session data for each participant. Session data was then collated for each session format across all participants and combined to provide a "*Grand Summary*". Prior to statistical analysis, all data were checked for normality of distribution. Sphericity of the data was investigated using the Mauchly test and the Greenhouse-Geisser adjustment was made when data was deemed non-spherical. Data are reported as mean and standard deviation (mean ± SD), and CVs are presented as a percentage unless specified otherwise. When assessing variability, low CVs indicating a consistent response and high CVs displaying variable response.

Repeated-measures ANOVA with Bonferroni corrections were used to analyse power output and physiological response data for exercise training formats. When significant differences were found, post-hoc tests were used to determine where differences occurred. Effect sizes were calculated using η_p^2 and were defined as small, medium, or large based upon 0.10, 0.25, and above 0.40, respectively (Cohen 1988).

Quantification of individual variation observed was completed by calculating CVs for the WAV, BAV, and TV of each parameter by expressing the standard deviation relative to the mean for each parameter. Linear mixed modelling was completed to analyse the variability in power output, work done, HR, BLa^{-1}, RPE, %MMP, $\%HR_{max}$, $\dot{V}_E$, $\dot{V}_E/\dot{V}O_2$, $\dot{V}_E/\dot{V}CO_2$, respiratory exchange ratio (RER), Time at $\%\dot{V}O_{2max}$, TSI%, O_2Hb, HHb, and tHb for each combination of duration and intensity.

Furthermore, linear mixed modelling was used to assess the relative contribution of both WAV and BAV to the total variability observed (Bliss 1967; Hansen *et al.* 1997; Bagger, Petersen and Pedersen 2003). Bland-Altman plots were used to display between-athlete variability of each session format by plotting the average difference in data between the three repeats (Average of R1-R2, R2-R3, R1-R3) and average session performance for each individual (Bland and Altman 1986). In addition, reference lines and values are presented for the mean ± 1.96 SD. Participants who were deemed '*variable*' had a session

difference SD greater than 2 times the mean session difference SD across all participants; encapsulating the 95 % confidence interval (Dai and Wang 1992).

Critical difference levels were used to indicate whether the difference observed between two work bout measurements was significant (Fraser, Hyltoft and Lytken 1990; Fraser and Harris 1989; Harris and Yasaka 1983; Bagger, Petersen and Pedersen 2003). The following equation was utilised to calculate critical difference:

$$first\ measurement \times Z \times \sqrt{2} \times WAV$$

where the first measurement is the measurement of interest, Z =1.65, which results in establishing a two-tailed probability of 10 %, and WAV is the observed within-subjects CV for the parameter involved.

Linear mixed models, ANOVA's, and post-hoc tests were conducted using the Statistical Package for the Social Sciences, version 26 for Mac OS X (SPSS, IBM®, Armonk, New York, USA), and an alpha level was set at $P < .05$ for the criteria for detection of significance in all cases. CV and critical differences were calculated in Microsoft Excel (Excel v16.3 Microsoft, Redmond, Washington, USA).

5.4 Results

5.4.1 Between-session differences

5.4.1.1 Power output, cardiovascular, perceptual, and blood lactate response to exercise sessions

Power output, work done, HR, HR_{max}, BLa^{-1}, RPE and End RPE are reported in Table 5.2. Differences were observed between sessions for power output ($F_{(1.154, 18.461)} = 248.035$; $\eta_p^2 = .939$, $P < .001$) and work done ($F_{(1.072, 17.153)} = 467.999$; $\eta_p^2 = .967$, $P < .001$). HR_{max} was lower in SHORT versus LONG ($P = .04$), but not between SHORT and CONT ($P = .428$) or LONG and CONT ($F_{(2, 32)} = 3.261$; $\eta_p^2 = .169$, $P > .999$). Both SHORT and LONG were higher versus CONT for BLa^{-1} ($F_{(2, 32)} = 43.232$; $\eta_p^2 = .730$, $P < .001$) and RPE ($F_{(2, 32)} = 23.560$; $\eta_p^2 = .596$, $P < .001$). No differences were observed between sessions for HR ($F_{(2, 32)} = .968$; $\eta_p^2 = .057$, $P = .391$) or End RPE ($F_{(2, 32)} = 1.773$; $\eta_p^2 = .100$, $P = .186$).

Variability analysis

In power output, work done, HR, HR_{max}, BLa^{-1}, RPE and End RPE, presented in Table 5.2, TV, BAV, and WAV were lowest in LONG (10.8 %, 10.9 %, 4.3 %) followed by SHORT (11.4 %, 11.5 %, 4.7 %) and highest in CONT (12.4 %, 12.5 %, 5.0 %). Levels of TV, BAV, and WAV across all session formats were lowest in HR_{max} (3.4 %) , followed by End RPE (3.9 %), and highest in BLa^{-1} (25.9 %).

Table 5.2 – Power output, cardiovascular, and perceptual response during RPE-clamped exercise bouts showing mean data, standard deviation, coefficients of variation, variance components, and critical difference levels.

				Coefficient of variation			Variance components			Critical difference
Variable		**Mean**	**SD**	**TV (%)**	**BAV (%)**	**WAV (%)**	**Var total**	**BAV (%)**	**WAV (%)**	**WAV (%)**
Power output (W)										
SHORT	*a*	382	55	14.4	14.6	3.4	3112.9	0.9	0.1	241.3
LONG	*a*	310	45	14.4	14.7	2.7	2073.1	1.0	0.0	158.2
CONT	*a*	262	40	15.2	15.4	2.6	1634.7	1.2	0.0	126.0
Work done (kJ)										
SHORT	*a*	1146	165	14.4	14.6	3.5	27995.7	92.3	7.7	723.9
LONG	*a*	744	107	14.4	14.7	2.8	11964.1	95.8	4.2	381.9
CONT	*a*	629	95	15.2	15.4	2.6	9426.3	121.6	3.8	299.0
HR (bpm)										
SHORT		164	10	6.0	6.0	2.5	95.9	84.1	15.9	77.1
LONG		163	10	5.9	5.9	2.3	92.7	83.8	16.2	71.5
CONT		164	10	6.3	6.4	2.6	111.4	69.7	19.5	78.1
HR_{max} (bpm)										
SHORT	*b*	176	8	4.5	4.6	1.2	66.4	91.2	8.8	38.7
LONG		179	8	4.3	4.3	1.9	58.8	78.9	21.1	62.7
CONT		178	8	4.3	4.4	1.3	60.0	77.3	8.5	44.1
BLa^{-1} ($mmol.L^{-1}$)										
SHORT	*c*	9.5	3.0	31.3	31.6	16.8	9.0	72.6	27.4	31.6
LONG	*c*	9.1	2.4	25.9	26.1	14.1	5.7	66.2	33.8	24.2
CONT		6.3	2.1	34.2	34.3	18.6	4.7	79.9	26.0	21.3
RPE										
SHORT	*c*	18	1	5.1	4.9	3.9	0.8	29.8	70.2	12.7
LONG	*c*	18	1	5.7	5.7	3.9	1.0	32.9	67.1	13.0
CONT		17	1	6.8	6.8	4.0	1.3	26.1	48.1	12.4
End RPE										
SHORT		19	1	4.1	4.0	1.8	0.6	48.5	51.5	6.6
LONG		19	1	4.8	4.9	2.3	0.9	50.3	49.7	8.4
CONT		19	1	5.0	4.9	3.1	0.9	48.4	58.8	11.0

a = Significant difference observed between all session formats (P < .001), b = P < .05 vs. LONG, c = P < .001 vs. CONT.

5.4.1.2 Expired gas response during exercise sessions

Absolute $\dot{V}O_2$, relative $\dot{V}O_2$, $\dot{V}_E/\dot{V}O_2$, $\dot{V}_E/\dot{V}CO_2$, RER, and $\dot{V}_E$ are reported in Table 5.3 and time spent above 70 %, 80 %, 85 %, and 90 % $\dot{V}O_{2max}$ are reported in Table 5.4. Absolute $\dot{V}O_2$ was higher in LONG versus SHORT ($P = .002$) and CONT ($F_{(1.470, 23.525)} = 7.292$; $\eta_p^2 = .313$, $P = .009$), with no difference between SHORT and CONT ($P > .999$). Relative $\dot{V}O_2$ was higher in LONG versus SHORT ($F_{(1.219, 19.502)} = 1.717$; $\eta_p^2 = .097$, $P = .004$), with no difference between SHORT and CONT ($P = .683$) or LONG and CONT ($P > .999$). All session formats were different for $\dot{V}_E/\dot{V}O_2$ ($F_{(2, 32)} = 82.301$; $\eta_p^2 = .837$, $P < .001$) and $\dot{V}_E/\dot{V}CO_2$ ($F_{(2, 32)} = 64.559$; $\eta_p^2 = .801$, $P < .001$). RER was higher in SHORT versus LONG ($P = .001$) and versus CONT ($P < .001$), with higher RER also in LONG versus CONT ($F_{(2, 32)} = 36.313$; $\eta_p^2 = .694$, $P = .014$). $\dot{V}_E$ was higher in both SHORT and LONG versus CONT ($F_{(2, 32)} = 35.565$; $\eta_p^2 = .690$, $P < .001$), with no difference between SHORT and LONG ($P = .614$). Time spent above 70 %$\dot{V}O_{2max}$ was higher during SHORT and CONT versus LONG ($F_{(2, 32)} = 28.211$; $\eta_p^2 = .638$, $P < .001$), with no difference between SHORT and CONT ($P = .194$). Time spent above 80 %$\dot{V}O_{2max}$ was higher in CONT versus SHORT ($P = .017$) and versus LONG ($F_{(2, 32)} = 6.912$; $\eta_p^2 = .302$, $P = .042$), with no difference between SHORT and LONG ($P > .999$). Time spent above 85 %$\dot{V}O_{2max}$ was lower in SHORT versus LONG ($P = .003$) and CONT ($F_{(2, 32)} = 6.544$; $\eta_p^2 = .290$, $P = .025$), with no difference between LONG and CONT ($P > .999$). Time spent above 90 %$\dot{V}O_{2max}$ was higher in LONG versus SHORT ($F_{(1.164, 18.630)} = 4.844$; $\eta_p^2 = .232$, $P < .001$), with no difference between SHORT and CONT ($P = .174$) or LONG and CONT ($P > .999$).

Variability analysis

In absolute $\dot{V}O_2$, relative $\dot{V}O_2$, $\dot{V}_E/\dot{V}O_2$, $\dot{V}_E/\dot{V}CO_2$, RER, and $\dot{V}_E$, presented in Table 5.3, TV and BAV were lowest in LONG (11.8 % and 11.9 %), followed by SHORT (12.5 % and 12.6 %), and CONT (13.3 % and 13.4 %). WAV was lowest in LONG (4.3 %), followed by CONT (4.4 %), and SHORT (5.1 %). Levels of TV, BAV, and WAV across all session formats were lowest in RER (4.2 %), and highest in $\dot{V}_E$ (14.0 %). In the parameters presented in Table 5.4, TV, BAV, and WAV were lowest in LONG (49.2 %, 49.4 %, 40.6 %), followed by CONT (81.5 %, 83.0 %, 42.6 %), and SHORT (120.9 %, 117.8 %, 57.3 %). Levels of TV, BAV, and WAV across all session formats were lowest in time spent above 70 %$\dot{V}O_{2max}$ (28.5 %), and highest in time spent above 90 %$\dot{V}O_{2max}$ (122.8 %).

Table 5.3 – Expired gas response during RPE-clamped exercise bouts showing mean data, standard deviation, coefficients of variation, variance components, and critical difference levels.

				Coefficient of variation			Variance components			Critical difference
Variable		**Mean**	**SD**	**TV (%)**	**BAV (%)**	**WAV (%)**	**Var total**	**BAV (%)**	**WAV (%)**	**WAV (%)**
$\dot{V}O_2$ (L.min^{-1})										
SHORT	*c*	3.31	0.47	14.1	14.4	4.5	0.2	84.5	15.5	2.8
LONG		3.46	0.51	14.7	15.0	5.0	0.3	73.1	26.9	3.2
CONT	*c*	3.26	0.52	15.8	16.0	5.0	0.3	71.9	23.4	3.1
$\dot{V}O_2$ (ml.kg.min^{-1})										
SHORT	*c*	47.12	7.39	15.7	16.0	4.8	56.9	85.3	14.7	42.8
LONG		49.20	7.84	15.9	16.2	5.1	63.7	80.3	19.7	46.7
CONT		47.41	7.58	16.0	16.2	5.0	59.2	86.6	18.3	44.7
$\dot{V}_E/\dot{V}O_2$										
SHORT	*a*	38.10	4.26	11.2	11.2	5.3	18.5	70.9	29.1	38.4
LONG	*a*	34.04	3.17	9.3	9.4	3.0	10.2	87.7	12.3	19.5
CONT	*a*	30.95	3.67	11.8	12.1	3.3	13.9	64.3	11.1	18.6
$\dot{V}_E/\dot{V}CO_2$										
SHORT	*a*	35.94	4.21	11.7	11.7	5.7	17.9	70.7	29.3	38.6
LONG	*a*	32.57	3.06	9.4	9.4	3.9	9.3	84.5	15.5	24.2
CONT	*a*	30.32	3.72	12.3	12.5	3.6	14.3	54.9	11.5	20.4
RER										
SHORT	*bc*	1.10	0.06	5.1	5.1	2.6	0.003	68.9	31.1	0.5
LONG	*d*	1.05	0.05	4.7	4.7	2.9	0.002	57.5	42.5	0.6
CONT		1.02	0.05	4.8	4.5	3.1	0.002	62.6	50.2	0.6
$\dot{V}_E$ (L.min^{-1})										
SHORT	*b*	129.06	22.16	17.2	17.4	7.5	505.6	75.9	24.1	184.3
LONG	*b*	125.72	20.87	16.6	16.9	6.0	453.6	72.4	27.6	142.0
CONT		107.67	20.49	19.0	19.2	6.6	431.2	76.1	27.5	130.7

a = Significant difference observed between all session formats (P < .001), b = P < .001 vs. CONT, c = P < .05 vs. LONG, d = P < .05 vs. CONT.

Table 5.4 – Time spent exercising relative to $\dot{V}O_{2max}$ during RPE-clamped exercise bouts showing mean data, standard deviation, coefficients of variation, variance components, and critical difference levels.

				Coefficient of variation			Variance components			Critical difference
Variable		**Mean**	**SD**	**TV (%)**	**BAV (%)**	**WAV (%)**	**Var total**	**BAV (%)**	**WAV (%)**	**WAV (%)**
Time at > 90 % V̇O2max (s)										
SHORT		140.59	312.80	222.5	206.7	83.4	101501.5	54.9	45.1	2602.2
LONG	*a*	507.84	394.35	77.7	78.7	68.8	160366.9	34.5	65.5	7009.7
CONT		438.24	645.47	147.3	150.7	69.6	429507.8	12.9	24.6	5347.7
Time at > 85 % V̇O2max (s)										
SHORT		399.22	549.75	137.7	139.0	70.2	314521.3	78.6	21.4	5311.2
LONG	*c*	808.43	426.87	52.8	53.3	42.9	188582.4	41.0	59.0	6276.1
CONT	*c*	918.04	811.97	88.4	89.8	46.3	682578.7	11.3	30.2	8123.4
Time at > 80 % V̇O2max (s)										
SHORT	*d*	864.71	766.62	88.7	90.5	50.0	610725.7	83.1	16.9	8550.1
LONG	*d*	1011.96	388.76	38.4	38.6	28.5	156391.9	34.3	65.7	5325.1
CONT		1457.06	849.10	58.3	59.4	33.2	748964.7	7.2	20.5	8724.2
Time at > 70 % V̇O2max (s)										
SHORT	*b*	1874.71	646.66	34.5	35.0	25.4	427787.3	47.1	52.9	8862.3
LONG		1273.92	354.73	27.8	26.8	22.0	130467.6	14.4	85.6	5297.9
CONT	*b*	2109.61	670.53	31.8	32.2	21.2	467037.0	4.0	39.1	8380.6

a = P < .001 vs. SHORT, b = P < .001 vs LONG, c = P < .05 vs. SHORT, d = P < .05 vs. CONT.

5.4.1.3 Muscle oxygenation response during exercise sessions

$\bar{X}$ Interval and Session ΔTSI%, ΔO_2Hb, ΔHHb, and ΔtHb are reported in Table 5.5 and Table 5.6, respectively. No differences were found between session formats in $\bar{X}$ΔTSI% ($F_{(2, 30)}$ = 3.229; η_p^2 = .177, *P* = .054), $\bar{X}$ΔHHb ($F_{(2, 30)}$ = 4.145; η_p^2 = .216, *P* = .057), $F_{(2, 30)}$ = .097; η_p^2 = .006, *P* = .908). $\bar{X}$ΔO_2Hb was higher in LONG versus CONT ($F_{(2, 30)}$ = 8.424; η_p^2 = .360, *P* = .004), with no difference between SHORT and LONG (*P* = .338), or SHORT and CONT (*P* = .063). No difference was found in ΔTSI% ($F_{(2, 30)}$ = 2.707; η_p^2 = .153, *P* = .083), ΔHHb ($F_{(2, 30)}$ = 1.686; η_p^2 = .101, *P* = .202), or ΔtHb ($F_{(2, 30)}$ = .281; η_p^2 = .018, *P* = .757) depending on session format. ΔO_2Hb was higher in LONG versus CONT ($F_{(2, 30)}$ = 8.082; η_p^2 = .350, *P* = .003), with no difference between SHORT and LONG (*P* = .325), or SHORT and CONT (*P* = .093).

Variability analysis

In $\bar{X}$ Interval and Session ΔTSI%, ΔO_2Hb, ΔHHb, and ΔtHb, presented in Table 5.5, TV and BAV were lowest in SHORT (-108.0 % and -14.6 %), followed by CONT (32.7 % and 68.0 %), and LONG (432.2 % and 128.4 %). WAV was lowest in LONG (-28.6 %), followed by CONT (45.3 %), and SHORT (64.1 %). Levels of TV, BAV, and WAV across all session formats were lowest in $\bar{X}$ΔO_2Hb (-118.2%), and highest in $\bar{X}$ΔtHb (288.1 %). In the parameters presented in Table 5.6, TV was lowest in SHORT (-53.8 %), followed by CONT (78.6 %), and LONG (376.0 %). BAV was lowest in LONG (-224.6 %), followed by SHORT (-56.5 %), and CONT (119.5 %). WAV was lowest in CONT (-264.9 %), followed by SHORT (46.7 %), and LONG (108.9 %). Levels of TV, BAV, and WAV across all session formats were lowest in ΔO_2Hb (-201.4 %), and highest in ΔtHb (192.5 %).

Table 5.5 – Muscle oxygenation response during RPE-clamped interval bouts showing mean data, standard deviation, coefficients of variation, variance components, and critical difference levels.

			Coefficient of variation			Variance components			Critical difference
Variable	**Mean**	**SD**	**TV (%)**	**BAV (%)**	**WAV (%)**	**Var total**	**BAV (%)**	**WAV (%)**	**WAV (%)**
X̄ Interval ΔTSI%									
SHORT	-1.83	3.88	-212.0	-223.1	-32.2	15.6	72.8	27.2	7.8
LONG	-1.15	5.16	-449.4	-76.9	429.8	26.7	34.5	65.5	-157.6
CONT	-3.36	6.32	-188.0	-151.0	-54.00	39.6	23.2	81.5	50.7
X̄ Interval ΔO2Hb									
SHORT	-0.56	2.16	-388.2	-403.0	-269.2	4.9	60.8	39.2	32.0
LONG	0.27	2.30	841.8	87.0	372.3	5.1	22.1	77.9	22.8
CONT *a*	-1.67	2.49	-149.3	-156.6	-48.6	6.4	17.6	68.0	10.8
X̄ Interval ΔHHb									
SHORT	1.88	2.99	159.5	162.9	24.0	9.3	51.8	48.2	7.5
LONG	0.72	4.56	637.0	148.4	-389.8	21.1	31.3	68.7	-63.7
CONT	2.88	4.81	167.1	151.7	74.3	24.1	27.4	93.3	45.3
X̄ Interval ΔtHb									
SHORT	1.32	3.25	246.3	258.7	71.9	11.0	49.4	50.6	14.0
LONG	0.99	4.33	438.0	429.2	-43.0	19.6	56.1	43.9	-9.6
CONT	1.21	4.79	396.1	427.2	41.1	23.6	46.6	52.4	15.9

a = P < .05 vs. LONG.

Table 5.6 – Muscle oxygenation response during RPE-clamped exercise sessions showing mean data, standard deviation, coefficients of variation, variance components, and critical difference levels.

			Coefficient of variation			Variance components			Critical difference
Variable	**Mean**	**SD**	**TV (%)**	**BAV (%)**	**WAV (%)**	**Var total**	**BAV (%)**	**WAV (%)**	**WAV (%)**
Session ΔTSI%									
SHORT	-2.72	5.52	-202.6	-216.2	-45.8	31.1	63.2	36.8	15.2
LONG	-1.47	6.55	-446.5	99.6	380.2	43.3	40.5	59.5	-186.5
CONT	-4.60	9.39	-204.0	-163.9	-36.2	86.0	20.4	72.2	52.6
Session ΔO2Hb									
SHORT	-0.88	3.16	-357.9	-366.0	0.5	10.4	63.0	37.0	-0.1
LONG	0.33	3.78	1145.7	-1331.6	-8.6	13.8	11.7	88.3	0.2
CONT *a*	-2.31	3.35	-144.9	-146.5	-602.9	11.7	13.8	71.0	246.9
Session ΔHHb									
SHORT	3.03	4.45	146.9	152.1	98.6	20.5	51.0	49.0	46.5
LONG	1.53	7.48	488.7	21.0	100.4	56.5	33.3	66.7	41.4
CONT	3.68	6.97	189.4	161.1	76.9	49.8	37.8	80.8	71.8
Session ΔtHb									
SHORT	2.15	4.27	198.6	204.2	133.5	19.0	53.4	46.6	44.3
LONG	1.86	5.89	316.0	312.6	-36.5	36.0	59.2	40.8	-14.3
CONT	1.37	6.47	474.0	627.4	-497.2	42.6	50.0	46.1	-261.0

a = P < .05 vs. LONG.

5.4.2 Within-session differences

Figure 5.2 displays differences within-session power output, HR, RPE, and BLa^{-1} for all three training session formats. There was no observed change in power output during training sessions for SHORT ($F_{(3, 14)} = 4.026$; $\eta_p^2 = .463$, $P \geq .099$), and LONG ($F_{(3, 14)} = 3.119$; $\eta_p^2 = .401$, $P = .087$), in CONT there was an increase in power output between each time point and T4 ($F_{(3, 14)} = 14.983$; $\eta_p^2 = .763$, $P < .001$). During SHORT, HR rose between T1-2 ($P < .001$) and T3-4 ($P < .001$), with no change between T2-3 ($P = .235$)($F_{(3, 14)} = 23.726$; $\eta_p^2 = .836$). During LONG, HR rose from T1-2 ($P = .005$) and T3-4 ($P < .001$), with no change between T2-3 ($P = .231$)($F_{(3, 14)} = 14.892$; $\eta_p^2 = .761$). HR rose through all time points in CONT ($F_{(3, 14)} = 24.073$; $\eta_p^2 = .838$, $P < .001$). There was an increase in RPE in SHORT between T1-2 and T3-4 ($F_{(3, 14)} = 68.765$; $\eta_p^2 = .936$, $P < .001$), and no change between T2-3 ($P = .077$). There was an increase in RPE in LONG through all time points ($F_{(3, 14)} = 46.881$; $\eta_p^2 = .909$, $P < .001$). There was an increase in RPE in CONT between T1-2 and T3-4 ($F_{(3, 14)} = 82.695$; $\eta_p^2 = .947$, $P < .001$), and no change between T2-3 ($P = .149$). During SHORT, BLa^{-1} rose from T1-2 ($P < .001$), and T3-4 ($P < 0.05$)($F_{(3, 14)} = 32.873$; $\eta_p^2 = .876$), and no change between T2-3 ($P > .999$). During LONG, BLa^{-1} rose from T1-2 ($P < .001$) and T2-3 ($P = .003$)($F_{(3, 14)} = 25.195$; $\eta_p^2 = .844$), and no change between T3-4 ($P = .135$). During CONT, BLa^{-1} rose from T1-2 ($P = .003$) and T3-4 ($P < .001$)($F_{(3, 14)} = 26.044$; $\eta_p^2 = .848$), and no change between T2-3 ($P = .132$).

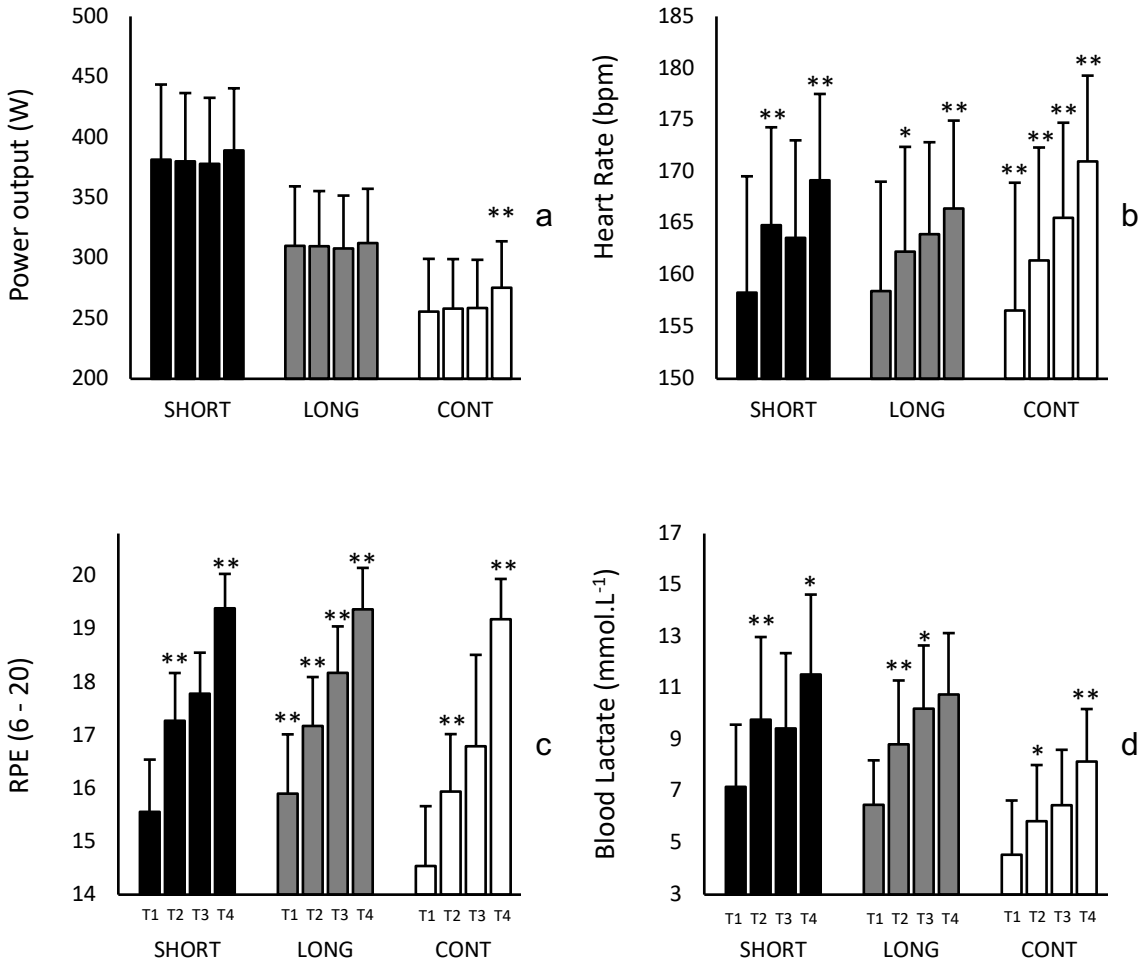


Figure 5.2 - Power output, heart rate, RPE, and blood lactate parameters during training sessions. Each segment is represented by a single column for each session format from T1-T4. Error bars display SD. * = $P < .05$ vs. Previous time point. ** = $P < .001$ vs. Previous time point.

Figure 5.3 displays differences within-session $\dot{V}O_2$, relative $\dot{V}O_2$, $\dot{V}_E/\dot{V}O_2$, $\dot{V}_E/\dot{V}O_2$, RER, and $\dot{V}_E$ for all three training session formats. There was no observed change in absolute or relative $\dot{V}O_2$ during training sessions for SHORT ($F_{(1.601, 25.621)} = 1.04$; $\eta_p^2 = .061$, $P = 383$), or LONG ($F_{(1.310, 20.961)} = .286$; $\eta_p^2 = .018$, $P = .660$), and within CONT there was an increase in $\dot{V}O_2$ between T1-T2 ($F_{(1.194, 19.107)} = 4.953$; $\eta_p^2 = .236$, $P < .001$). $\dot{V}_E/\dot{V}O_2$ response increased between T1-2, T3-4 ($P < .001$), and T2-3 ($F_{(1.169, 18.712)} = 39.957$; $\eta_p^2 = .714$, $P = .012$) in SHORT. In LONG, $\dot{V}_E/\dot{V}O_2$ response increased between T1-2, T2-3 ($P < .001$), and T3-4 ($F_{(1.623, 25.974)} = 51.930$; $\eta_p^2 = .764$, $P = .003$). In CONT, $\dot{V}_E/\dot{V}O_2$ response increased between T1-2, T3-4 ($P < .001$), and T2-3 ($F_{(1.587, 25.387)} = 69.421$; $\eta_p^2 = .813$, $P = .001$). $\dot{V}_E/\dot{V}CO_2$ was different between all time points during SHORT ($F_{(1.299, 20.785)} = 88.487$; $\eta_p^2 = .847$, $P < .001$), LONG ($F_{(1.392, 22.268)} = 124.859$; $\eta_p^2 = .886$, $P < .001$), and CONT ($F_{(1.613, 25.806)} = 95.416$; $\eta_p^2 = .856$, $P < .001$). RER during SHORT decreased between T1-2 ($P = .047$), T2-3 ($P = .027$), and increased between T3-4 ($F_{(1.556, 24.898)} = 9.429$; $\eta_p^2 = .371$, $P = .023$). During LONG, RER decreased between T1-2 ($P < .001$) and T2-3 ($F_{(1.363, 21.816)} = 62.154$; $\eta_p^2 = .795$, $P = .032$), and no change was observed between T3-4 ($P = .571$). In CONT, RER did not change between T1-2 ($P = .063$), T2-3 ($P = .462$), or T3-4 ($F_{(1.447, 23.149)} = 5.109$; $\eta_p^2 = .242$, $P = .120$). $\dot{V}_E$ during SHORT increased between T1-2 ($P < .001$), and T3-4 ($F_{(1.151, 18.419)} = 13.183$; $\eta_p^2 = .452$, $P = .002$), and no change between T2-3 ($P = .772$). During LONG, $\dot{V}_E$ increased between T1-2 ($P < .001$), T2-3 ($P = .013$), and T3-4 ($F_{(1.359, 21.750)} = 25.185$; $\eta_p^2 = .612$, $P = .023$) $\dot{V}_E$ during CONT increased between T1-2 ($P < .001$) and T3-4 ($F_{(1.216, 19.455)} = 29.175$; $\eta_p^2 = .646$, $P = .01$), with change between T2-3 ($P = .371$).

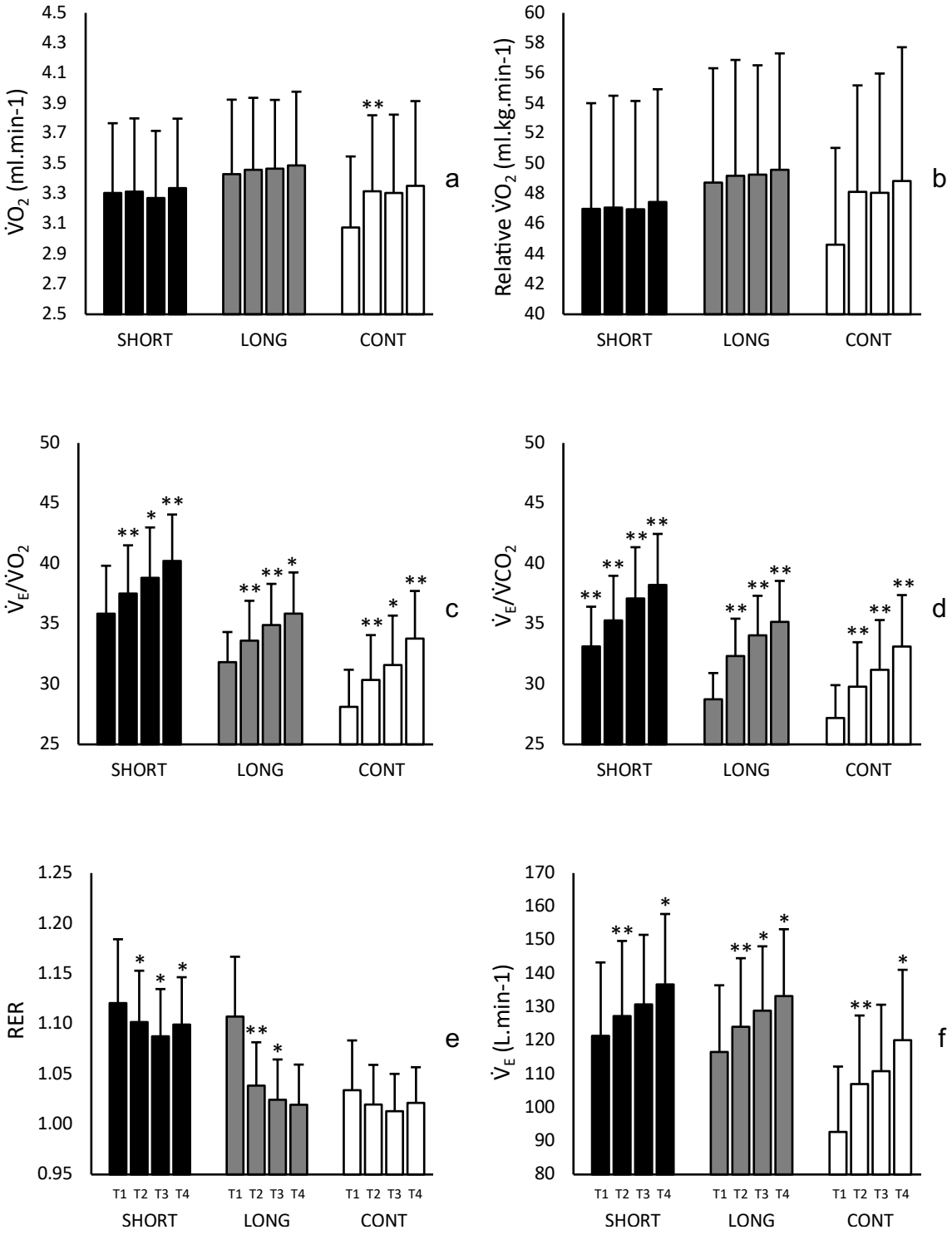


Figure 5.3 – Gas parameters during training sessions. Each segment is represented by a single column for each session format from T1-T4. Error bars display SD. * = $P < .05$ vs. Previous time point. ** = $P < .001$ vs. Previous time point.

Figure 5.4 displays differences within-session TSI%, O_2Hb, HHb, and tHb, all relative to baseline corrections for all three training session formats TSI% during SHORT decreased between T3-4 ($F_{(1.221, 18.318)} = 3.186$; $\eta_p^2 = .175$, $P = .019$), with no change between T1-2 ($P = .101$) or T2-3 ($P > .999$). During LONG, there was no change in TSI% between any time points ($F_{(1.143, 17.145)} = 1.214$; $\eta_p^2 = .075$, $P > .999$). TSI% during CONT decreased between T1-2 ($F_{(1.170, 17.554)} = 5.462$; $\eta_p^2 = .267$, $P = .004$), with no change between T2-3 ($P = .669$) or T3-4 ($P = .988$). O_2Hb during SHORT decreased between T1-2 ($P = .008$) and T3-4 ($P = .016$) and increased from T2-3 ($F_{(1.326, 19.886)} = 3.807$; $\eta_p^2 = .202$, $P = .008$). During LONG there was no change in O_2Hb between any time points ($F_{(1.757, 26.353)} = .293$; $\eta_p^2 = .019$, $P > .999$). O_2Hb during CONT decreased between T1-2 ($P = .034$) and T3-4 ($F_{(1.397, 20.960)} = 9.292$; $\eta_p^2 = .383$, $P = .025$), with no change between T2-3 ($P > .999$). HHb during SHORT increased between T3-4 ($F_{(1.272, 19.074)} = 3.574$; $\eta_p^2 = .192$, $P = .016$) with no change between T1-2 ($P = .190$) and T2-3 ($P > .999$). During LONG there was no change in HHb between any time points ($F_{(1.195, 17.924)} = 1.257$; $\eta_p^2 = .077$, $P > .999$). HHb during CONT increased between T1-2 ($F_{(1.358, 20.376)} = 6.626$; $\eta_p^2 = .306$, $P = . 009$), with no chance between T2-3 and T3-4 ($P > .999$). There was no change in tHb between any time points in SHORT ($F_{(1.276, 19.135)} = 3.201$; $\eta_p^2 = .176$, $P \geq .062$), LONG ($F_{(1.102, 16.535)} = 2.246$; $\eta_p^2 = .131$, $P \geq .156$), and CONT ($F_{(1.250, 18.745)} = .963$; $\eta_p^2 = .060$, $P > .999$).

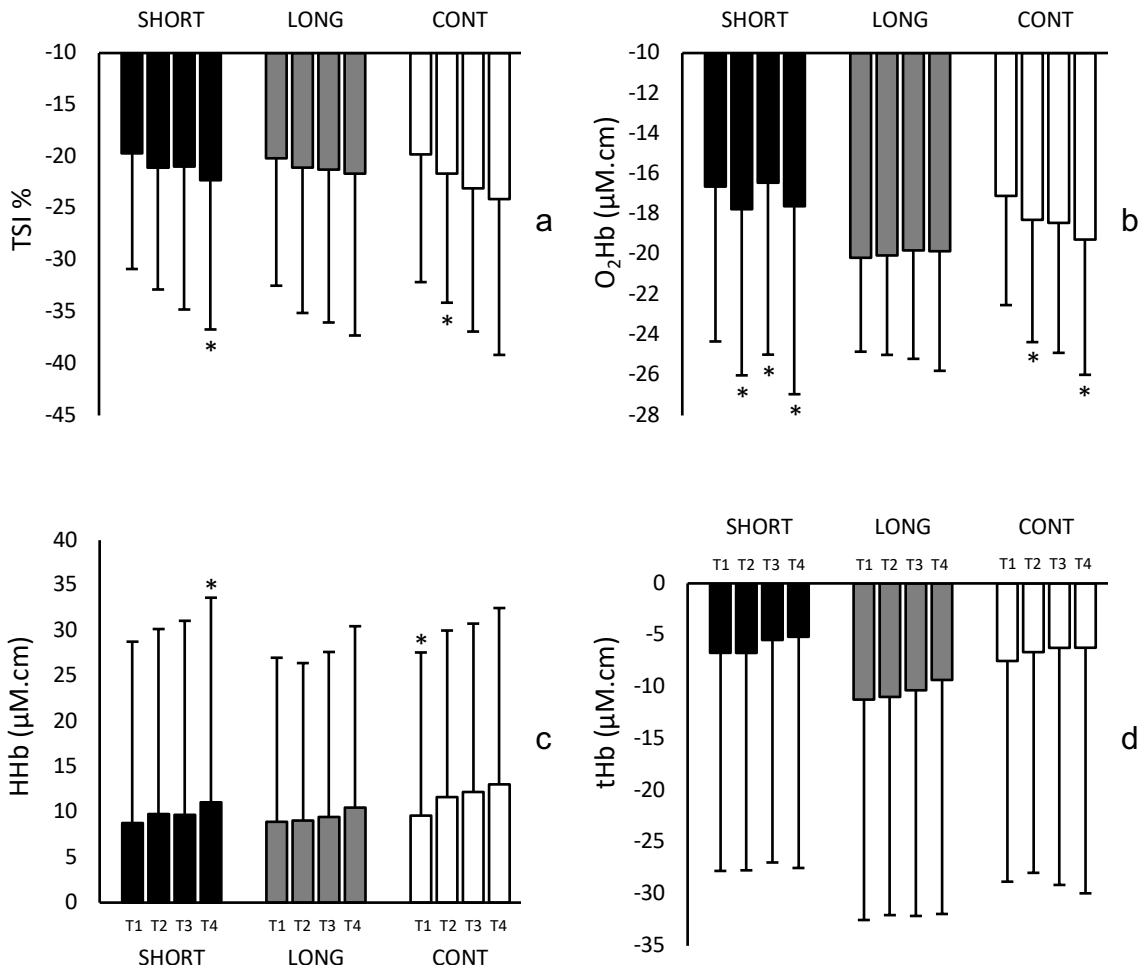


Figure 5.4 – NIRS data from training sessions; a) TSI%, b) tHb, c) O_2Hb, and d) HHb. Each segment is represented by a single column for each session format from T1-T4. Error bars display SD. * = $P < .05$ vs. Previous time point.

5.4.3 Within-athlete variability

Differences in each participant's training session power output relative to MMP are displayed in Figure 5.5, illustrating the degree of within-athlete variability dependent on session format, with participants deemed '*variable*' if individual session SD exceeds 2 times the mean session difference SD across all participants. In session format SHORT, Participants 5, 11, 15, and 17 were identified as being variable (SD ≥ 6.09 %). In format LONG, Participants 2, 9,13, 15, 16, and 17 were identified as being variable (SD ≥ 2.98 %). In format CONT, Participants 2, 3, and 13 were identified as being variable (SD ≥ 2.61 %). Similarly, differences in each participant's HR response relative to HR_{max} during sessions are displayed in Figure 5.6, showing the within-athlete variability dependent on session format. In session format SHORT, Participants 5 and 15 were identified as being variable (SD ≥ 3.25 %). In format LONG, Participants 5, 9, 11, and 12 were identified as being variable (SD ≥ 3.25 %). In format CONT, Participants 4, 5, 11, and 15 were identified as being variable (SD ≥ 4.48 %).

5.4.4 Between-athlete variability

Bland-Altmann plots in Figure 5.7 present session-by-session change in power output for every session completed by every participant (n = 51 sessions) for SHORT, LONG, and CONT. Bland-Altmann plots in Figure 5.8 display the average session difference in power output against the average power outputs for every participant (n = 17) for SHORT, LONG, and CONT, respectively.

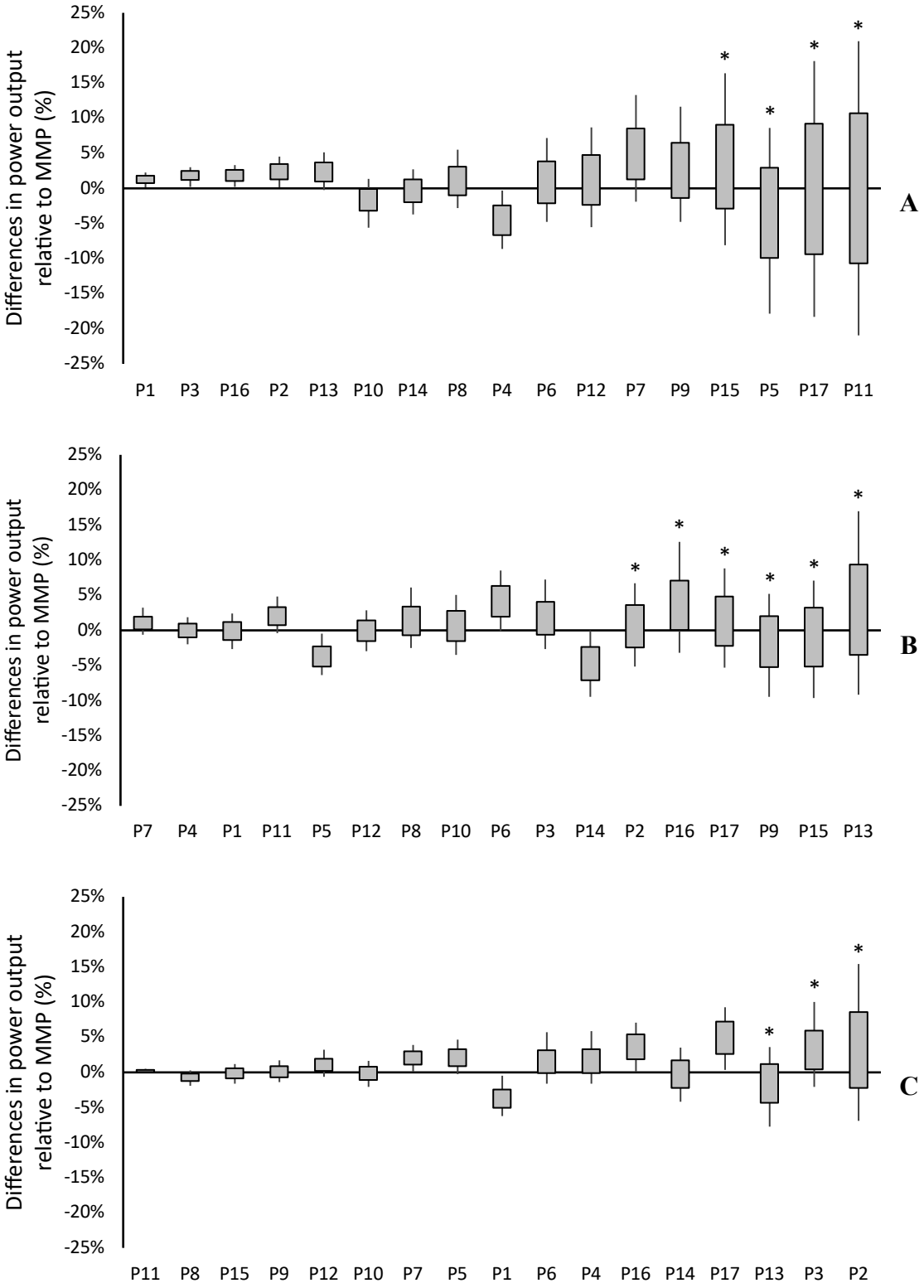


Figure 5.5 – Differences in performance power output during SHORT (a), LONG (b), and CONT (c) sessions, with participants displayed in order of magnitude of variability. Bars display the range in %MMP between session repeats for each participant, and error lines display 95% confidence intervals. * = greater than twice the standard deviation of the mean difference in %MMP.

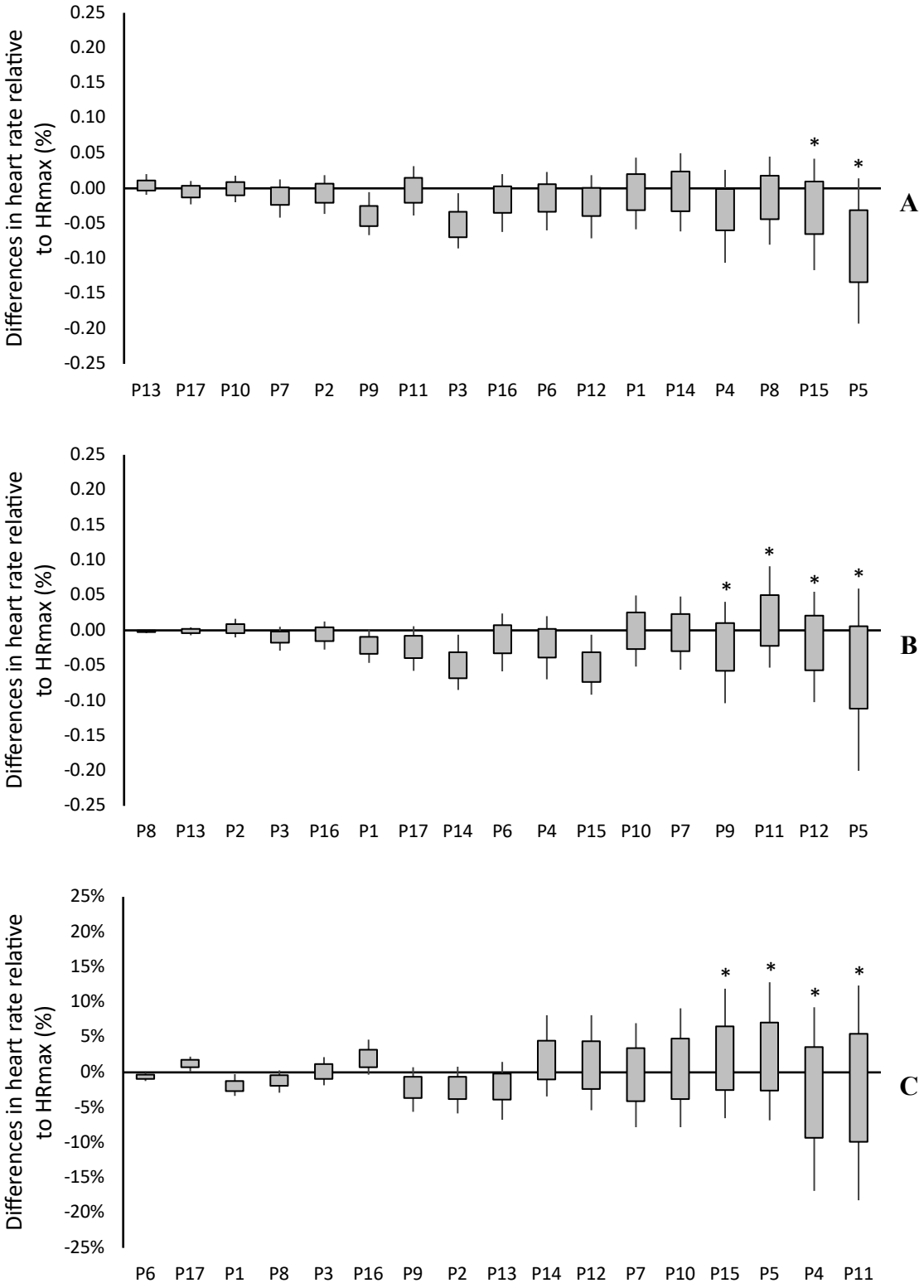


Figure 5.6 - Differences in heart rate relative to HR_{max} during SHORT (a), LONG (b), and CONT (c) sessions, with participants displayed. Bars display the range in $\%HR_{max}$ between session repeats for each participant, and error lines display 95% confidence intervals. * = greater than twice the standard deviation of the mean difference in $\%HR_{max}$.

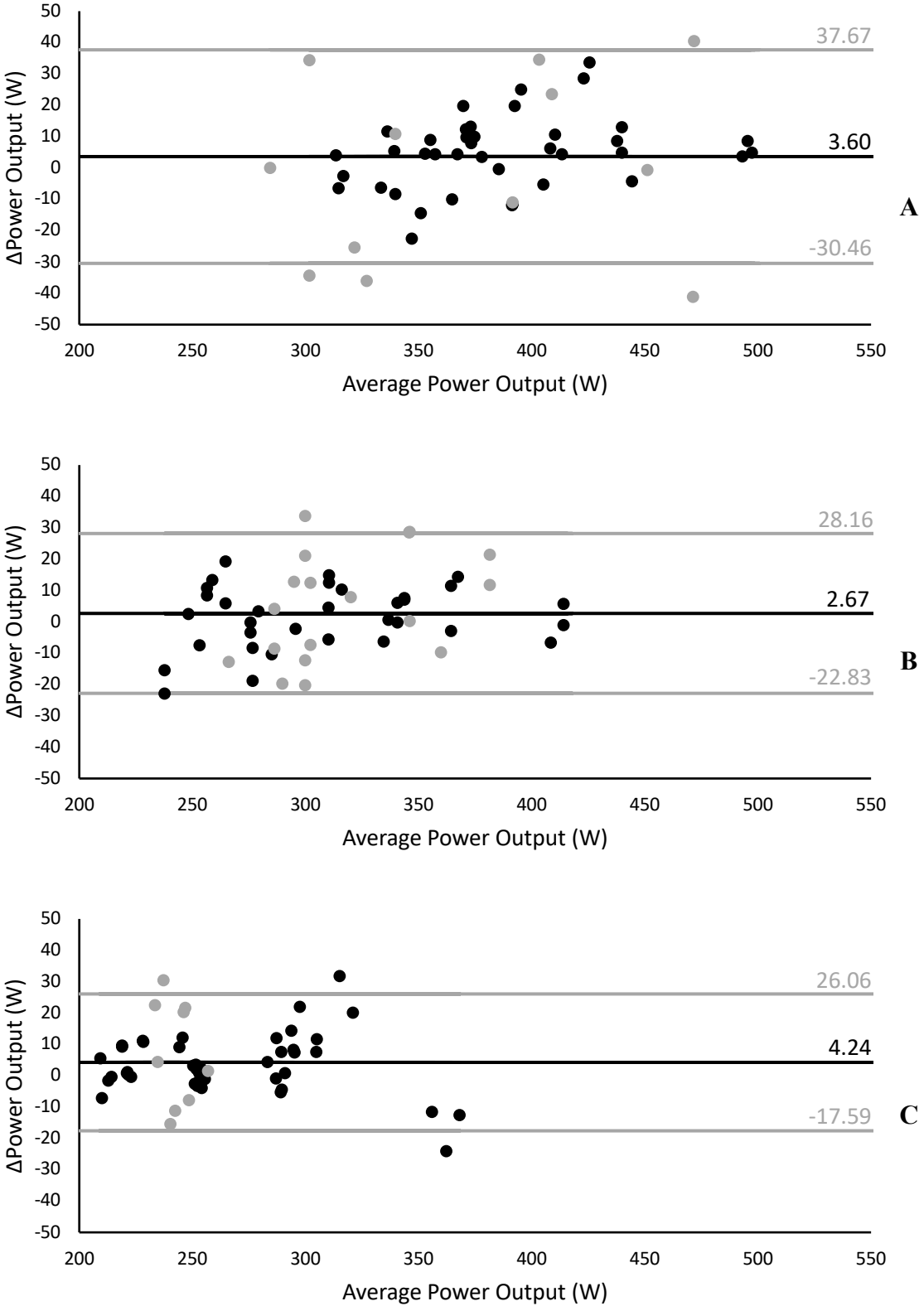


Figure 5.7 - Bland-Altmann Plots displaying session-by-session change in power output for every session completed by every participant (n = 51 sessions) during SHORT (a), LONG (b), and CONT (c) sessions. Black markers represent average and delta power output for sessions completed by each participant, with grey filled markers identifying variable participants (defined as within-session variability greater than twice the standard deviation of the mean difference in each group). Black horizontal lines represent delta bias, and grey lines display 95 % confidence intervals

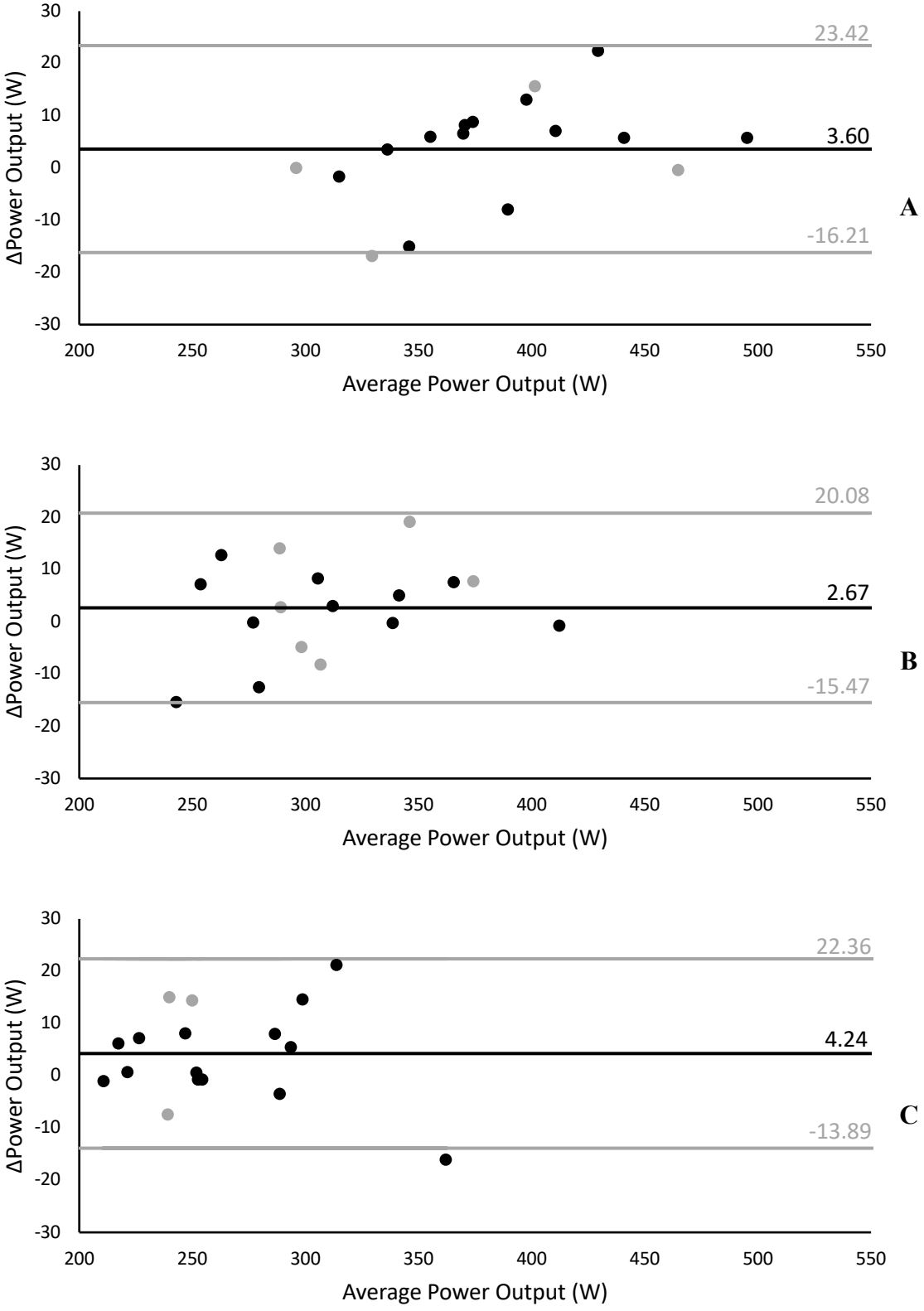


Figure 5.8 - Bland-Altmann Plots displaying average session difference in power output against the average power outputs for every participant (n = 17) during SHORT (a), LONG (b), and CONT (c) sessions. Black markers represent average and delta power output for sessions completed by each participant, with grey filled markers identifying variable participants (defined as within-session variability greater than twice the standard deviation of the mean difference in each group). Black horizontal lines represent delta bias, and grey lines display 95 % confidence intervals.

5.4.5 Psychological response comparisons

Number of negative DALDA responses was not different based on session format ($F_{(2, 28)}$ = 4.267; η_p^2 = .061, P = .412), or session order ($F_{(2, 28)}$ = .075; η_p^2 = .005, P = .928), and no interaction effect was observed ($F_{(2.052, 28.726)}$ = .118; η_p^2 = .008, P = .976).

NASA-TLX responses indicated a difference in mental demand based on session format based on tests of within-subject effects ($F_{(2, 28)}$ = 3.358; η_p^2 = .193, P = .049), but Bonferroni post-hoc testing was unable to identify differences between session formats (P ≥ .073), and no difference based on session repeat order ($F_{(2, 28)}$ = .1.484; η_p^2 = .096, P = .244) or interaction ($F_{(4, 56)}$ = .367; η_p^2 = .026, P = .831). No differences in physical demand were found based on session format ($F_{(2, 28)}$ = .669; η_p^2 = .046, P = .669), session repeat order ($F_{(2, 28)}$ = .440; η_p^2 = .03, P = .649), and no interaction effect was observed ($F_{(2.160, 30.246)}$ = .758; η_p^2 = .051, P = .487). No differences in temporal demand were found based on session format ($F_{(1.424, 19.94)}$ = .3.059; η_p^2 = .179, P = .084), session repeat order ($F_{(2, 28)}$ = .526; η_p^2 = .036, P = .597), and no interaction effect was observed ($F_{(4, 56)}$ = .919; η_p^2 = .062, P = .460). No differences in perceived performance were found based on session format ($F_{(2, 24)}$ = 2.949; η_p^2 = .197, P = .072), session repeat order ($F_{(2, 24)}$ = .912; η_p^2 = .071, P = .415), and no interaction effect was observed ($F_{(4, 48)}$ = .612; η_p^2 = .049, P = .656). A difference in task effort was observed based on session repeat order ($F_{(2, 28)}$ = .5.859; η_p^2 = .295, P = .007), being higher following Repeat 3 vs Repeat 1 (5.1 vs 7.2; P = .025), but no difference was found based on session format ($F_{(2, 28)}$ = .666; η_p^2 = .045, P = .522), and no interaction effect was observed ($F_{(4, 56)}$ = .462; η_p^2 = .032, P = .763). No differences in frustration were found based on session format ($F_{(2, 28)}$ = .226; η_p^2 = .016, P = .799), session repeat order ($F_{(2, 28)}$ = .2.837; η_p^2 = .169, P = .076), and no interaction effect was observed ($F_{(4, 56)}$ = .2.197; η_p^2 = .081, P = .136).

5.5 Discussion

The main findings of this study were that maximal isoeffort training sessions resulted in different levels of variability in the physiological response to LONG, SHORT and CONT exercise bouts. Within-athlete variability of session power output was greatest during SHORT, whereas between-athlete variability was greatest in CONT, and total variability was greatest in SHORT. The crucial aspect of matching between the training sessions was the *isoeffort* intensity prescription of a "maximal session effort", and this was achieved as RPE measured at the end of the session was recorded as 19 ± 1 across all formats.

In a study comparing continuous and intermittent (30 sec work/rest bouts, similar to SHORT) cycling protocols using an iso-effort training intensity prescription, Nicolò *et al.* (2014) reported lower levels of between-athlete variability than the present study for both power output and $\dot{V}O_2$, but BLa^{-1} response in the present study was observed to be more consistent during CONT in comparison to Nicolò *et al.* (2014). Within the study of Nicolò *et al.* (2014), there was higher work bout power output during CONT and SHORT compared to the present study (307 ± 36 W vs 262 ± 40 W, and 464 ± 51 W vs 382 ± 55 W, respectively). In addition, the participant $\dot{V}O_{2max}$ in the present study was 59.4 ± 8.2 $mL{\cdot}kg{\cdot}min^{-1}$, whereas the participants within Nicolò *et al.* (2014) were 67 ± 6 $mL{\cdot}kg{\cdot}min^{-1}$, which may explain the lower between-athlete variability in SHORT exercise due to the homogeneity of the work intensity between participants. Interestingly, the between-athlete variability of BLa^{-1} response in the data presented by Seiler and Sylta (2017) displayed increased consistency as interval duration decreases; 16 min (CV: 34 %), 8 min (CV: 26 %), and 4 min intervals (CV: 21 %). However, the data from the present study indicate that between-athlete variability increases with SHORT 30 sec intervals displaying higher between-athlete variability compared to LONG 5 min intervals. This possibly suggests that there may be an interval duration at which the between-athlete variability increases again as duration shortens, indicated by the increase in between-athlete variability from 5 min to 30 sec intervals. The above-described variability profile was also present in $\dot{V}_E/\dot{V}O_2$ and $\dot{V}_E/\dot{V}CO_2$ data in the current study with lowered within- and between-athlete variability between CONT and LONG, and then an observed increase between LONG and SHORT. With $\dot{V}_E/\dot{V}O_2$ and $\dot{V}_E/\dot{V}CO_2$ levels increasing as interval duration decreases, indicating higher levels of respiratory response and anaerobic contribution in terms of $\dot{V}_E/\dot{V}CO_2$ (Poole *et al.* 1991; Farias-Junior *et al.* 2019). In line with previous investigations comparing intermittent and continuous exercise protocols, LONG was observed to have the highest $\dot{V}O_2$ response (Zafeiridis *et al.* 2015; Zafeiridis *et al.* 2010; Zafeiridis *et al.* 2011). Observing the commonly utilised metric for overall exercise stimulus; time spent exercising > 90 %$\dot{V}O_{2max}$ (Wakefield and Glaister 2009; Rozenek *et al.* 2007; Rønnestad *et al.* 2020), the overall stimulus was significantly higher and less variable during LONG sessions in both within- and between-athlete variability. Interestingly, SHORT was found to have low within- and between-athlete variability in $\dot{V}O_2$ compared to LONG and CONT, so the high variability of time spent exercising > 90 %$\dot{V}O_2$max could indicate that the undulating nature of the exercise bout results in more variable and lower amplitude

$\dot{V}O_2$ response during the exercise session. This could indicate that overall, LONG provides a more consistent aerobic stressor compared to the other training formats.

At a peripheral level, the variability observed in NIRS data displays widely ranging variability profiles on an individual level in the present study. As local tissue hypoxia has been proposed as a stimulus that increases the activity of capillary proliferation, mitochondrial enzymes, and mitochondrial biogenesis in skeletal muscle (Fluck 2006; Zafeiridis *et al.* 2015; Prior, Yang and Terjung 2004; Terrados *et al.* 1990), this may be an important factor in assessing the individual response to standardised training sessions. The $\bar{X}$ Interval ΔTSI% was similar across groups, with similar within-athlete variability, but CONT displayed larger between-athlete variability than the two interval protocols. Differences in muscle deoxygenation (decreased $\bar{X}$ Interval ΔO_2Hb and increased $\bar{X}$ Interval ΔHHb) between continuous and intermittent protocols were observed, with CONT resulting in higher levels of deoxygenation compared to SHORT and LONG. $\bar{X}$ Interval ΔHHb displayed an extremely large amount of within-athlete variability during CONT. SHORT displayed the highest within-athlete variability for Session ΔTSI%, ΔO_2Hb, ΔHHb, indicating that the level of muscle deoxygenation varied more so compared to other formats when an individual undergoes the same session format. Session ΔtHb was most variable in CONT, compared to the interval protocols, which may be indicative of the differences in blood flow distribution between continuous and intermittent exercise protocols. Intermittent exercise results in increased blood flow to the exercising muscles and decreased blood flow to the skin and abdominal viscera (Neary, Hall and Bhambhani 2001; Åstrand and Rodahl 1986; Quaresima *et al.* 1996), whereas blood flows towards the skin during continuous protocols (Kenney and Johnson 1992), potentially highlighting differences observed in muscle oxygenation during the different exercise formats in the present study.

Large levels of between-athlete variability in Session ΔTSI%, ΔO_2Hb, ΔHHb, and ΔtHb were observed for all session formats, potentially displaying a pattern of individualisation of skeletal muscle deoxygenation when this data is looked at alongside the $\bar{X}$ Interval data. To highlight this example, levels of Session ΔHHb were observed to be similar between session formats, with low levels of within-athlete variability for SHORT, LONG, and CONT (7.4 %, 3.2 %, and 2.7 %, respectively). However, looking at the between-athlete variability associated with these measures, SHORT and CONT displayed high but similar

levels of variability (151.3 % and 124.7 %, respectively), whereas LONG displayed far greater levels of variability (410.3 %). The above indicates that all training sessions generally displayed consistency in skeletal muscle deoxygenation on a within-athlete level but highly variable on a between-athlete level.

The present study highlights the complex issue of variability when prescribing exercise training interventions and affords the classification of individuals as "*consistent*" or "*variable*" based on their within-athlete variability in session responses (Atkinson, Williamson and Batterham 2019; Mann, Lamberts and Lambert 2014). For example, considering the session power output relative to %MMP, participants P2, P13, P15, and P17 were identified as variable (± 2 x SD of mean session difference across all participants) in at least two session formats. With regards to HR relative to HR_{max}, participant P5 was identified as being variable in all session formats, and participants P11 and P15 were identified as variable in at least two training session formats. Interestingly, no participants who produced variable power outputs in CONT were variable with HR, whereas participant P9 was variable in both power and HR in LONG, and participants P5 and P15 were variable in both power and HR in SHORT. The presence of large degrees of between-athlete variability in physiological responses could influence the phenomena in which participants are non-responsive to a training intervention for one parameter (e.g. $\dot{V}O_{2max}$), but respond in another (e.g. lactate threshold; Vollaard *et al.* 2009). The interaction between the magnitude of a given physiological stimulus and the variability of said stimulus is likely to have a large influence on the overall physiological adaptation following an exercise training intervention. This can be demonstrated by observing the BLa^{-1} response to the three session formats; SHORT and LONG both had similar and significantly higher BLa^{-1} levels than CONT, but between SHORT and LONG within-athlete variability was 16.9 % and 14.1 %, respectively. This indicates that whilst the same levels of BLa^{-1} occur on average when the session is performed, the differences that an individual may experience each time they perform a training session are greater in SHORT, compared to LONG. With regards to the between-athlete variability, SHORT and LONG display 28.6 % and 23.1 % variability, respectively; this shows that the homogeneity of the BLa^{-1} response is much higher in LONG than in SHORT.

Recent research has suggested that variations in power output within a training interval bout can result in increased time at higher $\%\dot{V}O_{2max}$ (e.g. > 95 $\%\dot{V}O_{2max}$) compared to a

constant work rate at the same power output (Bossi *et al.* 2019; Billat *et al.* 2013). Similar findings were observed by Almquist *et al.* (2020), with greater time spent > 90 %$\dot{V}O_{2max}$ and > 90 %HR_{max} during short interval versus longer interval protocols. While the interval sessions within the present study utilised short and long interval protocols, the difference in interval structure may explain the lower time spent > 90 %$\dot{V}O_{2max}$ observed in comparison to previous findings (Almquist *et al.* 2020). For the short interval protocol, Almquist *et al.* (2020) utilised 3 sets of 13 x 30 sec work intervals with 15 sec recovery periods, in comparison with the present study which utilised 2 sets of 20 x 30 sec work intervals with 30 sec recovery periods. Almquist *et al.* (2020) also differed the prescription of long intervals, using 4 x 5 min work intervals with 2.5 min recovery periods, compared with the present study, which utilised 4 x 5 min work intervals with 5 min recovery periods. These differences may explain the observed divergent findings in time spent at higher percentages of $\dot{V}O_{2max}$ between studies.

Currently, it is unclear whether a training session with highly variable physiological stimuli between- and within-athletes will result in a training response to a group intervention with a large degree of individual variability. There are many potential aspects of training stimulus and response that are yet to be fully understood. For example, a response may have low within-athlete variability, showing that it is consistent when each individual repeats the exercise bout, but there may be high between-athlete variability, showing that the levels of response differ greatly between each individual. The importance may lie in response of the desired magnitude (e.g., high $\dot{V}O_2$ response) with low between-athlete variability, showing homogeneity across a group, and high between-athlete variability, showing individualisation of training stress on a given day. With coaches having used isoeffort training prescriptions to reach specified session efforts for many years (Seiler and Hetlelid 2005; Seiler and Sjursen 2004), the importance of the present data indicating the degrees of between-athlete and within-athlete variability can help our understanding about why these training methods are effective. As this present study focuses on the acute physiological response to exercise training sessions and the variability of these responses within and between athletes, future research may look to explore the levels of individual variability observed when maximal effort-based training sessions are repeated within a chronic training intervention. Whilst it has been suggested that acute exercise response does not always have a direct link with chronic training response (Cochran *et al.* 2014; Stepto *et al.* 2012; Nielsen *et al.* 2014), it is unclear whether the same can be said for levels of individual variability. Understanding the link between acute

training session variability and chronic training variability could provide researchers and practitioners with further insights into optimal individualisation of training.

5.6 Conclusion

In conclusion, the present study demonstrates that when sessions using short intervals are prescribed using a maximal isoeffort intensity prescription, a large degree of individual variability is observed compared to long or continuous training formats. It has also been identified that there may be training session formats that athletes are able to perform with less variability compared to other formats on an individual basis, presenting an opportunity for training individualisation.

Chapter 6 - Between-athlete variability in exercise training adaptations following 6 weeks of self-paced training using either long or short intervals

6.1 Abstract

Introduction: This study aimed to investigate the between-athlete variability associated with chronic training interventions using either long or short interval formats with maximal effort-based intensity prescriptions. **Methods**: Twenty-eight well-trained competitive cyclists (*n*=27 males, *n*=1 female; $\dot{V}O_{2max}$: 57.1 ± 8.3 ml·kg·min^{-1}) were randomly assigned to a control (CON) or a short (SHORT) or long (LONG) interval group and completed 6 weeks of training consisting of 3 maximal isoeffort sessions each week. $\dot{V}O_{2max}$, MMP, and 20 min TT performance were recorded before and after the training period. Participants were classified as responders or non-responders based on the within-athlete CV of the associated test measurement. **Results**: No differences in any measures were observed between groups at baseline ($P \geq .136$), and total training duration was similar in SHORT, LONG, and CON (48 h 25 min, 34 h 03min, and 40 h 41 min, respectively; $P = .672$). An interaction effect was observed between training group and MMP before and after the training intervention ($P = .027$, $\eta_p^2 = .251$). An interaction effect was observed in MMP W.kg^{-1} ($P = .003$, $\eta_p^2 = .366$) which increased in SHORT ($P = .019$, $\eta_p^2 = .629$), with no difference in LONG and CON. An interaction effect was observed in relative TT power ($P = .01$, $\eta_p^2 = .307$) which increased in SHORT ($P = .015$, $\eta_p^2 = .657$), along with absolute TT power which also increased in SHORT ($P = .042$, $\eta_p^2 = .526$). Participant response levels of 71 % (5/7), 40 % (4/10), and 27 % (3/11) in SHORT, LONG, and CON, respectively for $\dot{V}O_{2max}$, and 86 % (6/7), 60 % (6/10), and 27 % (3/11) in SHORT, LONG, and CON, respectively for absolute power during the TT. **Conclusion**: Training in SHORT increased MMP and both absolute and relative TT power, compared to no change in LONG or CON. Individual response levels indicate that effort-based training using SHORT intervals results in higher rates of training response compared to effort-based training using LONG intervals in both $\dot{V}O_{2max}$ and absolute TT power when total work duration is matched.

6.2 Introduction

Prescribing exercise training for improving endurance performance involves the deliberate manipulation of duration, frequency, and intensity of exercise, arranged in a manner that places physiological stress on the human body, inducing adaptations that can be associated with subsequent performance improvements (Borresen and Lambert 2008; Hawley and Burke 1998). Common methods of exercise intensity prescription, such as standardised percentages of various physiological markers, such as $\dot{V}O_{2max}$ or MMP, frequently result in

divergent responses between individuals (Mann, Lamberts and Lambert 2014). The concept of individual variability to exercise training has been apparent within research for many years (Astorino and Schubert 2014; Sisson, Katzmarzyk, Earnest, *et al.* 2009; Scharhag-Rosenberger *et al.* 2012; Bouchard, An, Rice, Skinner, *et al.* 1999; Rankinen *et al.* 2012; Bouchard 2012; Gurd *et al.* 2016; Pickering and Kiely 2019a; Pickering and Kiely 2019b; Williamson, Atkinson and Batterham 2016; Atkinson, Williamson and Batterham 2018; Montero and Lundby 2017; Sarzynski, Ghosh and Bouchard 2017; Chmelo *et al.* 2015; Pandey *et al.* 2015; Bonafiglia *et al.* 2016; Sparks 2017). It has been indicated that a large portion of this variability in training response may be influenced by differences in systematic stress that occurs when exercise is completed using standardised training prescriptions, due to factors such as differences in where lactate threshold occurs relative to $\dot{V}O_{2max}$ and MMP between individuals (Bouchard, Sarzynski, *et al.* 2011; Rankinen *et al.* 2012; Mann, Lamberts and Lambert 2014). As a result, high levels of individual variability in training response presents distinct challenges, particularly relating to the interpretation of research results, the statistical power of study designs, as well as the fundamental ability to prescribe training that has been individually optimised (Hecksteden *et al.* 2015). Even in studies showing significant group mean changes, it is common to observe high between-athlete variability in training response, which has recently led to a distinction being made between 'high responders' and 'low responders' and questioning the efficacy of applying findings based on group mean change to an individual (Mann, Lamberts and Lambert 2014). An individualised training prescription would therefore need to be standardised in a way that it could be applied across a group of athletes but also delivers a consistent level of systematic stress between individuals.

An effort-based approach has been used extensively in the prescription of athletic training and is implemented by instructing athletes to self-pace session exercise intensity to produce a "*maximal session effort*" (Seiler and Hetlelid 2005; Seiler and Sjursen 2004; Seiler *et al.* 2013). Effort-based prescription methods have been previously implemented to compare various intermittent exercise protocols (Seiler *et al.* 2013; Seiler and Hetlelid 2005; Seiler and Sjursen 2004), and later to compare intermittent and continuous exercise protocols (Nicolò, Bazzucchi, Haxhi, *et al.* 2014). When athletes self-pace, they regulate their work rate based on circumstantial factors with the goal of maintaining physiological homeostasis (Esteve-Lanao *et al.* 2008; Ulmer 1996) and avoiding premature fatigue or exhaustion (St Clair Gibson *et al.* 2006; Baron *et al.* 2011). The factors that the athlete relies on to adequately self-pace an exercise bout are a mixture of physiological (e.g., heart rate, ventilation/respiration rate), psychological (e.g., perception of time, motivation), and

biomechanical (e.g., the efficiency of movement and body posture) factors (Noakes 2011). The effort-based training intensity prescriptions may help to ensure consistency between-session effort (Seiler and Sjursen 2004), would allow athletes to self-regulate their work rate and adjust for their perceived pre-exercise readiness to perform, combining factors such as sleep, stress, wellness, or anxiety (Azevedo *et al.* 2021; Abbiss and Laursen 2008; Azevedo *et al.* 2019; Millet 2011; Yoon *et al.* 2009; Ungureanu *et al.* 2020), and therefore possibly aid in reducing between-athlete variability to chronic training interventions. A recent investigation has been conducted comparing long and short interval formats using effort-matched intensity prescriptions over a period of three weeks with three sessions per week (Rønnestad *et al.* 2020). The short interval session was comprised of 3 sets of 13 x 30 sec work bouts with 15 sec recovery between bouts and 3 min between sets, with the long interval session comprising of 4 sets of 5 min work bouts with 2.5 min recovery between sets. Work intensity was determined by the athletes being asked to maintain the highest average power during each interval session on a maximal effort basis. Rønnestad et al. (2020) found that short interval training resulted in greater improvements in peak aerobic power, %$\dot{V}O_{2max}$ at LT, and increased power output at LT, despite no difference in the change in the $\dot{V}O_{2max}$ observed between groups. Variability in training response was observed in both groups, with some athletes displaying high levels of response, non-response, as well as adverse response. This highlights the ability for effort-based intensity prescriptions in HIIT to effectively improve performance, although variability was present at the individual level.

So far, this thesis has demonstrated that the individual variability observed in the physiological response to isolated effort-based training session differs based on the interval format type. The findings of Chapter 5 indicated that when maximal effort-based intensity prescriptions are used, long interval (e.g., 5 min) session formats elicit a more homogenous training stimulus when compared to shorter intervals (e.g., 30 sec work/30 sec recovery). However, it is not clear whether the degree of individual variability observed in response to an acute session prescription translates to chronic training variability within groups. Therefore, the aim of this investigation is to compare the degrees of individual variability in training response to a 6-week training intervention comprising of two different interval session formats using maximal effort-based training intensity prescriptions.

Hypotheses

$H1_1$ – Lower levels of individual variability in training response is observed in the group utilising long intervals compared to short intervals.
$H1_0$ – There is no difference in levels of individual response between training groups.
$H2_1$ – The training response magnitude is higher in the group utilising long intervals compared to short intervals.
$H2_0$ – There is no difference in the training response magnitude between interval training groups.

6.3 Methodology

6.3.1 Participants

Twenty-eight well-trained male and female (27 males and 1 female; mean ± SD: age 34 ± 10 years, height 177.3 ± 9.1cm, mass 72.4 ± 10.6 kg, $\dot{V}O_{2max}$ 57.1 ± 8.3 mL.kg.min^{-1}, MMP 378 ± 61 W, HR_{max} 184 ± 10 bpm), cyclists with at least 3 years of cycling training and racing experience (corresponding to Performance Level 3 – 4; de Pauw *et al.* 2013; Decroix *et al.* 2016) provided written informed consent to voluntarily participate in the study which held full ethical approval from the local institutional ethics committee according to the Declaration of Helsinki.

6.3.2 Study design and experimental procedures

Participants visited the laboratory to perform baseline testing and then were randomly assigned to one of three groups: LONG, SHORT, or a control group (CON). Training groups LONG and SHORT completed 6 weeks of prescribed cycling training using maximal effort-based intensity prescriptions. Following the 6-week intervention, participants repeated the testing procedures in another visit to the exercise testing laboratory. An overview of the study design is presented in Figure 6.1.

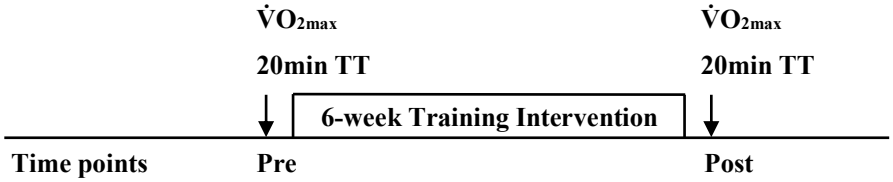


Figure 6.1 – Schematic of training intervention and testing time-points.

All testing visits for each participant were completed within the same 3-h period of the day, and participants were asked to attend in a euhydrated state and to maintain a consistent diet and lifestyle, and to avoid alcohol and strenuous exercise the day before the sessions. To allow for adequate familiarisation prior to data collection, participants were asked to attempt to incorporate effort-based training bouts in their own training before commencing the study. A cooling fan was present, and plain water was available for participants to drink ad libitum.

6.3.3 Maximal incremental test

Participants completed a maximal incremental test on a bicycle ergometer (Cyclus2, RBM Electronics, Leipzig, Germany) to identify MMP, $\dot{V}O_{2max}$, and HR_{max}. After riding at 100 W for a period of 10 min, the external load was increased by 20 W every 60 sec until volitional exhaustion, defined as the point where self-selected cadence dropped below 60 rpm despite strong verbal encouragement. Respiratory gas exchange data were assessed continuously throughout all testing procedures using an online gas analyser (Metalyzer 3B, CORTEX Biophysik GmbH, Leipzig, Germany) and an appropriately sized facemask covering the nose and mouth. A 10 sec rolling average was used when analysing respiratory gas exchange data. Expired gas data were analysed to quantify $\dot{V}O_2$, $\dot{V}CO_2$, $\dot{V}_E$, $\dot{V}_E/\dot{V}O_2$ and $\dot{V}_E/\dot{V}CO_2$. MMP was calculated as the highest power output achieved over a period of 60 sec, $\dot{V}O_{2max}$ was calculated as the highest $\dot{V}O_2$ averaged over a period of 30 sec, and HR_{max} was identified as the highest HR value reached in the incremental test.

6.3.4 20-min TT performance test

Following 30 min recovery from the maximal incremental test (10 min cool-down, 10 min seated rest, 10 min warm-up), participants were instructed to achieve the highest possible average power output for the duration (Evertsen, Medbø and Bonen 2001). Power output, HR, and respiratory gas exchange data were recorded in the same manner as in the *Maximal incremental test*. Participants were able to freely choose riding cadence, self-select power output, and view the duration remaining of the test.

6.3.5 Training intervention

Each week of 6-week training intervention incorporated three effort-based training sessions, completed at least 24 h apart to allow for adequate spacing and recovery between sessions. Sessions were instructed to be completed within the same 3 h period between subjects to reduce diurnal variability. Elapsed session time and performance data were available for participants during all training sessions, along with a graphical representation of the session format. Participants were able to complete training sessions on their home ergometer or attend the laboratory for supervised training sessions, with participants completing at least one supervised session to ensure correct completion of the training prescription. Supervised training sessions were performed on identical electromagnetically braked CompuTrainer LabTM ergometers (CompuTrainer, RacerMate, Seattle, WA, USA). Home training was permissible only if individuals trained using the power meter installed on their bike during the incremental and time-trial tests to check the matching of power readings. Training data was recorded and compiled on an online training monitoring platform (TrainingPeaks, Peaksware, Boulder, CO, USA). It was stressed that the volume of endurance riding completed should be maintained in a consistent manner throughout the training intervention period. Participants were instructed to replace three weekly training sessions with the prescribed training sessions and were able to complete extra training that was in line with their habitual training load leading into the intervention period. The athletes were instructed to not complete any other set high-intensity training sessions in addition to the prescribed training sessions.

Participants were divided into three training groups differing in prescribed training session format (details presented in Table 6.1 and Figure 6.2):

LONG – Long interval group; three weekly sessions comprising of 4 x 5 min efforts with 5 min active recovery between each effort, in addition to 2 – 3 additional endurance sessions,

SHORT – Short interval group; three weekly sessions comprising of 2 sets of 20 x 30 sec efforts/30 sec active recovery, with 5 min active recovery between sets, in addition to 2 – 3 additional endurance sessions,

CON – Control group; monitored unsupervised endurance training in line with habitual endurance load.

The intensity of the work bouts during the LONG and SHORT interval sessions was prescribed using an effort-based prescription. Participants were instructed to self-select a workload that would allow them to reach maximal exertion by the end of the training session. Recovery periods were instructed to be active recovery at an intensity that feels extremely easy to complete. A standardised warm-up consisting of 5 min cycling at 100 W and 5 min at 150 W was completed prior to each training session, and a standardised cool-down consisting of 5 min cycling at 150 W and 5 min at 100 W was completed following each session. Sessions were matched for overall maximal session effort basis (Nicolò *et al.* 2014; Seiler *et al.* 2013) with a total training period of the session at 40 min, excluding warm-up, cool-down, and a 5 min recovery period between the two sets in SHORT. Each exercise session *total effective period* was segmented into four time points for analysis.

Table 6.1 - Characteristics of the short interval (SHORT) and long interval (LONG) exercise protocols to be completed.

	SHORT	**LONG**
Duration of work interval (s)	30	300
Duration of rest period (s)	30	300
Number of repeats per set	20	4
Number of sets	2	1
Recovery time between sets (s)	300	0
Total work duration per session (s)	1,200	1,200
Total rest duration per session (s)	1,200	1,200
Total ‘training period’ duration (s)	2,400	2,400
Total session time (s)	4,200	3,600

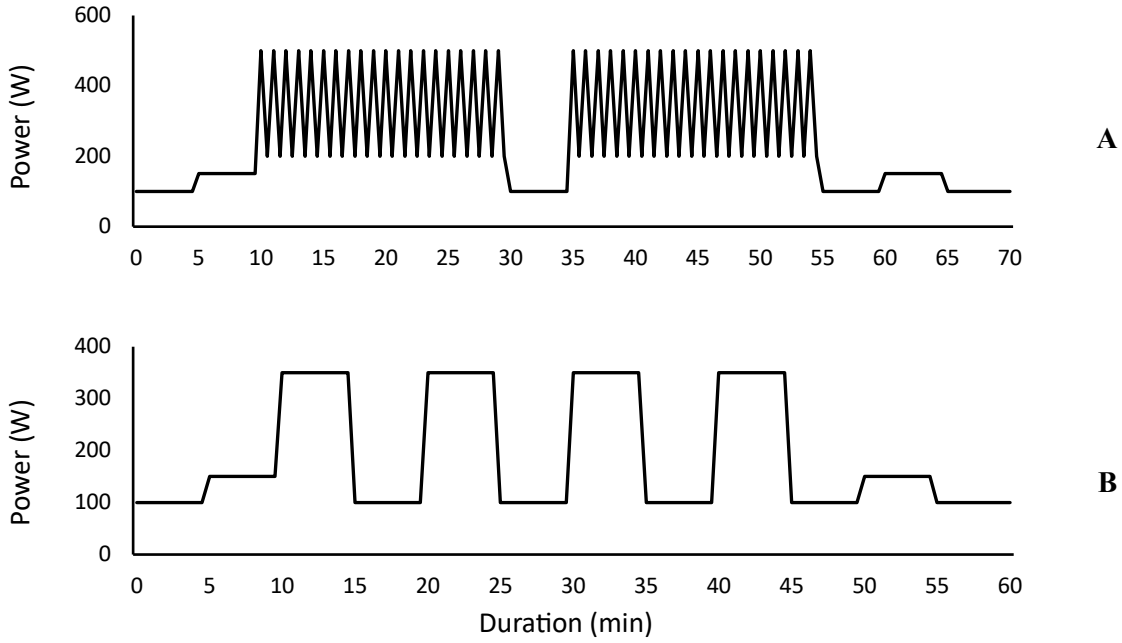


Figure 6.2 – Session format protocols; a) SHORT, b) LONG. Power outputs presented have been approximated for demonstration only.

6.3.6 Data processing and statistical analysis

Prior to statistical analysis, all data were checked for normality of distribution. Sphericity of the data was investigated using the Mauchly test, and the Greenhouse-Geisser adjustment was made when data was deemed non-spherical. Data are reported as mean ± SD, and CVs are presented as a percentage unless specified otherwise. A one-way ANOVA was used to analyse differences in pre-training baseline age, height, body mass, $\dot{V}O_{2max}$, MMP, HR_{max}, TT Power, TT HR, and TT $\dot{V}O_2$. Repeated-measures ANOVA was used to analyse the differences between groups in the changes in age, height, body mass, $\dot{V}O_{2max}$, MMP, HR_{max}, TT Power, TT HR, and TT $\dot{V}O_2$ following the training intervention. When significant differences were found, Bonferroni tests were used to determine where differences occurred. A repeated-measured one-way ANOVA was used to analyse the differences in training session power output and work done between training groups. Effect sizes were calculated using η_p^2 and were defined as small, medium, or large based upon 0.10, 0.25, and above 0.40, respectively (Cohen 1988).

To assess individual training response, within-athlete CV was identified from previous research for absolute $\dot{V}O_{2max}$ (CV = 5.6 % Katch, Sady and Freedson 1982), relative $\dot{V}O_{2max}$ (3.2 % Katch, Sady and Freedson 1982), MMP (4.0 % Montero and Lundby 2017), HR_{max} (1.4 % Bagger, Petersen and Pedersen 2003), 20 min TT power (1.4 % MacInnis,

Thomas and Phillips 2019), 20 min TT $\dot{V}O_2$ (3.3 % Bagger, Petersen and Pedersen 2003), 20 min TT HR (2.7 % Bagger, Petersen and Pedersen 2003). Training responses were then categorised into either: a training response, defined as an improvement greater than the within-athlete CV of the associated test measurement, a non-response defined as an improvement that is lower than the within-athlete CV of the associated test measurement, or a negative training response defined as a reduction in the associated test measurement that is larger than the within-athlete CV of the associated test measurement (Scharhag-Rosenberger *et al.* 2012).

Statistical analyses were conducted using the Statistical Package for the Social Sciences, version 26 for Mac OS X (SPSS, IBM®, Armonk, New York, USA), and an alpha level was set at $P < .05$ for the criteria for detection of significance in all cases. CV calculations and data processing were performed in Microsoft Excel (Excel v16.3 Microsoft, Redmond, Washington, USA).

6.4 Results

6.4.1 Participant details and baseline test results

Baseline testing data are shown in Table 6.2. There was no difference at the pre-training time point between groups in age ($F_{(2,25)} = .314$, $P = .734$), height ($F_{(2,25)} = .192$, $P = .826$), weight ($F_{(2,25)} = 1.337$, $P = .281$), absolute $\dot{V}O_{2max}$ ($F_{(2,25)} = .039$, $P = .961$), relative $\dot{V}O_{2max}$ ($F_{(2,25)} = 1.642$, $P = .214$), absolute MMP ($F_{(2,25)} = .162$, $P = .851$), relative MMP ($F_{(2,25)} = 1.871$, $P = .175$), HR_{max} ($F_{(2,25)} = 2.160$, $P = .136$), TT Power ($F_{(2,25)} = .07$, $P = .933$), relative TT Power ($F_{(2,25)} = 1.09$, $P = .352$), TT HR ($F_{(2,25)} = .869$, $P = .432$), or TT $\dot{V}O_2$ ($F_{(2,25)} = .503$, $P = .611$), as determined by one-way ANOVA. Participants did not change age over the intervention period in either SHORT ($P > .999$), LONG ($P = .168$), or CON ($P = .341$). No change was observed in height over the intervention period in either SHORT ($P = .433$), LONG ($P = .384$), or CON ($P = .655$), in addition to no change in weight in SHORT ($P = .064$), LONG ($P = .602$), or CON (P - .351).

6.4.2 Completed training

The total training duration for each participant separated into group is presented in Figure 6.3. There was no difference observed in total training duration in SHORT, LONG, and CON; 48 h 25 min, 34 h 03 min, and 40 h 41 min, respectively ($F_{(2,25)} = .404$, $P = .672$). The total training duration between individuals displays large individual variability,

ranging from 84 h 06 min – 12 h 00 min in SHORT, 98 h 56 min – 12 h 00 min in LONG, and 133 h 13 min – 2 h 20 min in CON. Average work bout power during interval sessions was higher in SHORT (391 ± 72 W) than in LONG (274 ± 52 W; $F_{(1,14)} = 15.888$, $P = .001$). Total work completed during interval training sessions was higher in SHORT (692.1 ± 107.2 kJ) than in LONG (546.1 ± 140.7 kJ; $F_{(1,13)} = 6.015$, $P = .029$). Significant differences in session power output between groups were observed in every training session repeat, highlighted in Figure 6.4A. Differences in session work done between groups were observed during Sessions 9 to 18, highlighted in Figure 6.4B.

Table 6.2 – Participant age, body mass, MMP, absolute and relative $\dot{V}O_{2max}$, HR_{max}, and TT performances before training (Mean ± SD). * = $P < .05$ vs. pre-training time point.

		ALL		SHORT		LONG	CON
	n =	28		7		10	11
PRE TRAINING	Age (years)	34 ± 10		34 ± 12		36 ± 9	31 ± 10
	Height (cm)	177.3 ± 9.1		178.3 ± 5.6		178.2 ± 12.2	176.0 ± 8.2
	Body mass (kg)	72.4 ± 10.6		75.8 ± 9.6		74.7 ± 12.4	68.3 ± 8.1
	$\dot{V}O_{2max}$ ($L.min^{-1}$)	4.1 ± 0.6		4.0 ± 0.5		4.0 ± 0.6	4.2 ± 0.6
	$\dot{V}O_{2max}$ ($ml.kg.min^{-1}$)	57.1 ± 8.3		54 ± 8.7		54.8 ± 7.9	61.6 ± 7.6
	MMP (W)	378 ± 61		367 ± 61		373 ± 50	396 ± 69
	MMP ($W.kg^{-1}$)	5.3 ± 0.9		5.0 ± 0.9		5.1 ± 0.8	5.7 ± 0.8
	HR_{max} (bpm)	184 ± 10		179 ± 10		182 ± 9	188 ± 10
	TT Power (W)	276 ± 56		274 ± 48		272 ± 56	281 ± 65
	TT Power ($W.kg^{-1}$)	3.9 ± 0.8		3.7 ± 0.7		3.7 ± 0.8	4.1 ± 0.8
	TT HR	169 ± 10		168 ± 8		166 ± 9	172 ± 11
	TT $\dot{V}O_2$	47.5 ± 7.8		46.7 ± 7.6		46.0 ± 7.6	49.3 ± 8.5
POST TRAINING	Age (years)	34 ± 10		34 ± 13		36 ± 9	31 ± 11
	Height (cm)	177.3 ± 9.2		178.6 ± 5.9		177.9 ±12.2	175.8 ±8.9
	Body mass (kg)	72.1 ± 10.4		73.2 ± 9.6		74.9 ± 12.4	68.8 ±9.7
	$\dot{V}O_{2max}$ ($L.min^{-1}$)	4.1 ± 0.6		4.2 ± 0.6		4.1 ± 0.6	4.1 ± 0.7
	$\dot{V}O_{2max}$ ($ml.kg.min^{-1}$)	58.0 ± 8.0		57.4 ± 7.4		55.6 ± 7.9	60.6 ± 8.6
	MMP (W)	382 ± 61		385 ± 59		383 ± 53	379 ± 76
	MMP ($W.kg^{-1}$)	5.3 ± 0.8		5.3 ± 0.9	*	5.2 ± 0.8	5.5 ± 0.9
	HR_{max} (bpm)	184 ± 11		179 ± 6		184 ± 12	187 ± 14
	TT Power (W)	283 ± 56	*	292 ± 52	*	279 ±53	281 ± 66
	TT Power ($W.kg^{-1}$)	3.95 ± 0.7		4.0 ± 0.7	*	3.8 ± 0.7	4.1 ± 0.8
	TT HR	170 ± 10		170 ± 8		167 ± 11	172 ± 12
	TT $\dot{V}O_2$	49.1 ± 7.4	*	49.5 ± 7.4		47.0 ± 6.6	50.6 ± 9.7

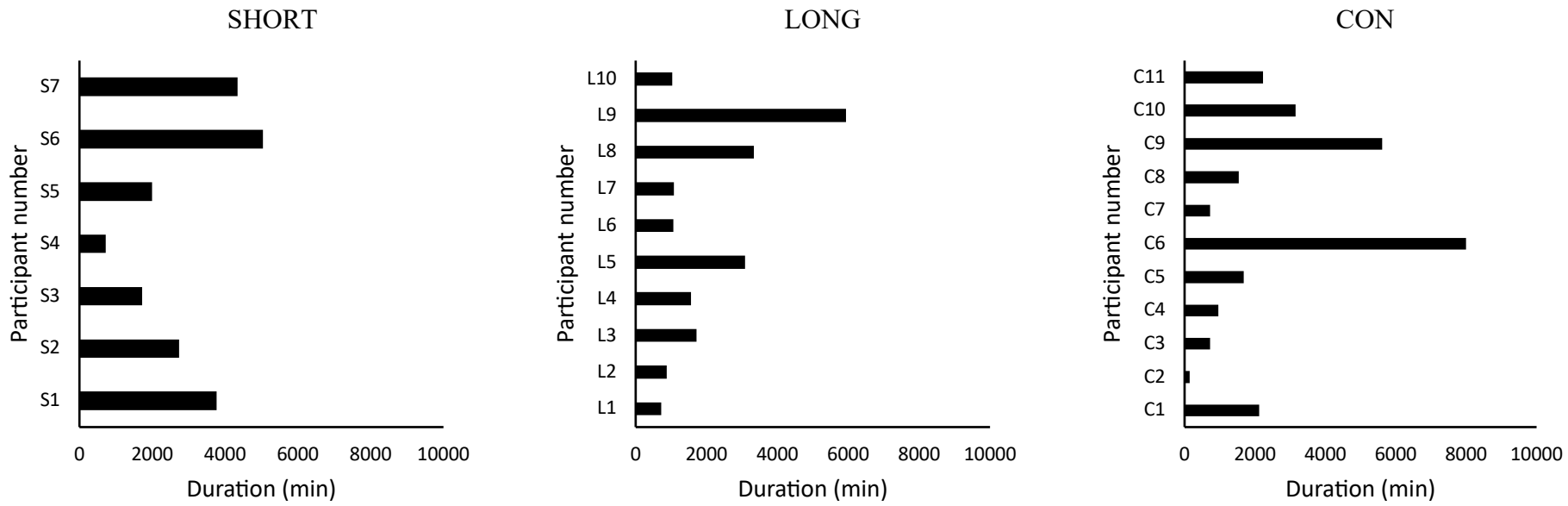


Figure 6.3 – Total training duration for each participant over the 6-week training intervention period.

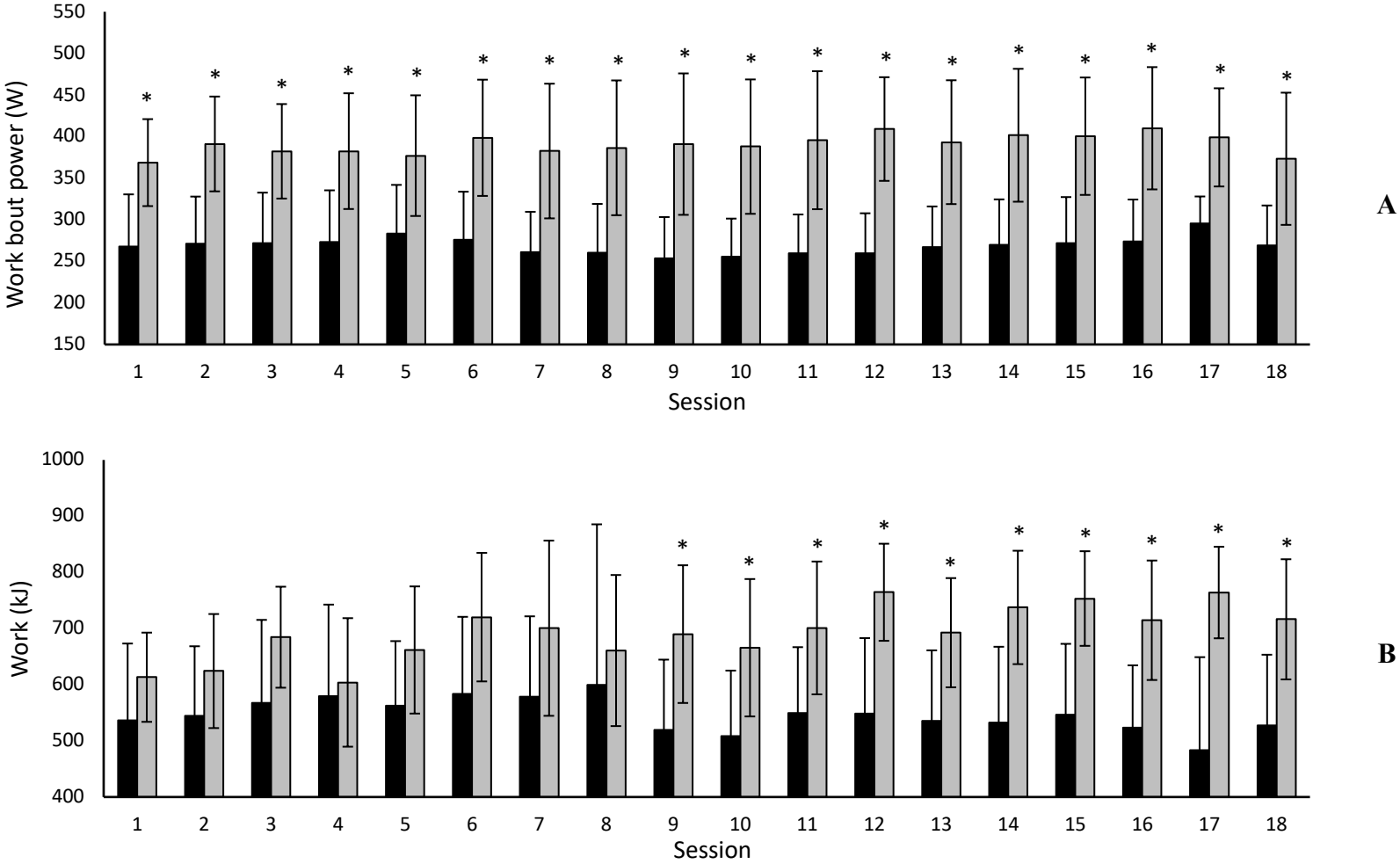


Figure 6.4 - Session power output (a) and work done (b) across the 18 sessions. Black bars represent LONG format sessions, and grey bars represent SHORT format sessions. Error bars ± SD. * = $P < .05$ vs. LONG.

6.4.3 Physiological and performance adaptations following training

6.4.3.1 Maximal incremental test

Absolute and relative $\dot{V}O_{2max}$, absolute and relative MMP, HR_{max} obtained during the maximal incremental test are presented graphically in Figure 6.5. There was no statistically significant interaction between the training group and the tests performed before and after training in relative $\dot{V}O_{2max}$ ($F_{(2, 25)} = 1.117$, $P = .343$, $\eta_p^2 = .082$), and no differences were observed between groups before ($P = .214$) or after the intervention ($P = .359$). No change was observed in relative $\dot{V}O_{2max}$ in LONG ($F_{(1, 9)} = .798$, $P = .395$, $\eta_p^2 = .081$), SHORT ($F_{(1, 6)} = 2.863$, $P = .142$, $\eta_p^2 = .323$), or CON ($F_{(1, 10)} = .023$, $P = .883$, $\eta_p^2 = .002$). There was no statistically significant interaction between the training group and the tests performed before and after training in absolute $\dot{V}O_{2max}$ ($F_{(2, 25)} = .092$, $P = .912$, $\eta_p^2 = .007$), and no differences were observed between groups before ($P = .961$) or after the intervention ($P = .989$). No change was observed in absolute $\dot{V}O_{2max}$ in LONG ($F_{(1, 9)} = 1.465$, $P = .257$, $\eta_p^2 = .140$), SHORT ($F_{(1, 6)} = .265$, $P = .625$, $\eta_p^2 = .042$), or CON ($F_{(1, 10)} = .151$, $P = .706$, $\eta_p^2 = .015$). There was a statistically significant interaction effect between the intervention and time for absolute MMP ($F_{(2, 25)} = 4.183$, $P = .027$, $\eta_p^2 = .251$;

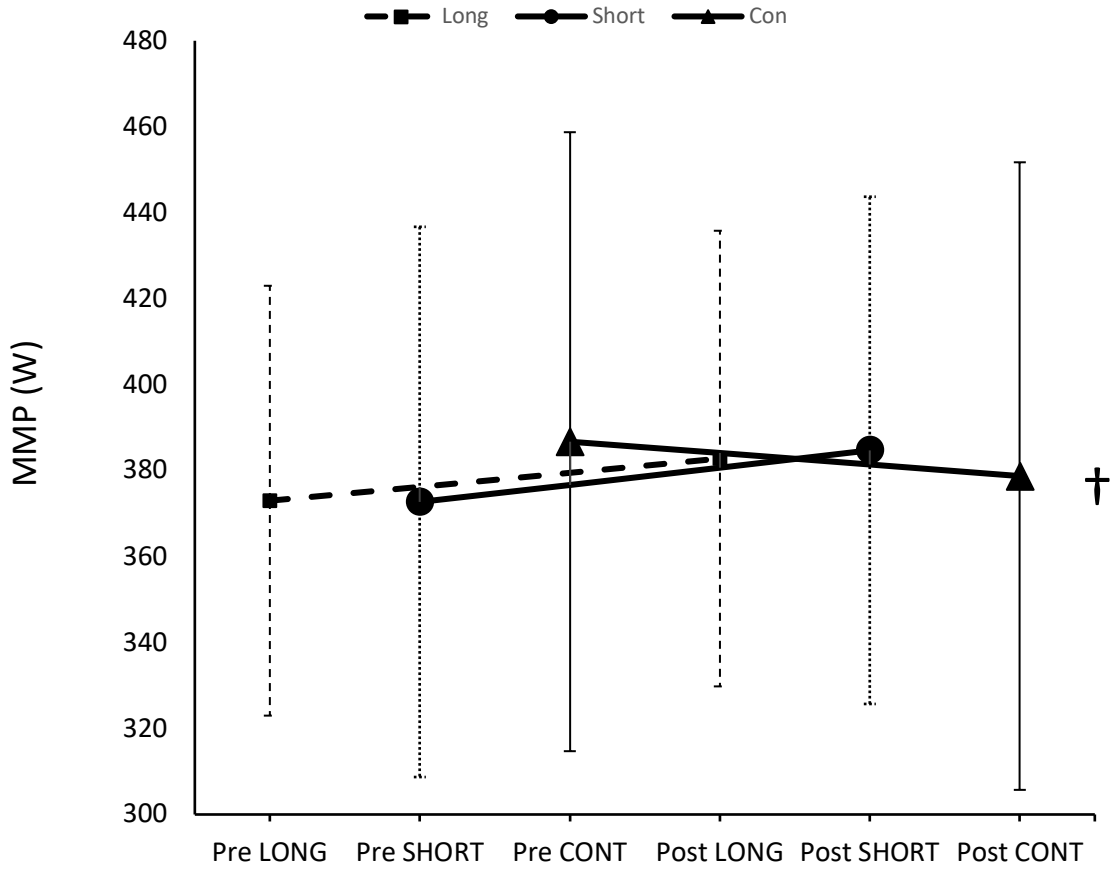


Figure **6.6**), but differences between groups were not detected using follow-up tests ($P > .05$). A statistically significant interaction effect was found between the intervention and time for relative MMP ($F_{(2, 25)} = 7.219$, $P = .003$, $\eta_p^2 = .366$), which was significantly increased in SHORT ($F_{(1, 6)} = 10.154$, $P = .019$, $\eta_p^2 = .629$), but no effect was observed in LONG ($F_{(1, 9)} = 1.782$, $P = .215$, $\eta_p^2 = .165$) or CON ($F_{(1, 10)} = 3.183$, $P = .105$, $\eta_p^2 = .241$). There was no statistically significant interaction between the intervention and time on HR_{max} ($F_{(2, 25)} = 1.404$, $P = .264$, $\eta_p^2 = .101$), and no differences were observed between groups before ($P = .136$) or after the intervention ($P = .341$). No effect of the intervention was observed in HR_{max} in LONG ($F_{(1, 9)} = 1.405$, $P = .266$, $\eta_p^2 = .135$), SHORT ($F_{(1, 6)} = .0$, $P > .999$, $\eta_p^2 = .0$), or CON ($F_{(1, 10)} = 2.181$, $P = .171$, $\eta_p^2 = .179$).

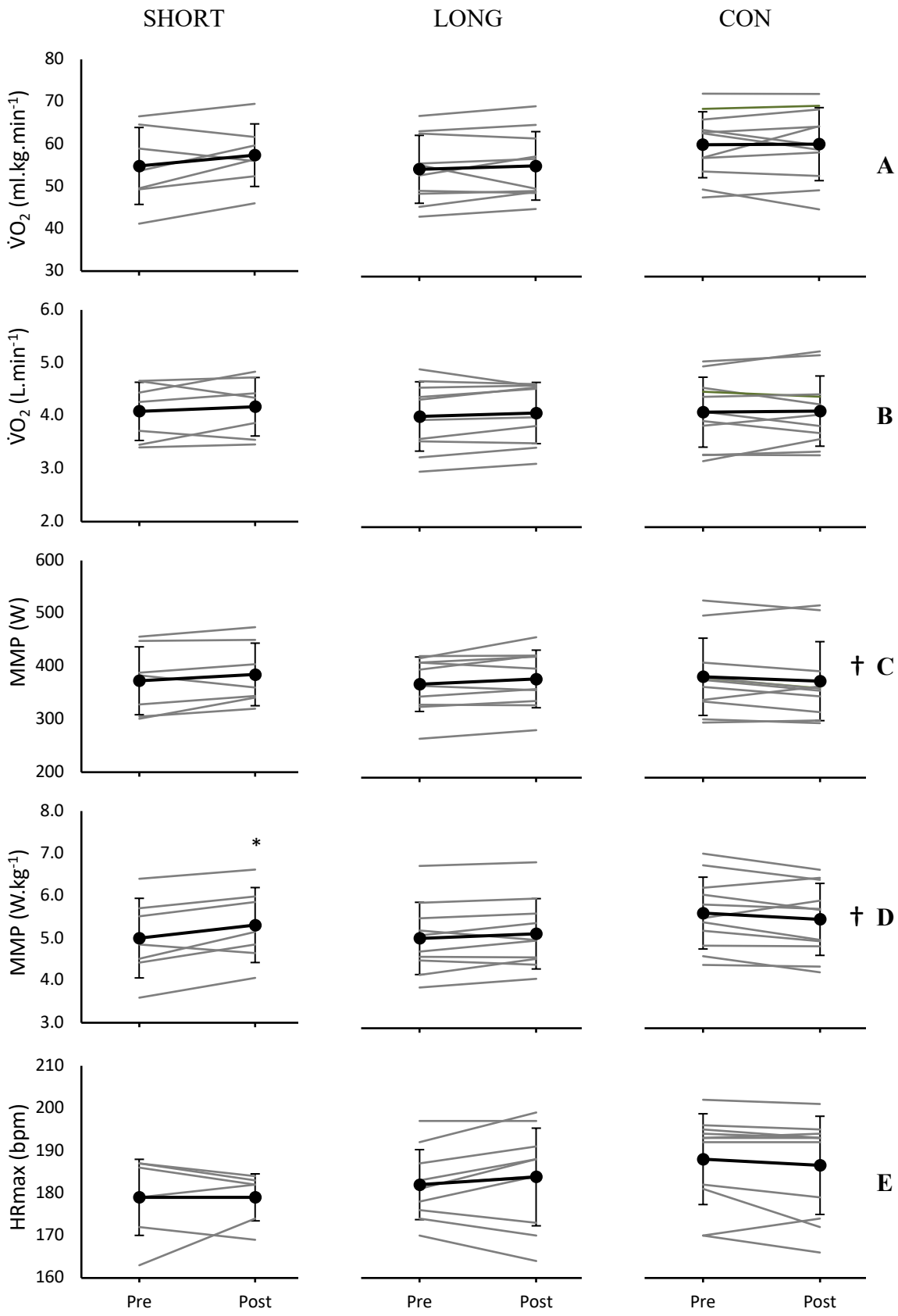


Figure 6.5 - Individual training responses from the maximal incremental test for a) relative $\dot{V}O_{2max}$, b) absolute $\dot{V}O_{2max}$, c) absolute MMP, d) relative MMP, and e) HR_{max}. * = $P < .05$ vs. pre-training time point. † = significant interaction effect between group and time $P < .05$.

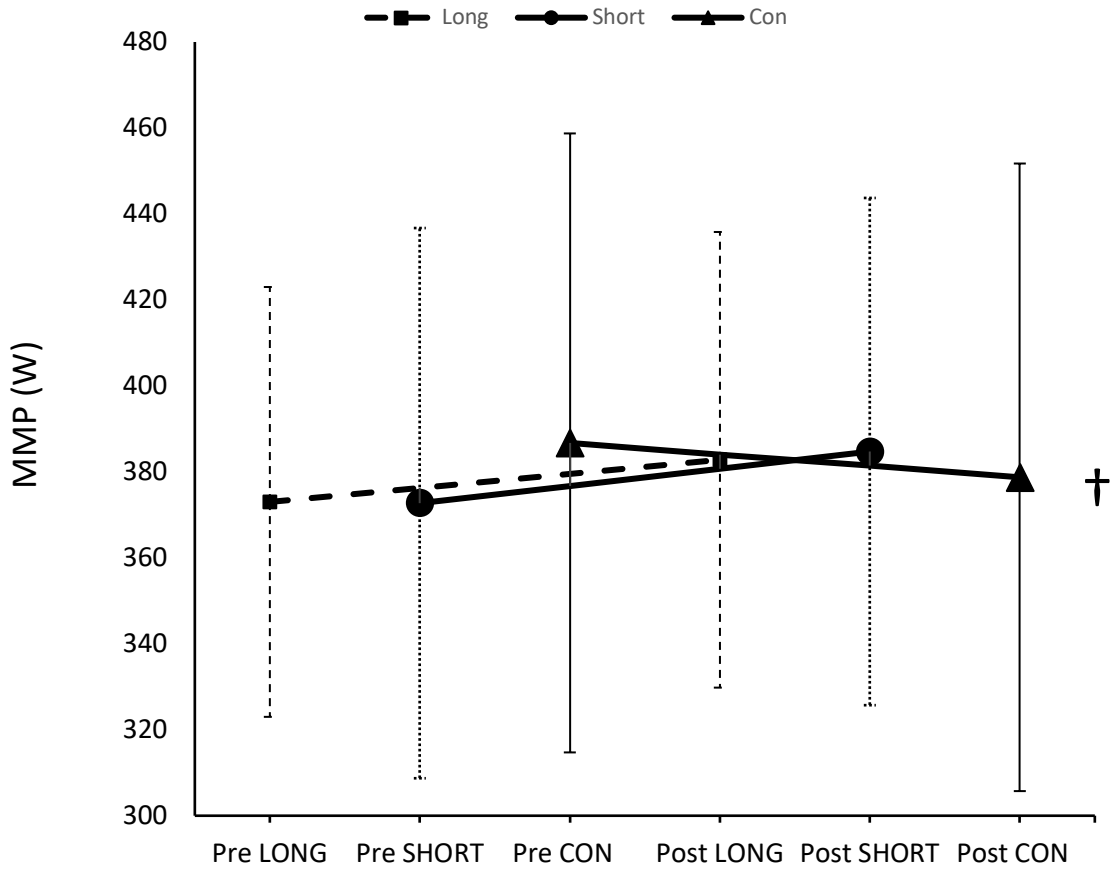


Figure 6.6 – Group mean differences in MMP before and after the training intervention across groups. Error bars display SD for each group. † = significant interaction effect between group and time ($P < .05$).

6.4.3.2 20-min TT performance test

Absolute and relative power, average HR, and $\dot{V}O_{2max}$ measured during the 20 min TT performance test are presented in Figure 6.7. There was no statistically significant interaction between the intervention and time on absolute TT power ($F_{(2, 25)} = 3.208$, $P = .058$, $\eta_p^2 = .204$), and no differences were observed between groups before ($P = .933$) or after the intervention ($P = .902$). Absolute TT power following the intervention was significantly increased in SHORT ($F_{(1, 6)} = 6.647$), $P = .042$, $\eta_p^2 = .526$), but no effect was observed in LONG ($F_{(1, 9)} = 3.684$, $P = .087$, $\eta_p^2 = .290$) or CON ($F_{(1, 10)} = .002$, $P = .967$, $\eta_p^2 = .0$). There was a statistically significant interaction between the intervention and time on relative TT power ($F_{(2, 25)} = 5.545$, $P = .01$, $\eta_p^2 = .307$), but no differences were observed between groups before ($P = .352$) or after the intervention ($P = .607$). Relative TT power following the intervention was significantly increased in SHORT ($F_{(1, 6)} = 11.5$, $P = .015$, $\eta_p^2 = .657$), but no effect was observed in LONG ($F_{(1, 9)} = 1.624$, $P = .235$, $\eta_p^2 = .153$) or CON ($F_{(1, 10)} = .448$, $P = .518$, $\eta_p^2 = .043$). There was no statistically significant

interaction between the intervention and time on TT HR ($F_{(2, 25)}$ = .466, P = .633, η_p^2 = .036), and no differences were observed between groups before (P = .432) or after the intervention (P = .618). No effect of the intervention was observed in TT HR in LONG ($F_{(1, 9)}$ = .826, P = .387, η_p^2 = .084), SHORT ($F_{(1, 6)}$ = 1.740, P = .235, η_p^2 = .225), or CON ($F_{(1, 10)}$ = .001, P = .971, η_p^2 = .0). There was no statistically significant interaction between the intervention and time on TT $\dot{V}O_2$ ($F_{(2, 25)}$ = .848, P = .440, η_p^2 = .064), and no differences were observed between groups before (P = .611) or after the intervention (P = .546). No effect of the intervention was observed in TT $\dot{V}O_2$ in LONG ($F_{(1, 9)}$ = 1.253, P = .292, η_p^2 = .122), SHORT ($F_{(1, 6)}$ = 3.104, P = .129, η_p^2 = .341), or CON ($F_{(1, 10)}$ = 3.365, P = .096, η_p^2 = .252).

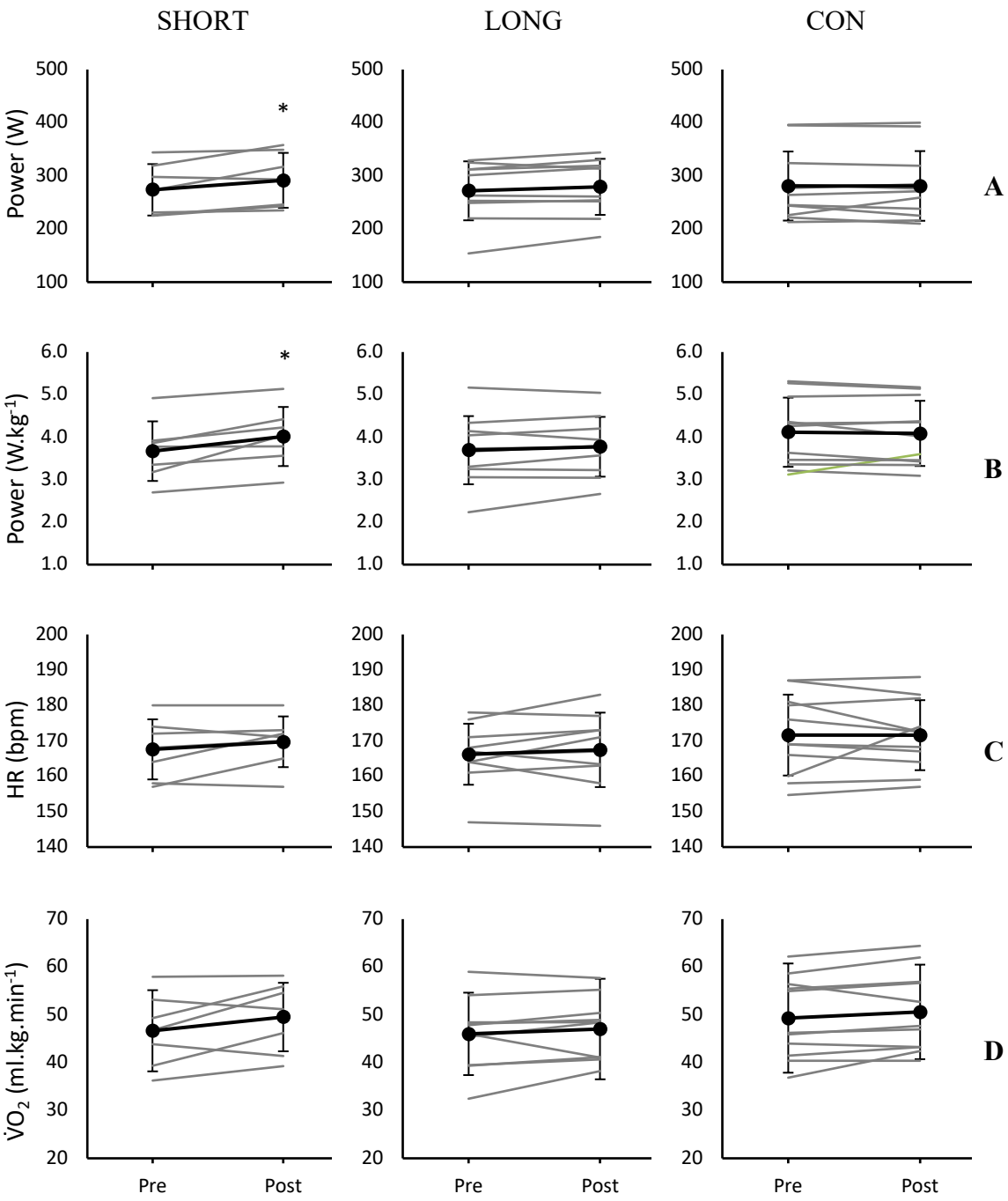


Figure 6.7 – Individual training responses from the 20 min TT performance test for a) absolute power, b) relative power, c) HR, and d) $\dot{V}O_2$. * = P < .05 vs. pre-training time point.

6.4.3.3 Variability in training responses

Different magnitudes of between-athlete variability were observed in training responses, as shown in Table 6.3, with SHORT displaying the largest between-athlete variability (70.5 %), followed by LONG (44.9 %), and CON (24.9 %). However, when training responses are marked as either a positive response, non-response, or negative response, all participants, with the exception of participants 6 and 9 from CON, improved in at least one measurement Table 6.4 and Table 6.5. Favourable changes in absolute $\dot{V}O_{2max}$ were identified in 71 % (5/7), 40 % (4/10), and 27 % (3/11) participants in, SHORT, LONG, and CON, respectively. Absolute power output during the 20 min TT performance test was improved in 86 % (6/7), 60 % (6/10), and 27 % (3/11) participants in, SHORT, LONG, and CON, respectively.

Table 6.3 - Between-athlete variability observed following intervention period in measures from maximal incremental exercise test and 20 min TT expressed as CV.

	SHORT	**LONG**	**CON**
n =	7	10	11
$\dot{V}O_{2max}$ (L.min^{-1})	34.9 %	47.8 %	12.4 %
$\dot{V}O_{2max}$ (ml.kg.min^{-1})	88.7 %	31.4 %	3.7 %
MMP (W)	3.5 %	34.8 %	42.2 %
MMP (W.kg^{-1})	63.1 %	68.0 %	48.0 %
HR_{max} (bpm)	106.9 %	58.1 %	53.6 %
TT Power (W)	103.7 %	52.2 %	2.7 %
TT Power (W.kg^{-1})	112.8 %	45.0 %	5.4 %
TT HR (bpm)	51.2 %	27.3 %	2.1 %
TT $\dot{V}O_2$ (ml.kg.min^{-1})	69.3 %	39.4 %	54.4 %
Average CV	70.5 %	44.9 %	24.9 %

Table 6.4 – Individual response matrix for changes in absolute and relative $\dot{V}O_{2max}$, MMP, and HR_{max} measured during the maximal incremental test. Green squares indicate response, white squares indicate non-response, red squares indicate negative response.

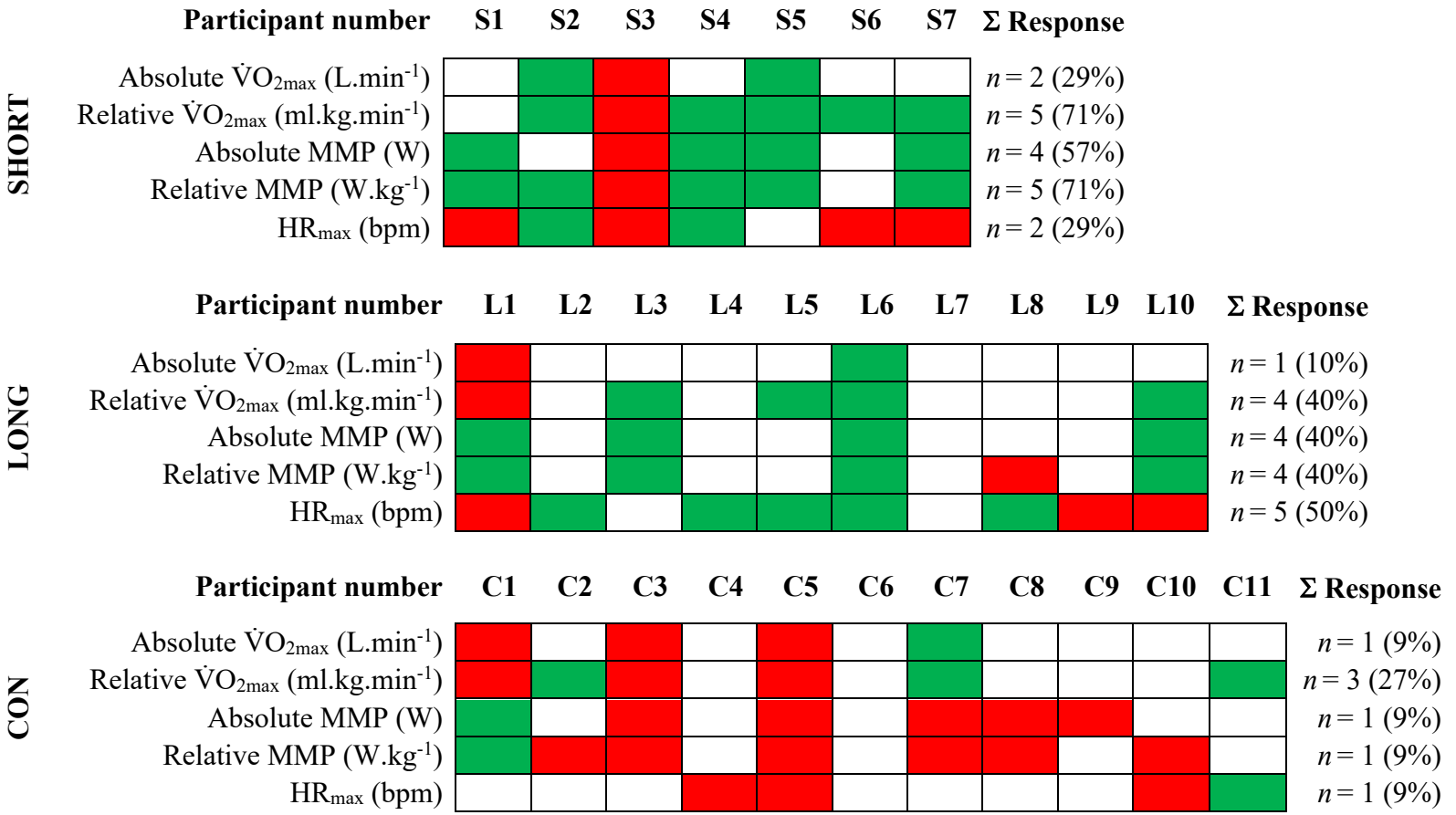


Table 6.5 – Individual response matrix for changes in absolute and relative power output, HR, and $\dot{V}O_2$ measured during the 20 min TT performance test. Green squares indicate response, white squares indicate non-response, red squares indicate negative response.

SHORT

Participant number	S1	S2	S3	S4	S5	S6	S7	Σ Response
$\dot{V}O_2$ (ml.kg.min^{-1})								*n* = 4 (57%)
Absolute Power (W)								*n* = 6 (86%)
Relative Power (W.kg^{-1})								*n* = 6 (86%)
HR (bpm)								*n* = 2 (29%)

LONG

Participant number	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	Σ Response
$\dot{V}O_2$ (ml.kg.min^{-1})											*n* = 4 (40%)
Absolute Power (W)											*n* = 6 (60%)
Relative Power (W.kg^{-1})											*n* = 5 (50%)
HR (bpm)											*n* = 3 (30%)

CON

Participant number	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	Σ Response
$\dot{V}O_2$ (ml.kg.min^{-1})												*n* = 5 (45%)
Absolute Power (W)												*n* = 3 (27%)
Relative Power (W.kg^{-1})												*n* = 2 (18%)
HR (bpm)												*n* = 1 (9%)

6.5 Discussion

The main finding of this study was that 6 weeks of effort-based training utilising a maximal session effort intensity prescription using a SHORT interval format resulted in significant improvements in relative MMP (5.0 ± 0.9 to 5.3 ± 0.9 W.kg^{-1}; *P* = .019) and both absolute (274 ± 48 to 292 ± 52 W; *P* = .042) and relative (3.7 ± 0.7 to 4.0 ± 0.7 W.kg^{-1}; *P* = .015) TT power output. Individual variability in training response was lower in SHORT than in LONG. Large heterogeneity in training response was observed across groups, ranging from 10 % (Absolute $\dot{V}O_{2max}$ in LONG) to 86 % response (Absolute and relative TT power in SHORT).

The magnitude of the training response observed within this study is surprising considering the duration of the study in comparison to previously published research. A large meta-analysis, incorporating 37 training studies with 334 untrained subjects in total, observed that using a mixture of HIIT and low-intensity training can result in an average increase of 0.5 L.min^{-1} in $\dot{V}O_{2max}$ (Bacon *et al.* 2013). In the present investigation, group-mean changes in $\dot{V}O_{2max}$ were observed to be much lower than this, with the significant change in SHORT reaching 0.2 L.min^{-1}, and the non-significant changes in LONG and CON being 0.1 L.min^{-1} and -0.1 L.min^{-1}, respectively. However, a large difference in the observed training response would likely be due to the difference in training status between the individuals in this study, and that of Bacon *et al.* (2013). Investigating the training durations completed by participants in the present study, the SHORT group completed ~ 8 h of training per week, with LONG completing ~ 5.6 h and CON completing ~ 6.6 h per week, indicating a large albeit non-significant range in volume. In addition to this, it is possible that the sessions completed within the LONG group were not performed at a high enough intensity to deliver a training stimulus of the desired nature. When compared to the average work bout power measured during Chapter 5, SHORT interval sessions were similar (Ch 5; 382 ± 55 W vs Ch 6; 391 ± 72 W), whereas the work bout power outputs during LONG interval sessions in the present chapter were lower than those performed in Chapter 5 (Ch 5; 310W ± 45 W vs Ch 6; 274 ± 52 W). It is of note that the work bout power output during SHORT is lower than has been previously reported, with 464 ± 51 W being reported during 30:30 format intervals by (Nicolò, Bazzucchi, Haxhi, *et al.* 2014). Despite the differences in work bout power between Chapter 5 and (Nicolò, Bazzucchi, Haxhi, *et al.* 2014), similar levels of BLa^{-1} (9.5 ± 3.0 mmol.L^{-1} vs 8.5 ± 2.4 mmol.L^{-1}, respectively), HR (164 ± 10 bpm vs 169 ± 5 bpm, respectively), and $\dot{V}O_2$ (3310 ± 470

ml.min^{-1} vs 3147 ± 234 ml.min^{-1}, respectively) were observed, showing that training stimulus may have been in-line with previously reported investigations. When power output was compared as a % of MMP, the SHORT interval session resulted in similar %MMP between Chapter 5 and the present chapter (101 %, and 107 %, respectively), whereas the LONG interval prescription resulted in lower %MMP between Chapter 5 and the present chapter (82 %, and 73 %, respectively). Interestingly, the work bout power output within the LONG interval sessions in the current study was extremely close to the 20 min TT power recorded before training for the LONG group (272 ± 56 W). It is therefore surprising that 6 weeks of training regularly at a power output measured for a 20 min TT would not result in an increase in 20 min TT power itself. However, this also suggests that the interval power output produced by participants was not high enough, given the similarity between the 20 min TT power output and the interval power in the LONG work bouts. It is also likely that due to the matching of total work duration between LONG and SHORT, the LONG group did not complete as many repetitions as would be commonly found within training programmes. It has been shown that ten weeks of training including three weekly training sessions which incorporate six repeats of 5 min at $\dot{V}O_{2max}$ has the potential to increase $\dot{V}O_{2max}$ by 1.2 L.min^{-1} in recreationally active individuals (Hickson, Bomze and Holloszy 1977). Having an insufficient number of repeated work bouts during LONG could also further explain the findings of significant improvement in MMP and TT performance within SHORT in the present study, as not only was the absolute work bout power output lower in LONG, but the number of repetitions may not have been sufficient.

Despite the limited number of significant changes following the training intervention in the present investigation, the use of effort-based training intensity prescriptions provides some insights into the potential for improved training individualisation. The effort-based intensity prescription was proposed to allow the exercising participants to self-regulate their exercise workload to a maximal tolerable stimulus, whilst taking into account internal and external stressors, continuing on from existing work on individualised training prescriptions (Kiviniemi *et al.* 2007; Capostagno, Lambert and Lamberts 2014). The levels of response to training in the present study were determined using the method utilised by Scharhag-Rosenberger *et al.* (2012), which involves identifying whether an individual has demonstrated changes that exceed the level of expected error (due to both biological variability and technical error of measurement) from laboratory testing. Despite the individualisation of training intensity by using effort-based prescriptions, large degrees of

individual variability are still present within the training adaptations following the intervention, as well as a lack of statistically significant changes in performance and physiological measures following training. Similar to the findings of Vollaard *et al.* (2009), the present study finds that response in one measurement does not guarantee a response in other measurements. Response levels of 86 %, 80 %, and 36 % in SHORT, LONG, and CON, respectively were identified for at least one laboratory measurement, supporting the notion that exercise response can be both modality- and measure-specific (Scharhag-Rosenberger *et al.* 2012; Pickering and Kiely 2019a; Pickering and Kiely 2019b). This finding supports the notion that including HIIT can reduce levels of non-response (Bacon *et al.* 2013), however, as this study did not utilise a crossover design it may be possible that the findings of the current study are influenced by genetic factors that influence baseline phenotype and associated training response to different exercise formats (Gaskill, Rice, *et al.* 2001; Bouchard, An, Rice, Skinner, *et al.* 1999; Perusse *et al.* 2001; Rice *et al.* 2001; Rice *et al.* 2002). Despite this, within the interval training groups, two individuals displayed adverse response in MMP measured during the incremental exercise test (S3 and L8), and two individuals displayed adverse response in TT performance (S3 and L9), showing that both groups display similar levels of training response and non-response.

6.6 Conclusion

Following 6 weeks of training using a short interval training format and a maximal session effort intensity prescription, a significant improvement in MMP and both absolute and relative TT power was found, compared to no change in these parameters when using long intervals. Individual response levels indicate that short interval formats are most likely to result in positive training response, compared to long intervals or a control group completing endurance training. The small sample size within the SHORT group in this study, as well as the low training intensity observed in the LONG group, are potential limiting factors.

Chapter 7 - Alteration in urinary metabolomic profile following acute maximal isoeffort training sessions

7.1 Abstract

Introduction: In this study, untargeted qualitative UPLC- MS was applied to provide a comprehensive global overview of the metabolic responses to exercise sessions in order to gain further insight into the mechanisms behind heterogeneity in exercise training response. **Methods:** Seventeen well-trained male competitive cyclists ($\dot{V}O_{2max}$: 59.4 ± 8.2 $ml \cdot kg^{-1} \cdot min^{-1}$) completed 9 exercise trials, each consisting of either short (SHORT) or long (LONG) interval, or continuous (CONT) effort-based training formats, each repeated 3 times using a maximal isoeffort intensity prescription. Urine samples were collected during visits to the laboratory prior to exercise (Pre), immediately following cessation of exercise (Post), and 1 hour following exercise (1h Post). Samples were analysed using UPLC- MS to construct a metabolite profile of three exercise training sessions. **Results:** Immediately following LONG and SHORT, decreases in uric acid, citric acid and increases in hypoxanthine and lactate were observed versus resting samples. Immediately following CONT, increases in hypoxanthine and lactate were observed versus resting samples. In the hour following all session formats, lactate decreased and in addition histidine decreased and creatine, tryptophan, and tyrosine increased following LONG. Comparing resting samples and samples collected one hour following the session, LONG and SHORT resulted in increased lactate and reduced uric acid excretion. Citric acid excretion decreased between pre-session and one hour following LONG and CONT. In addition, differences in urinary excretion of uric acid, lactate, hypoxanthine, tyrosine, citric acid, and hippuric acid were associated with variability in participant acute power output during the iso-effort format. **Conclusion:** The findings of the present investigation show that distinct metabolomic differences are present between LONG, SHORT, and CONT session formats using a maximal effort-based intensity prescription. The findings also display key differences in metabolomic response to exercise based on the level of individual variability in power output during acute exercise.

7.2 Introduction

The previous chapters have indicated that LONG training sessions provide a more consistent training stimulus compared to SHORT training sessions on an acute session basis, but the individual rate of response is greater when using SHORT sessions in response to a 6-week training intervention. This indicates that there may be some key differences in response to these specific training sessions, beyond those previously explored in this thesis.

Metabolomics is a method that allows researchers to use biofluids or cell tissue extracts to provide a snapshot of the whole-body metabolic profile at a specific time point (Beckonert *et al.* 2007). Human urine has gained much attention for use in metabolomics investigations in recent years, with the number of reports which use urine as sample material is continuously growing (Bernini *et al.* 2011; Zhang *et al.* 2012; Khamis, Adamko and El-Aneed 2017; Shao *et al.* 2016; Law *et al.* 2017; Deng *et al.* 2019; Yang *et al.* 2019). Metabolomic analysis of urine is able to provide a reflection of the state of the human system at a given timepoint (Yin, Lehmann and Xu 2015; Nicholson, Lindon and Holmes 1999). The current state of research into the impact of acute exercise on the human metabolome has a mixture of investigations seeking to understand the acute effects (Contrepois *et al.* 2020; Stathis, Carey and Snow 2005; Stathis *et al.* 1999; Sutton *et al.* 1980; Gerber *et al.* 2014; Dudzinska *et al.* 2018; Kurgan *et al.* 2019; Siopi *et al.* 2017; Sahlin, Tonkonogi and Söderlund 1999; Kistner *et al.* 2020; Pechlivanis *et al.* 2015; Nieman, Gillitt and Sha 2018; Zhao *et al.* 2020; Lehmann *et al.* 2010; Lee *et al.* 2010; Mukherjee *et al.* 2014; Nieman *et al.* 2012; Peake *et al.* 2014; Schranner *et al.* 2020; Davison *et al.* 2018; Hodgson *et al.* 2013; Zafeiridis *et al.* 2016; Daskalaki *et al.* 2015; C. Enea *et al.* 2010; Huang *et al.* 2010; Danaher, Gerber, Wellard, *et al.* 2016; Bally *et al.* 2017; Berton *et al.* 2017; Howe *et al.* 2018; Chorell *et al.* 2009; Nieman *et al.* 2014) as well as the adaptations to chronic training interventions lasting between 4 days and 1 year in duration (Stathis *et al.* 2006; Pechlivanis *et al.* 2013; San-Millán *et al.* 2020; Karl *et al.* 2017; Saude *et al.* 2007; Wientzek *et al.* 2014).

The synthesis of ATP from carbohydrate metabolism can be via the glycolytic pathway, resulting in pyruvate and lactate formation, or via oxidative phosphorylation. Lactate is an extremely common metabolite of investigation in exercise studies (Oliver Faude, Kindermann and Meyer 2009; Connor *et al.* 1982; Jang *et al.* 2018; Johnson and Edwards 1937; Kondoh, Kawase and Ohmori 1992; Lewis *et al.* 2010; Nikolaidis *et al.* 2016; Nikolaidis *et al.* 2018; Pechlivanis *et al.* 2013; Pechlivanis *et al.* 2015), but other metabolites such as fumarate (Danaher, Gerber, Wellard, *et al.* 2016; Hodgson *et al.* 2013; Huang *et al.* 2010; Nieman, Gillitt and Sha 2018; Pechlivanis *et al.* 2015), succinate (Brugnara *et al.* 2012; Hochachka and Dressendorfer 1976; Jang *et al.* 2018; Kelly, Kelly and Kelly 2020; Lewis *et al.* 2010; Reddy *et al.* 2020; Starling 2020), and citric acid (Krebs and Johnson 1980; Krebs, Salvin and Johnson 1938; Peake *et al.* 2014; Chorell *et*

al. 2009) give a further insight into the exercise-related changes in processes such as carbohydrate metabolism and energy production. Steroids such as cortisone or cortisol are derived from cholesterol (Miller and Auchus 2011), and whilst it has been shown that steroid profile is altered by exercise, there appears no clear pattern of change within the current literature (Schranner *et al.* 2020; Daskalaki *et al.* 2015; Zhao *et al.* 2020; Al-Khelaifi *et al.* 2018).

Amino acid alterations during and following exercise display a wide range of responses and are involved in many biological processes (Schranner et al. 2020), such as glucogenic amino acids such as alanine, glutamine, glycine, and histidine (Pechlivanis *et al.* 2013; Jang *et al.* 2018; Duft *et al.* 2017); ketogenic amino acids such as lysine (Berton *et al.* 2017; Chorell *et al.* 2009; Danaher, Gerber, Wellard, *et al.* 2016; Daskalaki *et al.* 2015; Howe *et al.* 2018; Lee *et al.* 2010; Siopi *et al.* 2017; Zhao *et al.* 2020); glucogenic/ketogenic amino acids such as isoleucine and tryptophan (Berton *et al.* 2017; Pechlivanis *et al.* 2013; Pechlivanis *et al.* 2015); and biogenic amines such as creatine and creatinine (Pechlivanis *et al.* 2015; Shi *et al.* 2007; Santone *et al.* 2014). Some amino acids are stable in their response to exercise, such as glycine, whereas tryptophan displays variable responses to exercise and depending on what body fluid was utilised for measurements (Strasser *et al.* 2016; Sheedy *et al.* 2014; Lustgarten *et al.* 2013; Daskalaki *et al.* 2015; Ito *et al.* 2003). Following exercise, tryptophan can be metabolized through a variety of pathways, such as the kynurenine pathway, which is stimulate when immune response is activated following exhaustive exercise (Strasser *et al.* 2016). Metabolites related to purine metabolism, such as creatine, hypoxanthine and uric acid, present a key opportunity to examine the exercise-induced alterations in ATP, AMP, and IMP utilisation and resynthesis and have been used previously in many exercise metabolomic studies (Sutton *et al.* 1980; Stathis, Carey and Snow 2005; Stathis *et al.* 1999; Gerber *et al.* 2014; Zieliński and Kusy 2012; Zieliński and Kusy 2015a; Zieliński and Kusy 2015b; Stathis *et al.* 2006; Hellsten-Westing *et al.* 1993; Zieliński, Kusy and Rychlewski 2011; Zieliński *et al.* 2009; Sahlin, Tonkonogi and Söderlund 1999; Hellsten-Westing, Sollevi and Sjödin 1991; Pechlivanis *et al.* 2015; Kaya *et al.* 2006; Lewis *et al.* 2010).

The aim of the current study was to compare the perturbations of metabolic profiles in key metabolites of interest following acute exercise of different session formats utilising a

maximal isoeffort training intensity prescription and investigate whether there are metabolites that are related to individual variability in acute exercise response can be identified. Many metabolites are measured during untargeted metabolomics analysis in response to exercise, many of which may be of unknown identity (Daskalaki, Easton and Watson 2015). To assist with the identification of compounds, selection of metabolites of interest prior to analysis can be conducted in order to putatively identify compounds discovered following OPLS-DA (Lee *et al.* 2006). The selected metabolites of interest in the current study are a mixture of metabolites associated with carbohydrate metabolism and the TCA cycle, amino acids, purines, and steroids (Schranner *et al.* 2020; Nieman, Gillitt and Sha 2018).

Hypotheses

$H1_1$ – Training session format groups display differences in metabolite profile across all time points (Pre, Post, 1hPost).

$H1_0$ – There are no differences in metabolite profile across training session format groups.

$H2_1$ – Differences in metabolite profile can be differentiated between variable and consistent participants.

$H2_0$ – No differences in metabolite profile can be differentiated between variable and consistent participants.

7.3 Methodology

7.3.1 Study design and experimental procedures

The samples utilised in this investigation were collected during the experimental data collection procedures within Chapter 5. Seventeen well-trained male competitive cyclists ($\dot{V}O_{2max}$: 59.4 ± 8.2 ml·kg·min^{-1}) completed 9 exercise trials, each consisting of either short (SHORT) or long (LONG) interval, or continuous (CONT) effort based training formats, each repeated 3 times using a maximal isoeffort intensity prescription. Three separate sample analysis runs were completed containing samples from a single exercise training session format: LONG, SHORT, and CONT (further details are presented in Section 5.3.2).

7.3.2 Urine sample collection and preparation

Urinary sample collection was completed by collecting spot urine samples prior to (Pre), immediately afterwards (Post), and 1 h following training sessions (1h Post; see Figure 7.1), processed following the guidelines of Want *et al.* (2010), and were analysed using UPLC-MS (Danaher, Gerber, Wellard, *et al.* 2016; Enea *et al.* 2010; Lehmann *et al.* 2010). Further urine sample collection details are described in section Chapter 3 (Section 3.11.1).

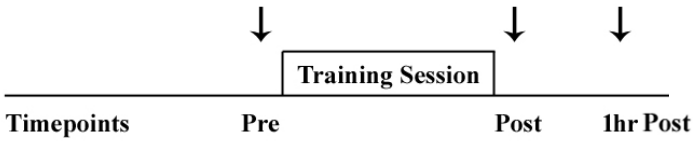


Figure 7.1 - Urine sampling timepoints

7.3.3 UPLC-MS analysis

A detailed overview of the UPLC-MS system setup, materials, and run details are presented in section Chapter 3 (Section 3.11.2) and details of run durations and injection numbers are presented in Table 7.1. Test mixes contained 17 (ESI^+) and 15 (ESI^-) known compounds from pure sources to putatively identify metabolites of interest in the samples (Table 7.2). Within each analysis batch (LONG, SHORT, and CONT), sample order was randomised to limit the impact of systematic error due to any potential degradation of the UPLC-MS system on the dataset over the acquisition time.

Table 7.1 – Experimental design of the UPLC-MS System.

	LONG	**SHORT**	**CONT**
Acquisition Time (h)	43.5	45.8	50.2
Injections	210	221	214
Test Mixes	6	5	3
Blanks	4	13	8
cQCs	20	20	20
QCs	16	15	15
Samples	150	150	132
Ionisation mode	ESI^+/ESI^-	ESI^+/ESI^-	ESI^+/ESI^-

Table 7.2 - The $[M+H]^{+/-}$ and RT of each compound in the test mix samples used for the assessment of UPLC-MS reproducibility in ESI^+ and ESI^-.

ESI^+				
Metabolite	**Formula**	**Mass**	**$[M+H]^+$**	**RT (min)**
Glycine	$C_2H_5NO_2$	75.0320	76.0399	0.38
L-Alanine	$C_3H_7NO_2$	89.0477	90.0555	0.44
Creatinine	$C_4H_7N_3O$	113.0589	114.0667	0.42
Succinate	$C_4H_6O_4$	118.0266	119.0344	1.16
Creatine	$C_4H_9N_3O_2$	131.0695	132.0773	1.26
L-Isoleucine	$C_6H_{13}NO_2$	131.0946	132.1025	0.44
Hypoxanthine	$C_5H_4N_4O$	136.0385	137.0463	0.88
L-Glutamine	$C_5H_{10}N_2O_3$	146.0691	147.0770	0.34
L(+)-Lysine	$C_6H_{14}N_2O_2$	146.1055	147.1134	0.38
L-Histidine	$C_6H_9N_3O_2$	155.0695	156.0773	0.36
Uric acid	$C_5H_4N_4O_3$	168.0283	169.0362	0.82
Hippuric acid	$C_9H_9NO_3$	179.0582	180.0661	4.10
L-Tyrosine	$C_9H_{11}NO_3$	181.0739	182.0817	1.16
Citric acid	$C_6H_8O_7$	192.0270	193.0348	0.80
L-Tryptophan	$C_{11}H_{12}N_2O_2$	204.0899	205.0977	3.48
Cortisone	$C_{21}H_{28}O_5$	360.1937	361.2015	6.35
Cortisol	$C_{21}H_{30}O_5$	362.2093	363.2093	6.37
ESI^-				
Metabolite	**Formula**	**Mass**	**$[M+H]^-$**	**RT (min)**
Lactate	$C_3H_6O_3$	90.0317	89.0317	0.68
Creatinine	$C_4H_7N_3O$	131.0695	112.0589	0.80
Fumarate	$C_4H_2O_4^{-2}$	113.9953	112.9953	0.95
Succinate	$C_4H_6O_4$	118.0266	117.0266	1.06
Creatine	$C_4H_9N_3O_2$	131.0695	130.0695	0.44
Hypoxanthine	$C_5H_4N_4O$	136.0385	135.0385	0.88
L-Glutamine	$C_5H_{10}N_2O_3$	146.0691	145.0691	0.39
L-Histidine	$C_6H_9N_3O_2$	155.0695	154.0695	0.36
Uric acid	$C_5H_4N_4O_3$	168.0283	167.0283	0.82
Hippuric acid	$C_9H_9NO_3$	179.0582	178.0582	4.10
L-Tyrosine	$C_9H_{11}NO_3$	181.0739	180.0739	1.16
Citric acid	$C_6H_8O_7$	192.0270	191.0270	0.80
L-Tryptophan	$C_{11}H_{12}N_2O_2$	204.0899	203.0899	3.48
Cortisone	$C_{21}H_{28}O_5$	360.1937	359.1937	6.35
Cortisol	$C+H_{30}O_5$	362.2093	361.2093	7.73

7.3.4 Data analysis

7.3.4.1 Univariate analysis of raw data

Details on the univariate analysis of raw data retention time, mass accuracy and variance are presented in Chapter 3 (Section 3.11.5).

7.3.4.2 Data pre-processing

Details of data pre-processing parameters are presented in Chapter 3 (Section 3.11.6) and utilised the XCMS package for R software (Smith *et al.* 2006) which allowed the chromatographic dataset to be reduced into a single matrix for further analysis.

7.3.4.3 Multivariate analysis

Details of multivariate data analyses, specifically PCA and OPLS-DA performed within SIMCA-P (Version 12, Umetrics, Sweden) are presented in Chapter 3 (Section 3.11.7). For each exercise group, pairwise OPLS-DA was performed to investigate acute changes in metabolic profile (e.g., Pre, Post, 1h Post). Additional OPLS-DA analyses were conducted with participants classified as either "*variable*" or "*consistent*" for each exercise session within Chapter 5 (Section 5.4.3). Variable participants were classified based on their exercise variability for %MMP (LONG = 6; SHORT = 4; CONT = 3) and $\%HR_{max}$ (LONG = 4, SHORT = 3, CONT = 4). The rationale for investigating variability relating to both MMP and HR_{max} is to isolate internal and external training load variability.

7.4 Results

7.4.1 Analysis of raw data

Following all data pre-processing steps, a range of 123 – 183 metabolic features were detected across all ionisation modes and sample groups (Table 7.3). Comparisons of all cQC BPI chromatograms indicated that the UPLC-MS system used had been conditioned sufficiently for all experiments, in both ESI^+ and ESI^-, and cQC reached stability after 10 injections.

Table 7.3 - Metabolic features present in dataset following each stage of filtering during XCMS pre-processing.

		Raw Data	Post MinFrac Filtering	Post CV Filtering
ESI^+	**LONG**	234	171	141
	SHORT	247	168	136
	CONT	210	142	123
ESI^-	**LONG**	366	247	183
	SHORT	361	235	142
	CONT	369	235	162

The metabolites presented in Table 7.4 were excluded from identification following mass accuracy and peak intensity threshold failures. Inspection of QC samples was performed using the identified metabolites from test mix injections to assess the analytical reproducibility of the UPLC-MS systems' mass accuracy (Table 7.5), retention time drift (Table 7.6), and alterations to the peak intensities (Table 7.7 and Table 7.8). L-Alanine and succinate failed to meet the ≤ 30 ppm mass accuracy threshold in ESI^+, while all other metabolites met the threshold and remained stable for all sample runs for both ESI^+ and ESI^- (Table 7.5). The maximum retention time drift observed was 3.0 seconds in both ESI^+ and ESI^-. Retention time was also relatively stable across ESI^+ and ESI^- for LONG, SHORT, and CONT experimental runs (1.3 vs 1.3 sec, 0.9 vs 1.1 sec, and 1.1 vs 1.4 sec, respectively: Table 7.6). Peak intensity change data are presented in Table 7.7 and Table 7.8, and indicated metabolites were excluded due to peak intensity CV values exceeding the accepted thresholds of 30 %.

Table 7.4 – Metabolites excluded from identification based on failure of mass accuracy and peak intensity CV thresholds. SHORT ESI^+ not included based on the lack of QC clustering fit from PCA analysis.

	LONG	SHORT	CONT
ESI^+	L-Alanine L-Isoleucine L-Lysine Succinate	-	L-Glutamine L-Isoleucine Succinate
ESI^-	L-Histidine	Cortisol	Creatine L-Histidine Cortisol

Table 7.5 - Mass accuracy for QC samples in ESI^+ and ESI^- UPLC-MS conditions.

			ESI^+			ESI^-		
Metabolite	**[M+H]**	**[M-H]**	**Standard Compound**	**Ppm Error**	**Theoretical Mass Diff.**	**Standard Compound**	**Ppm Error**	**Theoretical Mass Diff.**
Glycine	76.0399	-	76.0	0	0.0399	-	-	-
Lactate	-	89.0317	-	-	-	89.0	0	0.0317
L-Alanine	90.0555	-	90.04	**-2207.51**	0.0155	-	-	-
Creatinine	114.0667	112.0589	114.1	0	-0.0333	112.1	0	-0.0411
Fumarate	-	112.9953	-	-	-	113.0	0	-0.0047
Succinate	119.0344	117.0266	119.1	**840.3**	-0.0656	117.0	0	0.0266
Creatine	132.0773	130.0695	132.1	0	-0.0227	130.1	0	-0.0305
L-Isoleucine	132.1025	-	132.1	0	0.0025	-	-	-
Hypoxanthine	137.0463	135.0385	137.0	0	0.0463	135.0	0	0.0385
L-Glutamine	147.077	145.0691	147.1	0	-0.0230	145.1	0	-0.0309
L(+)-Lysine	147.1134	-	147.1	0	0.0134	-	-	-
L-Histidine	156.0773	154.0695	156.1	0	-0.0227	154.1	0	-0.0305
Uric acid	169.0362	167.0283	169.0	0	0.0362	167.0	0	0.0283
Hippuric acid	180.0661	178.0582	180.1	0	-0.0339	178.1	0	-0.0418
L-Tyrosine	182.0817	180.0739	182.1	0	-0.0183	180.1	0	-0.0261
Citric acid	193.0348	191.027	193.0	0	0.0348	191.0	0	0.0270
L-Tryptophan	205.0977	203.0899	205.1	0	-0.0023	203.1	0	-0.0101
Cortisone	361.2015	359.1937	361.2	0	0.0015	359.2	0	-0.0063
Cortisol	363.2093	361.2093	363.2	0	0.0093	361.2	0	0.0093

Table 7.6 – Retention time deviation for QC samples in ESI^+ and ESI^- UPLC-MS conditions.

ESI^+						
	LONG		SHORT		CONT	
Metabolite	Average (min)	Drift (sec)	Average (min)	Drift (sec)	Average (min)	Drift (sec)
Glycine	0.41	1.20	0.41	1.20	0.41	0.60
L-Alanine	0.44	0.00	0.44	0.00	0.44	0.00
Creatinine	0.42	0.60	0.42	0.00	0.42	0.00
Succinate	1.17	1.20	1.16	1.20	1.17	1.80
Creatine	0.44	1.80	0.44	0.00	0.44	0.00
L-Isoleucine	1.17	1.80	1.16	1.20	1.17	1.20
Hypoxanthine	0.89	0.60	0.88	1.20	0.88	1.20
L-Glutamine	0.34	1.20	0.34	0.60	0.34	0.60
L(+)-Lysine	0.39	1.20	0.38	0.60	0.39	1.20
L-Histidine	0.36	0.60	0.37	0.60	0.36	1.20
Uric acid	0.82	0.60	0.82	0.60	0.82	0.60
Hippuric acid	4.11	1.80	4.09	1.80	4.10	1.80
L-Tyrosine	1.17	1.80	1.16	1.20	1.17	1.80
Citric acid	0.79	1.20	0.79	0.00	0.79	1.20
L-Tryptophan	3.49	1.80	3.48	1.80	3.48	3.00
Cortisone	6.36	2.40	6.35	1.80	6.35	1.80
Cortisol	6.38	1.80	6.36	1.80	6.37	1.20
ESI^-						
	LONG		SHORT		CONT	
Metabolite	Average (min)	Drift (sec)	Average (min)	Drift (sec)	Average (min)	Drift (sec)
Lactate	0.69	0.60	0.68	0.60	0.69	0.60
Creatinine	0.80	1.20	0.79	1.20	0.79	1.20
Fumarate	0.95	0.60	0.95	0.60	0.95	0.60
Succinate	1.06	1.20	1.05	1.20	1.05	1.20
Creatine	0.44	1.20	0.43	1.20	0.44	1.20
Hypoxanthine	0.88	1.20	0.88	0.60	0.88	1.20
L-Glutamine	0.38	0.60	0.39	0.60	0.38	0.00
L-Histidine	0.37	1.20	0.36	1.20	0.37	1.20
Uric acid	0.82	0.60	0.82	0.60	0.82	1.20
Hippuric acid	4.10	2.40	4.10	1.20	4.10	2.40
L-Tyrosine	1.17	1.80	1.16	1.80	1.17	1.20
Citric acid	0.80	1.20	0.79	0.60	0.79	1.20
L-Tryptophan	3.49	2.40	3.47	1.80	3.48	2.40
Cortisone	6.36	1.80	6.34	2.40	6.35	3.00
Cortisol	7.73	1.2	7.72	1.2	7.72	1.80

Table 7.7 - Peak intensity, variation, and percentage reduction for QC samples in ESI^+. Bold CV values indicate failure of 0.3 CV threshold.

Metabolite	LONG (N=18)			SHORT (N=17)			CONT (N=17)		
	Average	CV	% Loss	Average	CV	% Loss	Average	CV	% Loss
Glycine	2.90E + 06	18.64	-46 %	2.98E + 06	15.63	-38 %	3.25E + 06	15.80	-42 %
L-Alanine	2.56E + 05	**41.58**	-66 %	1.97E + 05	**63.93**	-80 %	3.82E + 05	24.32	-63 %
Creatinine	9.34E + 07	12.46	-43 %	8.69E + 07	16.61	-66 %	9.24E + 07	13.78	-39 %
Succinate	3.17E + 04	13.55	-41 %	2.50E + 04	18.73	-55 %	2.89E + 04	14.22	-41 %
Creatine	9.51E + 05	7.33	-21 %	7.99E + 05	13.86	-38 %	9.44E + 05	12.04	-34 %
L-Isoleucine	3.61E + 06	**46.78**	-69 %	2.43E + 06	**52.07**	-79 %	6.43E + 06	**40.77**	-69 %
Hypoxanthine	1.43E + 07	8.33	-24 %	1.21E + 07	13.93	-38 %	6.05E + 06	12.38	-37 %
L-Glutamine	4.93E + 05	26.10	-57 %	7.10E + 04	29.31	-63 %	8.70E + 04	**49.86**	-75 %
L(+)-Lysine	1.02E + 05	**39.20**	-66 %	5.30E + 05	15.50	-52 %	4.20E + 05	27.42	-53 %
L-Histidine	4.90E + 04	8.70	-28 %	4.43E + 04	20.31	-52 %	4.21E + 04	26.56	-64 %
Uric acid	8.62E + 06	9.73	-28 %	7.02E + 06	12.24	-40 %	8.59E + 06	8.64	-30 %
Hippuric acid	2.13E + 07	4.84	-18 %	1.89E + 07	7.39	-24 %	1.92E + 07	8.55	-27 %
L-Tyrosine	1.63E + 06	8.94	-26 %	1.29E + 06	9.94	-29 %	1.54E + 06	11.88	-36 %
Citric acid	1.29E + 06	10.05	-32 %	1.08E + 06	13.59	-38 %	9.87E + 05	16.93	-43 %
L-Tryptophan	1.82E + 06	10.60	-29 %	1.32E + 06	7.96	-27 %	1.53E + 06	17.88	-48 %
Cortisone	1.49E + 04	16.11	-45 %	1.28E + 04	15.65	-47 %	1.06E + 04	13.69	-46 %
Cortisol	1.24E + 04	14.28	-38 %	1.20E + 04	13.07	-38 %	7.85E + 03	15.00	-37 %

Table 7.8 - Peak intensity, variation, and percentage reduction for QC samples in ESI$^-$. Bold CV values indicate failure of 0.3 CV threshold.

Metabolite	LONG (N=18)			SHORT (N=17)			CONT (N=17)		
	Average	CV	% Loss	Average	CV	% Loss	Average	CV	% Loss
Lactate	1.84E + 06	7.21	-22 %	1.34E + 06	8.33	-22 %	2.43E + 05	17.30	-37 %
Creatinine	4.36E + 04	12.69	-50 %	3.94E + 04	11.87	-35 %	3.43E + 04	12.48	-34 %
Fumarate	1.04E + 04	14.81	-43 %	7.17E + 03	14.97	-44 %	3.88E + 03	15.92	-38 %
Succinate	2.60E + 05	5.63	-21 %	2.37E + 05	10.79	-32 %	2.61E + 05	4.66	-19 %
Creatine	6.59E + 03	20.23	-51 %	5.64E + 03	20.81	-48 %	6.48E + 03	**36.40**	-68 %
Hypoxanthine	9.63E + 05	12.06	-45 %	9.44E + 05	11.01	-44 %	3.68E + 05	12.32	-47 %
L-Glutamine	2.19E + 04	19.00	-50 %	1.70E + 04	20.37	-52 %	1.72E + 04	14.18	-50 %
L-Histidine	8.93E + 04	**41.31**	-68 %	4.27E + 04	21.14	-58 %	4.05E + 04	**80.71**	-85 %
Uric acid	3.55E + 06	6.02	-17 %	3.16E + 06	7.12	-22 %	3.11E + 06	5.18	-17 %
Hippuric acid	1.27E + 07	5.74	-17 %	1.13E + 07	5.91	-22 %	1.13E + 07	4.55	-14 %
L-Tyrosine	2.69E + 04	20.34	-56 %	2.06E + 04	29.22	-68 %	1.76E + 04	17.14	-55 %
Citric acid	5.44E + 06	7.79	-23 %	5.00E + 06	8.81	-25 %	4.57E + 06	9.65	-26 %
L-Tryptophan	2.31E + 04	15.50	-44 %	1.70E + 04	22.56	-59 %	1.34E + 04	20.68	-55 %
Cortisone	3.65E + 03	19.03	-58 %	4.10E + 03	14.75	-39 %	3.15E + 03	19.23	-54 %
Cortisol	4.96E + 03	19.34	-47 %	9.85E + 03	**48.77**	-92 %	6.96E + 03	**44.29**	-75 %

7.4.2 PCA analysis and assessment of QC stability

PCA analysis was conducted to observe any trends or patterns present in the datasets related to each training session format. The stability of the UPLC-MS system was assessed using repeated QC sample injections interspersed throughout the sample runs. The PCA model statistics are displayed in Table 7.9, outlining the predictive component of the PCA, Q^2, the variance (R^2X), and percentage of variance in PC1 and PC2. The PCA scores plots for the first two PCs are shown in Figure 7.2. Generally, all PCA models, except for SHORT in ESI^+, show tight clustering of QC, indicating good analytical reproducibility throughout all analytical runs. However, two clusters of QC samples were observed for ESI^+ for SHORT, and these two clusters were based on the two days of the analytical run being completed over a duration of a 6-day period, compared to the 2-day period of the other sample runs. Despite various corrections on the QC samples by applying appropriate normalisation, the QC cluster in the SHORT ESI^+ remained split and no further analysis will be performed on this dataset. As the purpose of this investigation was to ascertain individual variability in metabolomic response to different training sessions, data points falling outside the 95 % Hotelling's T^2 ellipsis were included for further supervised analysis by OPLS-DA. In addition, the outlier samples were investigated and were due to biological variations in metabolites, not any analytical issue. The PCA score plots for ESI^+ in LONG and SHORT revealed no identifiable clusters or trends based on participant, but a skewing of the data could be observed in both plots based on time point of collection (Pre, Post, 1hPost). Less of a trend based on the time point of collection could be observed in the PCA scores plot for ESI^+ in CONT. No clusters or trends were observed based on participants in the PCA score plots for ESI^- for LONG, SHORT, and CONT, but trends can be observed based on time point of collection in all three plots, respectively, but the direction of these trends was not similar between groups.

Table 7.9 - PCA scores details. R^2X = regression coefficient, Q^2 = predictive component of the PCA to assess model validity, PC1/PC2 = principal component 1 (PC1) and principal component 2 (PC2) for each PCA model. SHORT ESI^+ highlighted in red based on the lack of QC clustering fit from PCA analysis.

	ESI^+				**ESI^-**			
	R^2X	**Q^2**	**PC1**	**PC2**	**R^2X**	**Q^2**	**PC1**	**PC2**
LONG	.274	.210	.212	.0062	.310	.256	.245	.065
SHORT	.334	.286	.261	.073	.273	.196	.203	.070
CONT	.252	.180	.176	.076	.314	.261	.234	.080

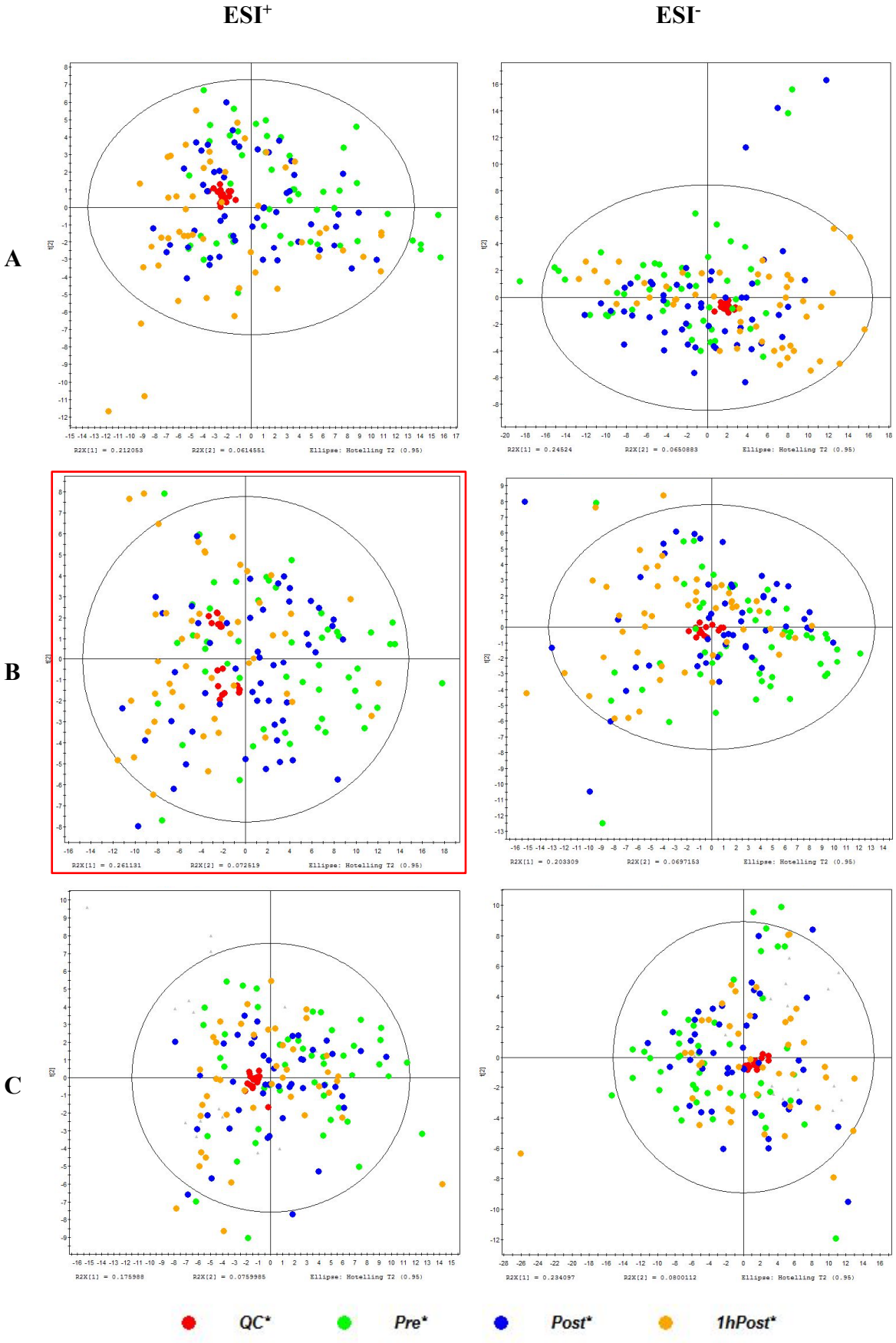


Figure 7.2 - PCA scores plots displaying good analytical reproducibility of the UPLC-MS as shown by tight QC clustering for LONG (A), SHORT (B), and CONT (C) in ESI^+ and ESI^-, with the exception of SHORT ESI^+ highlighted with a red border that indicates the lack of QC clustering on the PCA scores plot.

7.4.3 OPLS-DA analysis

7.4.3.1 Acute response to exercise

To evaluate the time-dependent metabolomic perturbations following the training sessions, OPLS-DA models were generated comparing sample collection time points for each session format in a pairwise manner. The initial OPLS-DA model generated for CONT in ESI^+ for Post vs 1hPost was deemed to be invalid ($P = .21$ from permutation testing), highlighted in red within Table 7.10, and was not included in any further analyses. All other OPLS-DA models generated positive Q^2 values and passed permutation testing ($n = 100$; $P < .05$). Figure 7.3, Figure 7.4, and Figure 7.5 display OPLS-DA scatter plots for both ESI^+ and ESI^- for LONG, SHORT, and CONT, respectively. Table 7.10 also presents model diagnostics, outlining variability in each model due to separation between groups, and due to within-group variability.

Table 7.10 - OPLS-DA scores based on acute exercise effects. Model diagnostics presented based on separation of scatter plots between groups, and within-group variability as a % CV. SHORT ESI^+ not included based on the lack of QC clustering fit from PCA analysis. OPLS-DA analyses which failed to establish valid models have been excluded.

		Time	R^2X	R^2Y	Q^2	A	Between-group separation based on predictive component	Within-group variability based on orthogonal component	P-Value
ESI^+	**LONG**	Pre vs Post	.644	.772	.696	2	18%	31%	< .01
		Post vs 1h Post	.590	.379	.098	2	8%	32%	.01
		Pre vs 1h Post	.599	.638	.524	2	18%	32%	< .01
	CONT	Pre vs Post	.676	.656	.593	2	10%	36%	< .01
		Pre vs 1h Post	.629	.546	.441	2	9%	38%	< .01
ESI^-	**LONG**	Pre vs Post	.654	.757	.704	2	17%	24%	< .01
		Post vs 1h Post	.612	.577	.435	2	10%	38%	< .01
		Pre vs 1h Post	.605	.683	.576	2	11%	41%	< .01
	SHORT	Pre vs Post	.657	.680	.594	2	17%	21%	< .01
		Post vs 1h Post	.602	.525	.367	2	9%	26%	< .01
		Pre vs 1h Post	.641	.660	.521	2	13%	24%	< .01
	CONT	Pre vs Post	.416	.684	.527	2	8%	16%	< .01
		Post vs 1h Post	.584	.375	.133	2	5%	40%	< .01
		Pre vs 1h Post	.635	.688	.598	2	8%	41%	< .01

R^2X = variation in X explained by the model, R^2Y = variation in Y explained by the model, Q^2 Y= goodness of prediction, A = number of orthogonal components, P-value = permutation test probability of spurious model generation.

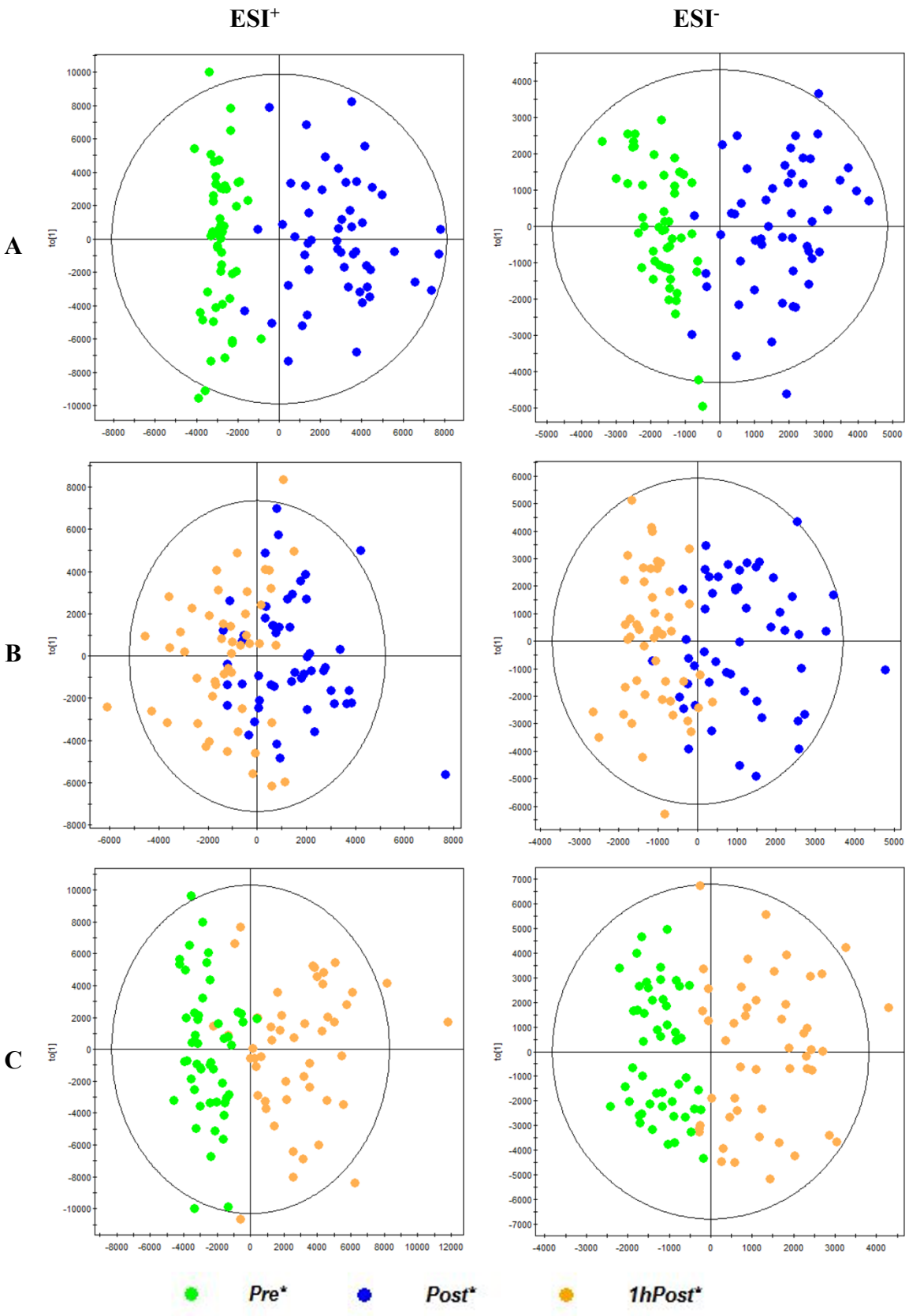


Figure 7.3 – OPLS-DA scores plots displaying separation of the LONG experimental run for Pre-Post (A), Post-1hPost (B), and Pre-1hPost (C) in ESI^+ and ESI^- modes. X-axis displays OPLS-DA predictive component, and Y-axis displays OPLS-DA orthogonal component.

ESI⁻

A

B

C

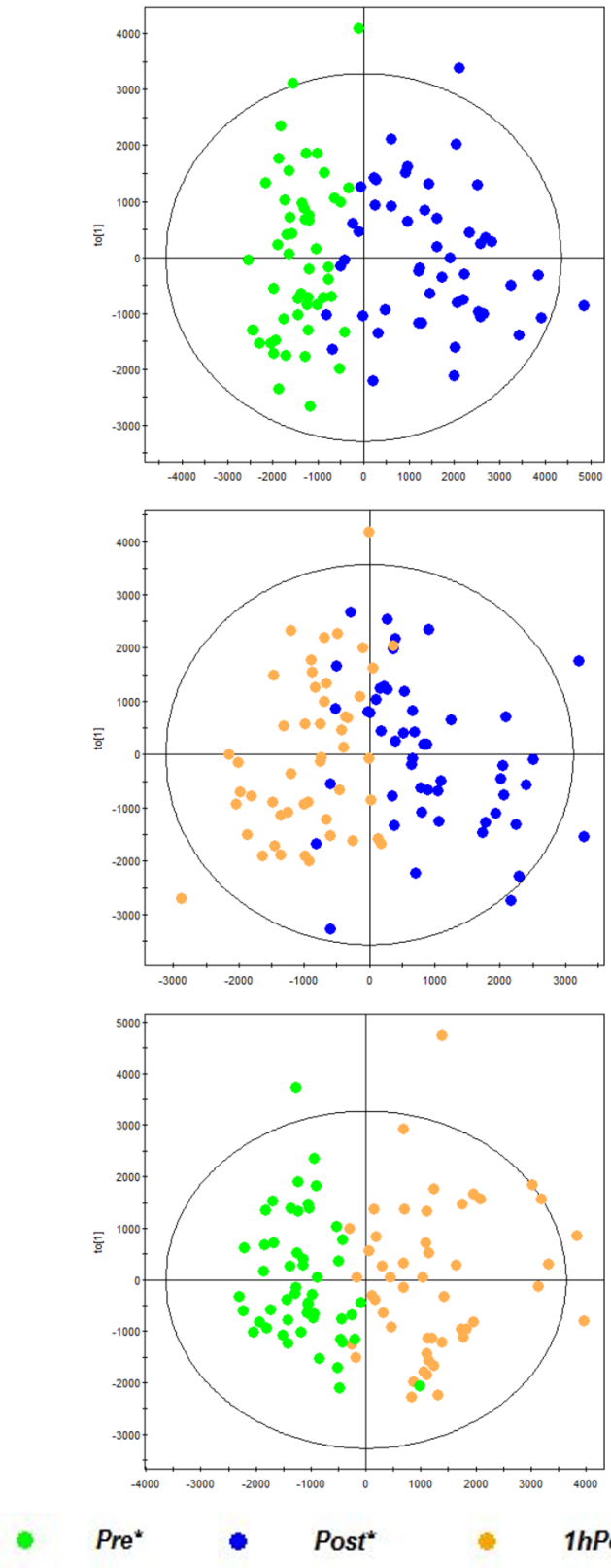


Figure 7.4 – OPLS-DA scores plots displaying separation of the SHORT experimental run for Pre-Post (A), Post-1hPost (B), and Pre-1hPost (C) in ESI⁻. SHORT ESI⁺ not included based on the lack of QC clustering fit from PCA analysis. X-axis displays OPLS-DA predictive component, and Y-axis displays OPLS-DA orthogonal component.

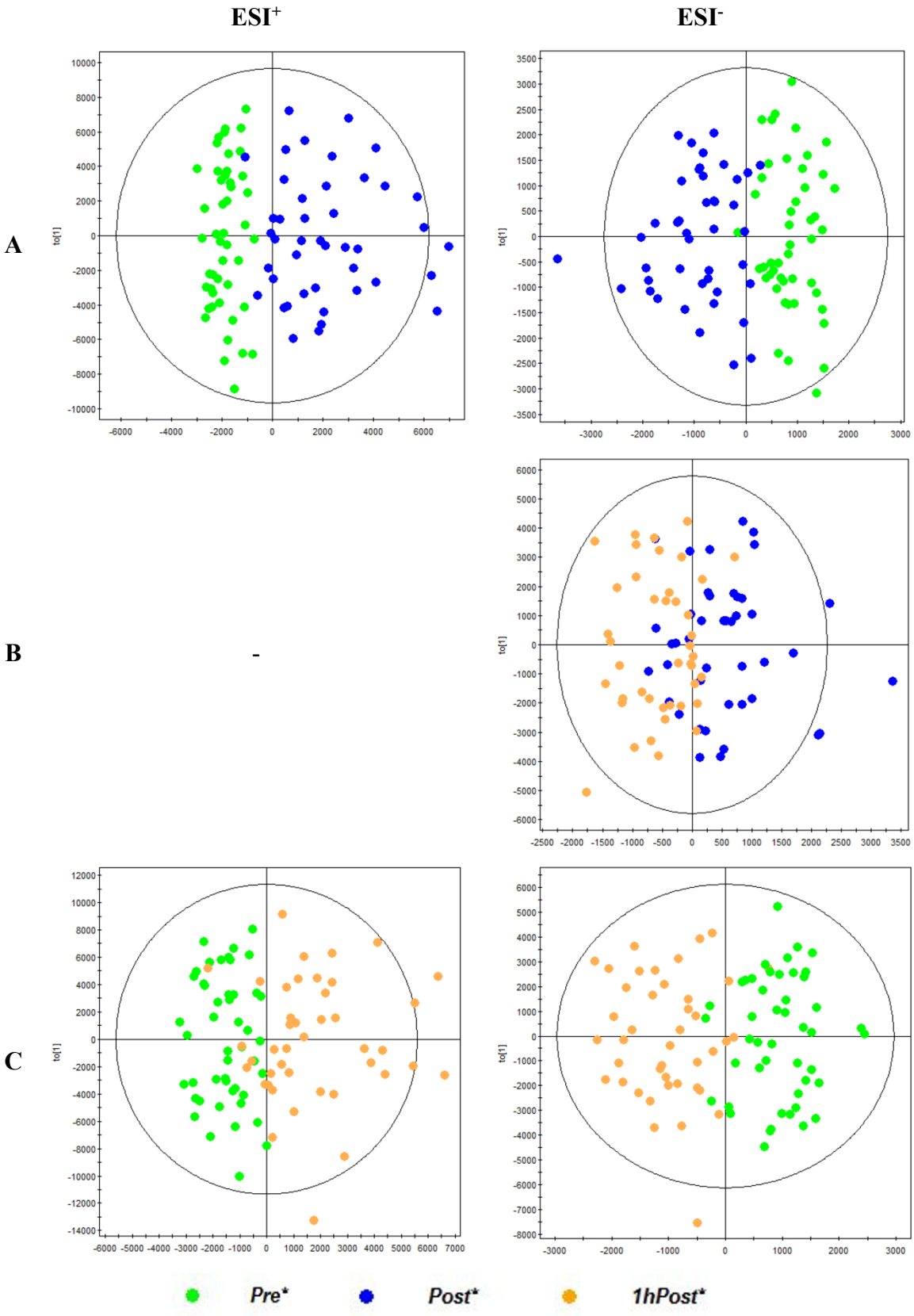


Figure 7.5 – OPLS-DA scores plots displaying separation of the CONT experimental run for Pre-Post (A), Post-1hPost (B), and Pre-1hPost (C) in ESI^+ and ESI^-. CONT ESI^+ Post-1hPost not included based on an invalid OPLS-DA model. X-axis displays OPLS-DA predictive component, and Y-axis displays OPLS-DA orthogonal component.

Metabolites contributing to OPLS-DA separation

For each valid OPLS-DA model, S-plots were used to extract metabolites that contribute to the separation in each model and these involved a range of metabolite classes, including alkaloids, amino acids, carboxylic acids, purine derivatives, organic acids, in addition to several unknown compounds (Table 7.11, Table 7.12, and Table 7.13).

Urinary excretion of uric acid was observed to decrease compared to baseline (Pre vs Post) following LONG (ESI^+ and ESI^-) and SHORT (ESI^-). Urinary excretion of citric acid was observed to decrease compared to baseline (Pre vs Post) following LONG (ESI^+) and SHORT (ESI^-). Urinary excretion of lactate was observed to increase compared to baseline (Pre vs Post) following LONG (ESI^-), SHORT (ESI^-), and CONT (ESI^-). Urinary excretion of hypoxanthine was observed to increase compared to baseline (Pre vs Post) following LONG (ESI^+ and ESI^-), SHORT (ESI^-), and CONT (ESI^+ and ESI^-). A decrease in the excretion of histidine, and an overall increased in creatine, tryptophan, and tyrosine was observed in the hour following the session (Post vs 1hPost) following LONG (ESI^+). A decrease in the excretion of lactate was observed in the hour following the session (Post vs 1hPost) following LONG (ESI^-), SHORT (ESI^-), and CONT (ESI^-). An overall decrease in the excretion of uric acid was over the course of the session (Pre vs 1hPost) following LONG (ESI^+) and SHORT (ESI^-). An overall decrease in the excretion of citric acid was over the course of the session (Pre vs 1hPost) following LONG (ESI^+) and CONT (ESI^+ and ESI^-). An overall increase in the excretion of lactate was observed in the hour following session (Pre vs 1hPost) following LONG (ESI^-) and SHORT (ESI^-).

Table 7.11 - Exercise induced acute metabolic alterations based on OPLS-DA and S-Plot analysis between Pre and Post time points in ESI^+ and ESI^- modes. SHORT ESI^+ not included based on the lack of QC clustering fit from PCA analysis. Unknown metabolites are presented by *m/z* and retention time.

Ionisation mode	Biological Class	Metabolite	*m/z (M)*	RT (min)	LONG	SHORT	CONT
ESI^+	Alkaloids	Uric acid	169.0	0.82	↓	-	
	Carboxylic acids & derivatives	Citric acid	193.0	0.78	↓	-	
	Purine derivatives	Hypoxanthine	137.0	0.88	↑	-	↑
	Unknown	137/133	137.0	2.22	↑	-	
		169/35	169.0	0.58	↑	-	
ESI^-	Alkaloids	Uric acid	167.0	0.82	↓	↓	
	Carboxylic acids & derivatives	Citric acid	191.0	0.78		↓	
	Organic acids	Lactate	89.0	0.68	↑	↑	↑
	Purine derivatives	Hypoxanthine	135.0	0.88	↑	↑	↑
	Unknown	145.1/36	145.1	0.60			↑
		145.1/37	145.1	0.62	↑		
		191/32	191.0	0.53			↑

↑ indicates increased urinary excretion of metabolite at Post compared to Pre. ↓ indicates decreased urinary excretion of metabolite at Post compared to Pre.

Table 7.12 - Exercise induced metabolic alterations determined by OPLS-DA and S-Plot analysis between Post and 1h-Post time points. SHORT ESI^+ not included based on the lack of QC clustering fit from PCA analysis. CONT ESI^+ not included based on the result of invalid OPLS-DA model. Unknown metabolites are presented by *m/z* and time.

Ionisation mode	Biological Class	Metabolite	*m/z (M)*	RT (min)	LONG	SHORT	CONT
ESI^+	Amino acids & derivatives	Creatine	132.1	1.27	↑	-	-
		L-Histidine	156.1	0.33	↓	-	-
		L-Tryptophan	205.1	3.50	↑	-	-
		L-Tyrosine	182.1	1.17	↑	-	-
	Unknown	137/133	137.0	2.22	↓	-	-
		169/208	169.0	3.47	↑	-	-
		169/222	169.0	3.70	↑	-	-
ESI^-	Organic acids	Lactate	89.0	0.68	↓	↓	↓
	Unknown	89/146	89.0	2.43	↓		
		89/181	89.0	3.02	↓		
		117/145	117.0	2.42		↑	
		117/166	117.0	2.77			↑
		145.1/83	145.1	1.38			↑
		145.1/84	145.1	1.40	↑		
		145.1/94	145.1	1.57			↑
		145.1/95	145.1	1.58	↑	↑	
		145.1/117	145.1	1.95			↑
		145.1/118	145.1	1.97	↑		
		191/32	191.0	0.53		↑	
		191/70	191.0	1.17	↑		
		203.1/221	203.1	3.68		↑	
		359.2/83	359.2	1.38		↑	
		359.2/84	359.2	1.40	↑		

↑ indicates increased urinary excretion of metabolite at 1h-Post compared to Post. ↓ indicates decreased urinary excretion of metabolite at 1h-Post compared to Post.

Table 7.13 - Exercise induced metabolic alterations determined by OPLS-DA and S-Plot analysis between Pre and 1h-Post time points. SHORT ESI^+ not included based on the lack of QC clustering fit from PCA analysis. Unknown metabolites are presented by *m/z* and time.

Ionisation mode	Biological Class	Metabolite	*m/z (M)*	RT (min)	LONG	SHORT	CONT
ESI^+	Alkaloids	Uric acid	169.0	0.82	↓	-	
	Carboxylic acids & derivatives	Citric acid	193.0	0.78	↓	-	↓
	Purine derivatives	Hypoxanthine	137.0	0.88	↑	-	↑
	Unknown	137/127	137.0	2.12	↑	-	
		147.1/48	147.1	0.80		-	↓
ESI^-	Alkaloids	Uric acid	167.0	0.82		↓	
	Carboxylic acids & derivatives	Citric acid	191.0	0.78			↓
	Purine derivatives	Hypoxanthine	135.0	0.88	↑	↑	↑
	Organic acids	Lactate	89.0	0.68	↑	↑	
	Unknown	135/649	135.0	10.82	↓	↓	
		145.1/37	145.1	0.62	↑		
		145.1/83	145.1	1.38			↑
		145.1/84	145.1	1.40	↑		
		145.1/95	145.1	1.58	↑	↑	
		191/33	191.0	0.55	↑	↑	↑
		191/53	191.0	0.88	↑		
		359.2/84	359.2	1.40	↑	↑	↑

↑ indicates increased urinary excretion of metabolite at 1h-Post compared to Pre. ↓ indicates decreased urinary excretion of metabolite at 1h-Post compared to Pre.

7.4.3.2 Within-athlete variability

To evaluate the variability-dependent metabolomic perturbations following the training sessions, OPLS-DA models were generated comparing participants who were identified as variable or consistent within Chapter 5 (Section 5.4.3) based on %MMP and $\%HR_{max}$. The initial OPLS-DA model generated for CONT in ESI^+ for %MMP was deemed to be invalid ($P = .07$ from permutation testing) and is highlighted in red within Table 7.14, and was not included in any further analyses. All other OPLS-DA models generated positive Q^2 values and passed permutation testing ($n = 100$; $P < .05$). Figure 7.6 and Figure 7.7 display scores plots for valid OPLS-DA models in ESI^+ and ESI^- variable and consistent participant groups based on participant variability of training session power as %MMP, and training session HR as $\%HR_{max}$, respectively. The OPLS-DA models for LONG %MMP in ESI^-, LONG $\%HR_{max}$ in ESI^-, and CONT $\%HR_{max}$ in ESI^- displayed separation between variable and consistent groups in scatter plots, whereas other models did not show clear separation between groups despite valid models. Table 7.14 also presents model diagnostics, outlining variability in each model due to separation between groups, and due to within-group variability.

Table 7.14 - OPLS-DA scores based on variability analysis. Model diagnostics presented based on separation of scatter plots between groups, and within-group variability as a % CV. SHORT ESI^+ not included based on the lack of QC clustering fit from PCA analysis. OPLS-DA analyses which failed to establish valid models have been excluded.

		Time	R^2X	R^2Y	Q^2	A	Between-group separation based on predictive component	Within-group variability based on orthogonal component	P-Value
ESI^+	**LONG**	$\%HR_{max}$	.566	.385	.210	2	8%	31%	< .01
	CONT	%MMP	.594	.193	.098	2	7%	23%	.02
		$\%HR_{max}$	.616	.292	.124	2	7%	30%	.01
ESI^-	**LONG**	%MMP	.589	.400	.263	2	5%	36%	< .01
		$\%HR_{max}$	.576	.407	.241	2	5%	19%	< .01
	SHORT	%MMP	.592	.257	.130	2	8%	37%	.01
		$\%HR_{max}$	.606	.187	.068	2	8%	37%	< .01
	CONT	%MMP	.552	.308	.171	2	7%	37%	< .01
		$\%HR_{max}$	.592	.413	.186	2	12%	31%	< .01

R^2X = variation in X explained by the model, R^2Y = variation in Y explained by the model, Q^2 Y= goodness of prediction, A = number of orthogonal components, P-value = permutation test probability of spurious model generation.

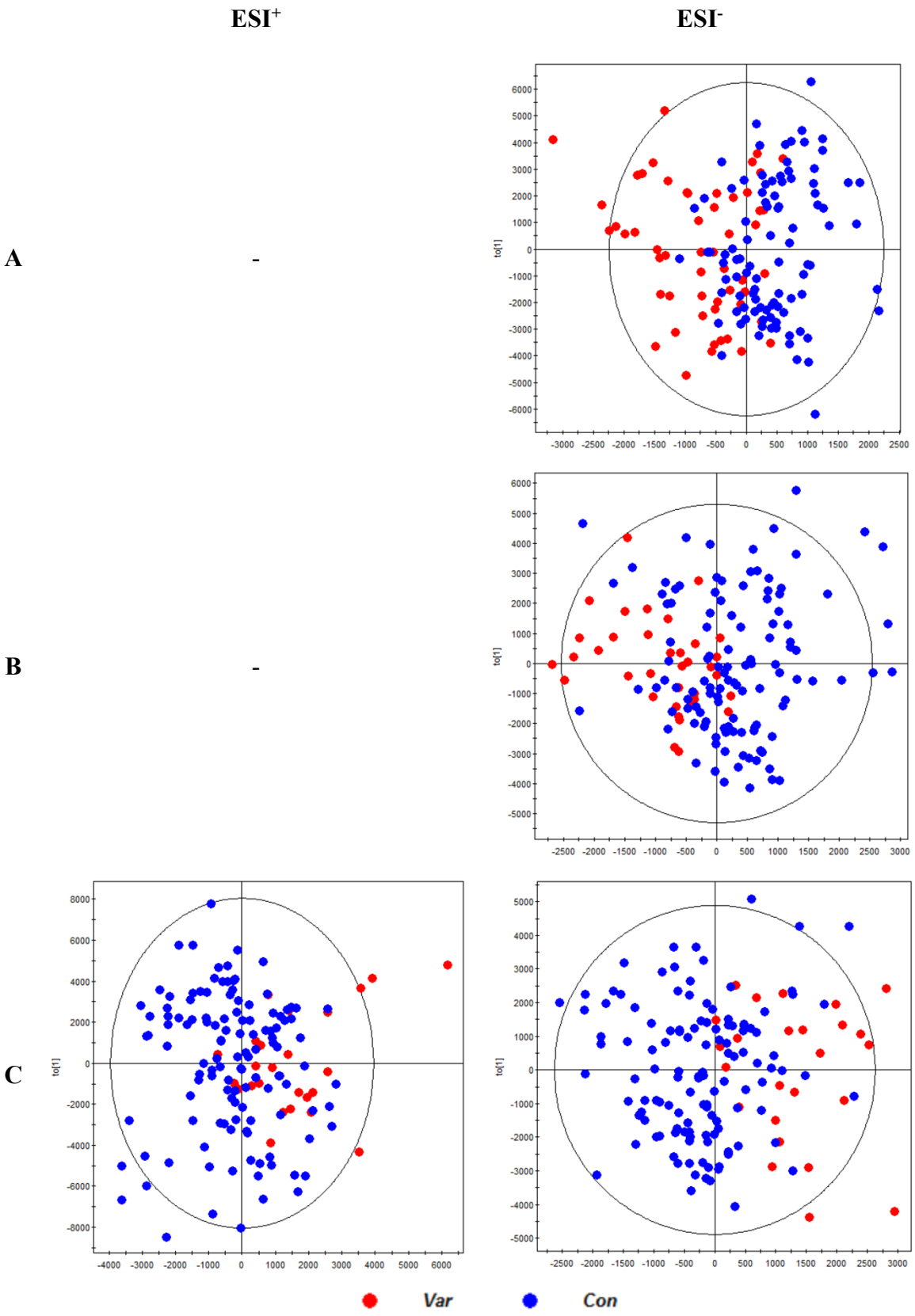


Figure 7.6 – OPLS-DA plots displaying separation based on participant variability of training session power as %MMP for LONG (A), SHORT (B), and CONT (C) in ESI^+ and ESI^-. LONG ESI^+ %MMP not included based on an invalid OPLS-DA model and SHORT ESI^+ not included based on the lack of QC clustering fit from PCA analysis.

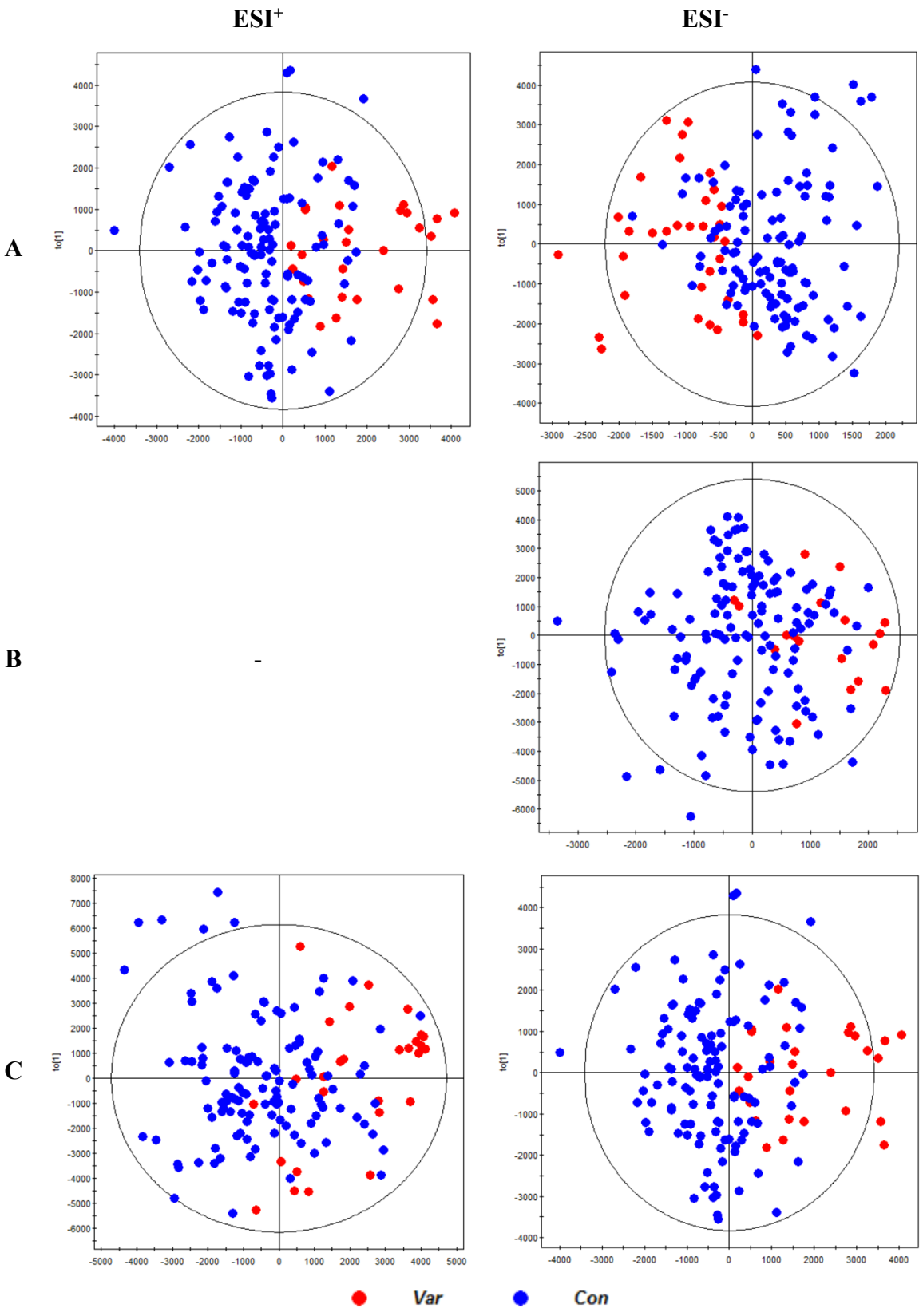


Figure 7.7 – OPLS-DA plots displaying separation based on participant variability of training session power as $\%HR_{max}$ for LONG (A), SHORT(B), and CONT (C) in ESI^+ and ESI^-. SHORT ESI^+ not included based on the lack of QC clustering fit from PCA analysis.

Metabolites contributing to OPLS-DA separation

For each valid OPLS-DA model, S-plots were used to extract metabolites that contribute to the separation in each model and these involved a range of metabolite classes, including amino acids, purine derivatives, organic acids, and several unknown compounds, and results are presented in Table 7.15, Table 7.16, and Table 7.17.

Hypoxanthine excretion was decreased, and L-tyrosine and citric acid excretion was increased within the variable group versus the consistent group in LONG (ESI^+) when based on session heart rate relative to $\%HR_{max}$. Hypoxanthine excretion was increased within the variable group versus the consistent group in SHORT (ESI^+) when based on both session power output relative to %MMP, as well as heart rate output relative to $\%HR_{max}$. Uric acid was increased within the variable group versus the consistent group in SHORT (ESI^+) when based on heart rate output relative to $\%HR_{max}$, and lactate was increased within the variable group versus the consistent group in SHORT (ESI^+) when based on session power output relative to %MMP. Uric acid and hippuric acid (ESI^+), and lactate (ESI^-) excretion was increased within the variable group versus the consistent group in CONT when based on session power output relative to %MMP. Citric acid excretion was increased within the variable group versus the consistent group in CONT (ESI^+) when based on both session power output relative to %MMP and heart rate relative to $\%HR_{max}$.

Table 7.15 - Exercise induced metabolic differences between variable and consistent participants determined by %MMP and $\%HR_{max}$ in LONG as determined by OPLS-DA and S-Plot analysis. %MMP ESI^+ not included based on the result of invalid OPLS-DA model.

Ionisation mode	Biological Class	Metabolite	*m/z (M)*	RT (min)	%MMP	$\%HR_{max}$
ESI^+	Amino acids & derivatives	L-Tyrosine	182.1	1.17	-	↑
	Carboxylic acids & derivatives	Citric Acid	193.0	0.78	-	↑
	Purine derivatives	Hypoxanthine	137.0	0.87	-	↓
	Unknown	137/127	137.0	2.12	-	↓
		169/26	169.0	0.43	-	↓
		169/35	169.0	0.58	-	↓
		182.1/145	182.1	2.42	-	↑
		193/25	193.0	0.42	-	↑
ESI^-	Unknown	145.1/37	145.1	0.62		↓
		167/76	167.0	1.27		↑
		191/374	191.0	6.23	↑	
		203.1/246	203.1	4.10		↑
		203.1/253	203.1	4.22	↑	
		359.2/266	359.2	4.43		↑
		359.2/277	359.2	4.62		↑
		359.2/320	359.2	5.33		↑
		359.2/326	359.2	5.43		↑
		361.2/232	361.2	3.87		↑
		361.2/240	361.2	4.00		↑
		361.2/266	361.2	4.43		↑
		361.2/273	361.2	4.55		↑
		361.2/281	361.2	4.68		↑

↑ indicates increased urinary excretion of metabolite in variable group versus consistent group. ↓ indicates decreased urinary excretion of metabolite in variable group versus consistent group.

Table 7.16 - Exercise induced metabolic differences between variable and consistent participants determined by %MMP and $\%HR_{max}$ in SHORT as determined by OPLS-DA and S-Plot analysis.

Ionisation mode	Biological Class	Metabolite	*m/z (M)*	RT (min)	%MMP	$\%HR_{max}$
ESI⁻	Alkaloids	Uric Acid	167.0	0.82		↑
	Purine derivatives	Hypoxanthine	135.0	0.87	↑	↑
	Organic acids	Lactate	89.0	0.68	↑	
	Unknown	115/68	115.0	1.13	↓	↓
		117/172	117.0	2.87	↑	
		117/186	117.0	3.10	↑	↑
		180.1/261	180.1	4.35	↓	↓
		191/32	191.0	0.53		↓
		191/159	191.0	2.65	↓	↓
		203.1/221	203.1	3.68	↓	↓
		203.1/268	203.1	4.47	↓	↓

↑ indicates increased urinary excretion of metabolite in variable group versus consistent group. ↓ indicates decreased urinary excretion of metabolite in variable group versus consistent group.

Table 7.17 - Exercise induced metabolic differences between variable and consistent participants determined by %MMP and $\%HR_{max}$ in CONT as determined by OPLS-DA and S-Plot analysis.

Ionisation mode	Biological Class	Metabolite	*m/z (M)*	RT (min)	%MMP	$\%HR_{max}$
ESI⁺	Alkaloids	Uric Acid	169.0	0.82	↑	
	Benzenoids	Hippuric Acid	180.1	4.10	↑	
	Carboxylic acids & derivatives	Citric Acid	193.0	0.80	↑	
	Unknown	205.1/29	205.1	0.48	↑	
ESI⁻	Carboxylic acids & derivatives	Citric Acid	191.0	0.78		↑
	Organic acids	Lactate	89.0	0.67	↑	
	Unknown	117/145	117.0	2.42		↑
		145.1/198	145.1	3.30		↑
		191/32	191.0	0.53		↑
		203.1/4.08	203.1	4.08	↓	

↑ indicates increased urinary excretion of metabolite in variable group versus consistent group. ↓ indicates decreased urinary excretion of metabolite in variable group versus consistent group.

7.5 Discussion

This study assessed the metabolomic perturbations incurred by the completion of three different training session formats using a maximal effort-based intensity prescription. The acute responses were assessed using baseline urine samples obtained prior to the training session, to those obtained immediately following the session, and one hour immediately following completion of the session. Comparisons of the urinary metabolome of variable and consistent participant groups based on within-athlete variability of session power as %MMP or session HR as $\%HR_{max}$ were also performed.

The present investigation observed increased levels of excreted urinary lactate following all training session formats compared to baseline resting samples, similar to previous studies comparing high intensity and low-intensity training sessions (Peake *et al.* 2014). This increase in lactate excretion is commonly seen following exhaustive exercise (Hood *et al.* 1988; Cairns 2006) and can be reflective of the increase in lactate production and clearance following physically demanding exercise (Johnson and Edwards 1937; Cairns 2006; Allen, Lamb and Westerblad 2008). Despite the excretion of lactate decreasing in the hour following all session formats, there was only an overall increase in the urinary excretion of lactate one hour after exercise versus pre-session samples in LONG and SHORT, which may reflect the differing levels of energy demand between the LONG, SHORT, and CONT exercise formats. There was also increased urinary excretion of hypoxanthine immediately following all sessions and remained elevated in the hour following the training sessions, a finding supported by previous research studies (Hargreaves *et al.* 1998; Green and Fraser 1988; Hellsten-Westing, Sollevi and Sjödin 1991; Houston and Thomson 1977; Enea *et al.* 2010; Pechlivanis *et al.* 2010; Muhsen Ali *et al.* 2016; Mukherjee *et al.* 2014; Pechlivanis *et al.* 2015). Increased excretion of urinary hypoxanthine may reflect the increase in ATP metabolism due to the likely increased rate of ATP utilisation during the training sessions. The increased hypoxanthine can be linked to the reformation of ATP from two adenosine diphosphate (ADP) molecules, which results in inosine monophosphate (IMP). Following exercise, IMP is quickly restored to ATP (Lowenstein 1972), but a proportion of IMP is dephosphorylated to inosine, which is then oxidised to form hypoxanthine and then either excreted or oxidised in the kidneys to form xanthine and uric acid.

Over a period of 24 hours following bouts of high intensity exercise uric acid is commonly observed to increase, mainly due to the oxidisation of hypoxanthine in resting conditions (Green and Fraser 1988). The findings of the present investigation are contrary to this, with decreased uric acid excretion across the whole session, including the hour following exercise, in both LONG and SHORT sessions, along with the increased hypoxanthine excretion during these sessions. The divergent findings between the present investigation and (Green and Fraser 1988) may be due to the difference in training status and exercise performance between the sample groups, as well as a time frame of 24 hours between sample collections. Furthermore, despite increased hypoxanthine excretion being observed during CONT, there was no accompanying decrease in uric acid, which may be the result of a lower rate of ATP utilisation within this exercise format, allowing the process of oxidation of hypoxanthine into uric acid able to occur. The urinary excretion of amino acids has been explored greatly, with widely ranging results dependent on factors such as exercise modality, exercise intensity, dietary control, and participant fitness levels (Kistner *et al.* 2020; Pechlivanis *et al.* 2010; Muhsen Ali *et al.* 2016; Mukherjee *et al.* 2014; Pechlivanis *et al.* 2015; Siopi *et al.* 2017). Increased tyrosine excretion has been observed following 24 weeks of combined aerobic and strength training, correlating strongly with total strength and $\dot{V}O_{2max}$ (Duft *et al.* 2017). The finding of increased excretion of tyrosine following LONG is supported by previous findings of elevated serum levels of tyrosine following an incremental exercise test until exhaustion (Strasser *et al.* 2016). Interestingly, reduced tyrosine excretion was observed following brief maximal exercise (Pechlivanis *et al.* 2015), which possibly suggests that shorter duration maximal exercise is not able to stimulate the phenylalanine metabolism into tyrosine, also supported by the time-dependent increase in tyrosine excretion observed following exercise lasting between 15 and 765 min in athletes (Haralambie and Berg 1976). This could explain the lack of tyrosine excretion following SHORT exercise format.

It has been suggested that histidine could be used as a marker of exercise-induced muscle damage, based on the finding that prolonged and intense exercise generating free radicals and oxidative damage to cells (Reid *et al.* 1992; Powers and Jackson 2008), and histidine being utilised as a source of antioxidant to cells (Son, Satsu and Shimizu 2005), therefore indicating that elevated histidine may contribute to relieving oxidative stress following exercise. This notion is supported by the finding of elevated urinary excretion of histidine generally being observed following acute maximal exercise (Pechlivanis *et al.* 2013). However, this appears to contrast with the finding in the present investigation that histidine

excretion decreased following LONG. One possible explanation may be that the levels of exercise-induced muscle damage in the exercise sessions in the present study were not high enough to result in increased histidine excretion for use as an antioxidant. Interestingly, (Pechlivanis *et al.* 2013) reported decreased histidine excretion in the training group who had longer recovery duration between maximal efforts during training, which supports the notion that a higher level of exercise-induced muscle damage results in increased histidine excretion. The trained nature of the participants in this investigation would also likely impact the histidine response to exercise, as cycling training has been shown to not induce exercise-induced muscle damage (Nalcakan 2014).

Tryptophan has been observed to decrease following variable length sub-maximal exercise (Thysell *et al.* 2012), as well as maximal HIIT using short duration sprints (Pechlivanis *et al.* 2010). This is in apparent contradiction to the findings of the present investigation, where urinary excretion of tryptophan increased in the one hour after exercise following LONG. However, following exercise there is a high demand for nicotinamide adenine dinucleotide (NAD^+; Martin et al., 2020), which remains elevated for a short period (Sahlin *et al.* 1976), and then returns to a baseline or lower level (Coelho *et al.* 2016). It is possible that the reduction in tryptophan excretion one hour following LONG be due to tryptophan metabolism increasing transiently and then returning to a level lower than baseline. Urinary creatine was found to be increased in the one hour following exercise in LONG in the present study. Previously, reductions in urinary creatine have been observed that following repeated maximal sprint exercise, both in trained sprinters and untrained individuals (Bezrati-Benayed *et al.* 2014). The findings of the present study may reflect increased phosphocreatine recovery kinetics within the study sample population due to their training status, resulting in excess creatine for excretion (Forbes, Slade and Meyer 2008)

Citric acid, and the derivative citrate, are key intermediates in the Krebs Cycle, which is essential for oxidative energy production. Citrate is synthesised from acetyl CoA and oxaloacetate under the presence of citrate synthase (CS) and controlled by the presence of ATP (Wiegand and Remington 1986). Increased CS activity has been observed following training (Vigelsø, Andersen and Dela 2014), which would result in more citrate synthesis and therefore increase the urinary excretion of citrate (López *et al.* 2010; Nuñez *et al.* 2012). Modest increases in plasma citrate following exercise have been observed (Lewis *et*

al. 2010). However, it has also been observed that acidosis results in decreased urinary citrate excretion (Unwin, Capasso and Shirley 2004; Simpson 1983). The findings of decreased citric acid excretion following LONG and SHORT would support this notion, with the high levels of metabolic acidosis during these formats. Interestingly, citric acid excretion remained supressed in the hour following only LONG, possibly reflecting post-exercise metabolism differences between the two exercise formats. Furthermore, citric acid excretion was observed to be decreased between pre-exercise samples and the hour following exercise in CONT, which could reflect alterations in CS activity due to lower levels of ATP turnover within the longer duration and lower-intensity training session format.

The urinary excretion of lactate was able to differentiate variable and consistent participants based on %MMP in SHORT and CONT formats, with increased excretion being found in variable participants. This may reflect the varying levels of utilisation of the glycolytic pathway and resultant lactate excretion within variable participants in SHORT, and subtle changes in workload relative to the participant's individual lactate threshold. An increased level of urinary hypoxanthine excretion was observed in participants who display variable exercise performance during the SHORT interval format based on both %MMP and $\%HR_{max}$. This may reflect the differing levels of ATP utilisation between sessions per participant. However, the higher levels of hypoxanthine excretion may potentially be a marker that reflects a higher level of overall performance level of participants within the variable group. There have been numerous investigations into the post-exercise excretion of hypoxanthine (Enea *et al.* 2010; Pechlivanis *et al.* 2010; Sahlin, Tonkonogi and Söderlund 1999; Hellsten *et al.* 2001; Chorell *et al.* 2012), and it has been suggested that hypoxanthine excretion may be a predictor of performance in highly trained athletes (Zieliński, Krasińska and Kusy 2013), but is also proposed as an indicator of exercise-related energetic stress (Zieliński and Kusy 2015a; Zieliński and Kusy 2015b; Sahlin, Tonkonogi and Söderlund 1999). Interestingly, hypoxanthine excretion was decreased in the variable group based on $\%HR_{max}$ in LONG, contrary to the findings within the SHORT group, and indicating that hypoxanthine may not be a suitable target for variability-based phenotyping.

Higher levels of uric acid excretion was observed in the variable group based on $\%HR_{max}$ in SHORT and %MMP in CONT, which could represent higher levels of hypoxanthine

oxidation and variable levels of ATP utilisation (Green and Fraser 1988). The effects of exercise on the metabolism of hippuric acid have not been extensively explored, with (Neal *et al.* 2013) reporting decreased hippuric acid following threshold-based training, and (Davison *et al.* 2018) reporting no change in hippuric acid to exercise. Within the present investigation, hippuric acid was observed to be increased in the variable group based on %MMP in CONT. The reasons for this increased hippuric acid excretion in variable participants would require further exploration to identify regulatory pathways. Hippuric acid is responsive to different dietary sources such as fruit, coffee, or tea, as well as influences of the gut microbiome, which could impact the findings of the present study (Toromanović *et al.* 2008; Clifford *et al.* 2000; Pero 2010).

The observation of decreased histidine in variable participants based on % HR_{max} in CONT is in accordance with the findings of decreased histidine excretion following exercise in general (Thysell *et al.* 2012; Lewis *et al.* 2010). It is thought that histidine within microbes is a potential source of antioxidants on a cellular level (Son, Satsu and Shimizu 2005). This could imply that there is an increased need to relieve oxidative stress from exercise within participants who display variable exercise response. Excretion of tyrosine was increased in the variable group based on %HR_{max} in LONG. Increased tyrosine excretion may indicate the higher level of exercise intensity of the variable training sessions versus consistent groups. Increased excretion of tyrosine has been shown following HIIT exercise (Peake *et al.* 2014), as well as longer duration endurance exercise (Refsum, Gjessing and Strømme 1979). Tyrosine is also influenced by the gut microbiome in a similar manner to hippuric acid, possibly supporting the finding of increased tyrosine and hippuric acid in these groups (Pero 2010). Interestingly, citric acid was increased in the variable group based on both %MMP and %HR_{max} in CONT, and only %HR_{max} in LONG. This could possibly indicate an increase in CS activity following the session, despite the increased metabolic acidosis, as well as TCA cycle activity generally being increased following exercise to regain homeostasis and energy balance (Leek *et al.* 2001).

The lowest ranges of variability can be observed in SHORT, with 21% - 26% variability in OPLS-DA models from the orthogonal component being related to within-group variability when analysing the acute response to exercise, possibly reflecting a more homogenous exercise-induced metabolic perturbation. CONT displayed the highest within-group variability in both session execution and post-exercise metabolomic variability, with 40% for Post vs 1h Post and 41% for Pre vs 1h Post. Therefore, higher variability in how a training session is performed may result in higher variability in metabolomic profile.

However, despite this being observed for the Pre vs 1h Post and Post vs 1hPost time points, the Pre vs Post time points do not display the same relationship, with CONT displaying the lowest within-group variability (16%). This is possibly due to the lower magnitude of metabolic disturbance due to the lower power output session, as well as the likelihood that the metabolic disturbances from these shorter duration sessions resolving quickly following cessation of exercise (Chorell *et al.* 2009). The variability observed in metabolomic profile when samples are analysed grouped by whether individual participants were variable or consistent based on %MMP or $\%HR_{max}$, indicates similar pattern of variability to the observed TV variability from Chapter 5; with LONG showing the lowest within-group variation (31% [HR_{max} in ESI^+], 36% [MMP in ESI^-], and 16% [HR_{max} in ESI^-]), followed by CONT (37% [MMP in ESI^-] and 31% [HR_{max} in ESI^-]), and highest in SHORT (37% [MMP in ESI^-] and 37% [HR_{max} in ESI^-]). This highlights that there appears to be a similar pattern of variability observed in metabolomic profiles compared to variability in session performance in some measures, but how this equates to chronic training response has yet to be examined in the literature.

A limitation of the present study relates to the limited number of identified metabolites, which resulted in many unknown metabolites, potentially resulting in biomarkers of exercise variability being overlooked or incomplete pathways of metabolism being identified. Future investigations may look to identify these unknown compounds; however, this identification of measured unknown metabolites remains one of the most challenging aspects of non-targeted metabolomics (Dunn, Broadhurst, *et al.* 2011b). An extensive list of metabolomic compounds that respond to exercise interventions, both acute and chronic, has been produced as part of a review (Daskalaki, Easton and Watson 2015), but constraints on the number of compounds available for selection in the current thesis were due to budget for purchasing the known compounds and commercial availability of these compounds. Another limitation of the present study relates to the lack of QC clustering within SHORT samples analysed in ESI^+ mode resulted in the loss of some data for analysis. This separation of the QC samples could have been due to factors that affect system stability during run such as contaminant build-up in the system, or small alterations in the mobile phase composition.

7.6 Conclusion

The findings of the present investigation show that distinct metabolomic differences are present between LONG, SHORT, and CONT session formats using a maximal effort-based intensity prescription. The findings also display key differences in metabolomic response to exercise based on the level of individual variability to the different acute exercise session formats. The findings suggest a similar pattern of variability observed in metabolomic profiles when compared to variability in training session performance. The importance of variability of acute metabolomic profile response on the magnitude and variability observed in chronic training response has yet to be established.

Chapter 8 - Changes in urinary metabolome following a 6-week training intervention using maximal isoeffort training sessions

8.1 Abstract

Introduction: The present study utilised untargeted qualitative UPLC-MS to provide a global overview of the changes in the urinary metabolome following 6-weeks of maximal isoeffort training sessions from 3 different training groups. **Methods:** Twenty-eight well-trained competitive cyclists (*n*=27 males, *n*=1 female; $\dot{V}O_{2max}$: 57.1 ± 8.3 $ml \cdot kg^{-1} \cdot min^{-1}$) were randomly assigned to a control group (CON), or groups assigned short (SHORT) or long (LONG) interval sessions and completed 6 weeks of training consisting of 3 maximal isoeffort sessions each week. Urine samples were collected prior to (pre) and following the 6-week training intervention, and samples were analysed using UPLC-MS to construct a metabolite profile for the two exercise intervention groups and the control group. OPLS-DA analysis was performed comparing metabolomic profile prior to and following 6 weeks of the training intervention, as well as comparing the metabolomic profiles of responders and non-responders to each training intervention group based on classifications from Chapter 6. **Results:** Following the 6-week training intervention, OPLS-DA models were not able to identify differences between samples collected before and after the training intervention for any training groups. When baseline and post-training samples were analysed based on responder status regardless of training group, lactate, hypoxanthine, and succinate were able to differentiate between responders and non-responders to the training intervention. Within SHORT intervention group, uric acid, citric acid, succinate, and hippuric acid were able to differentiate between responders and non-responders. **Conclusion:** The findings of the present investigation highlight metabolite markers that are associated with chronic training responder status irrespective of training group, and markers that differentiate between LONG and SHORT interventions.

8.2 Introduction

The prescription of a standardised training intervention commonly results in divergent responses between individuals (Mann, Lamberts and Lambert 2014; Bouchard, An, Rice, Skinner, *et al.* 1999; Bouchard and Rankinen 2001; Rankinen *et al.* 2012; Morss *et al.* 2004; Kraus *et al.* 2001; Bouchard, Blair, *et al.* 2012; Boule, Weisnagel, *et al.* 2005; Bouchard *et al.* 1994), which may be in part due to how exercise intensity is prescribed across individuals (Bouchard, Sarzynski, *et al.* 2011; Rankinen *et al.* 2012; Mann, Lamberts and Lambert 2014). The effort-based intensity prescription could present an option to individualise exercise intensity, such as prescribing a "*maximal session effort*" (Seiler and Hetlelid 2005; Seiler and Sjursen 2004; Seiler *et al.* 2013), requiring

participants to regulate their workload based on circumstantial factors with the goal of maintaining a target level of physiological stress (Esteve-Lanao *et al.* 2008; Ulmer 1996) to achieve a specified level fatigue or exhaustion (St Clair Gibson *et al.* 2006; Baron *et al.* 2011). It has been suggested that the variability of performance might be intensity-dependent when using effort-based training, with variability increasing as intensity increases (Nicolò et al. 2014; Seiler and Sylta 2017). However, based on the findings of Chapters 4 and 5, the present thesis has generally observed individual variability to decrease as exercise intensity increases. On a chronic training basis, the findings within Chapter 6 of this thesis indicate that using a maximal session effort intensity prescription and SHORT interval formats leads to a greater incidence of positive exercise training response compared to LONG interval formats. While it has been observed that factors such as baseline $\dot{V}O_{2max}$ influences training response (Sisson, Katzmarzyk, Earnest, *et al.* 2009; Kohrt, Malley, Coggan, *et al.* 1991; Bouchard and Rankinen 2001), genetic factors can explain approximately 47 % of the observed variance in $\dot{V}O_{2max}$ response (Bouchard, An, Rice, Skinner, *et al.* 1999). Further investigation has identified 39 single-nucleotide polymorphisms which were associated with exercise training response; of which, 21 were responsible for 49 % of the variability in $\dot{V}O_{2max}$ response to training (Bouchard, Leon, *et al.* 1995; Morss *et al.* 2004; Kraus *et al.* 2001).

Many investigations into the human metabolome response to chronic effects of exercise interventions have been conducted (Bragazzi *et al.* 2020; Bassini and Cameron 2014; Zieliński and Kusy 2012; Hellsten-Westing *et al.* 1993; Zieliński, Kusy and Rychlewski 2011; Zieliński *et al.* 2009; van Velzen *et al.* 2008; Duft *et al.* 2017; Pla *et al.* 2020; Kistner *et al.* 2019; Kuehnbaum, Gillen, Kormendi, *et al.* 2015; Neal *et al.* 2013; Kuehnbaum, Gillen, Gibala, *et al.* 2015). The findings of Chapter 7, which investigated the acute metabolomic response to effort-based training sessions, displayed differences between variable and consistent individuals in the urinary excretion of uric acid, L-histidine, L-tyrosine, citric acid, hypoxanthine, and lactate. However, whether there are metabolite markers that are associated with variability in chronic training response and whether these markers are also associated with acute variability is yet to be established. Therefore, the aim of the current study was to investigate the changes in metabolomic profile following a chronic training intervention using the same exercise formats and whether any metabolomic features are associated with chronic exercise training response.

Hypotheses

$H1_1$ – Identified metabolomic profiles display differences between LONG, SHORT and CON training groups following six weeks of isoeffort based exercise.

$H1_0$ – There are no differences in identified metabolomic profiles between LONG, SHORT, and CON training groups.

$H2_1$ – Identified metabolomic profiles display differences between responders, non-responders, and adverse-responders, both globally and within each training group.

$H2_0$ – There are no differences in metabolomic profiles between responders, non-responders, and adverse-responders both globally and within each training group.

8.3 Methodology

8.3.1 Study design and experimental procedures

The samples utilised in this investigation were collected during the experimental data collection in Chapter 6. Twenty-eight well-trained competitive cyclists (*n*=27 males, *n*=1 female; $\dot{V}O_{2max}$: 57.1 ± 8.3 ml·kg·min^{-1}) were randomly assigned to a control (CON) or a short (SHORT) or long (LONG) interval group and completed 6 weeks of training consisting of 3 maximal isoeffort sessions each week. $\dot{V}O_{2max}$, MMP, and 20 min TT performance was recorded before and after the 6-week training period. Participants were classified as responders or non-responders based on whether their response falls above or below the within-athlete CV of $\dot{V}O_{2max}$, MMP, and 20 min TT, respectively. All samples were analysed in one analysis batch, which contained all experimental samples in a randomised order (further details are presented in Section 6.3.2).

8.3.2 Urine sample collection and preparation

Urinary sample collection was completed by collecting spot urine samples prior to the maximal incremental test procedure in Chapter 6, before the 6-week training intervention (Pre), and again following the 6-week intervention (Post; see Figure 8.1) processed following the guidelines of Want *et al.* (2010), and were analysed using UPLC- MS (Danaher, Gerber, Wellard, *et al.* 2016; Enea *et al.* 2010; Lehmann *et al.* 2010). Further urine sample collection details are described in section Chapter 3 (Section 3.11.1).

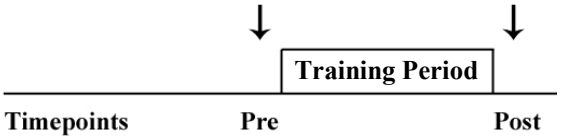


Figure 8.1 - Urine sampling timepoints

8.3.3 UPLC-MS analysis

A detailed overview of the UPLC-MS system setup, materials, and run details are presented in section Chapter 3 (Section 3.11.2), and details of run durations and injection numbers are presented in Table 8.1. Test mixes contained 12 (ESI^+) and 12 (ESI^-) known compounds from pure sources to identify metabolites of interest (Table 8.2). Randomisation of the sample order was performed to limit the impact of systematic error due to any potential degradation of the UPLC-MS system on the dataset over the acquisition time.

Table 8.1 –Experimental design of the UPLC-MS System.

	ALL
Acquisition Time (h)	20.7
Injections	100
Test Mixes	7
Blanks	10
cQCs	20
QCs	6
Samples	58
Ionisation mode	ESI^+/ESI^-

Table 8.2 - $[M+H]^{+/-}$ and RT of each compound in the test mix samples used for the assessment of UPLC-MS reproducibility in ESI^+ and ESI^-.

ESI^+				
Metabolite	**Formula**	**Mass**	**$[M+H]^+$**	**RT (min)**
L-Alanine	$C_3H_7NO_2$	89.0477	90.0555	0.43
Creatinine	$C_4H_7N_3O$	113.0589	114.0667	0.42
Creatine	$C_4H_9N_3O_2$	131.0695	132.0773	1.14
L-Isoleucine	$C_6H_{13}NO_2$	131.0946	132.1025	0.43
Hypoxanthine	$C_5H_4N_4O$	136.0385	137.0463	0.83
Uric acid	$C_5H_4N_4O_3$	168.0283	169.0362	0.78
Hippuric acid	$C_9H_9NO_3$	179.0582	180.0661	3.90
L-Tyrosine	$C_9H_{11}NO_3$	181.0739	182.0817	2.91
Citric acid	$C_6H_8O_7$	192.0270	193.0348	0.75
L-Tryptophan	$C_{11}H_{12}N_2O_2$	204.0899	205.0977	3.32
Cortisone	$C_{21}H_{28}O_5$	360.1937	361.2015	5.88
Cortisol	$C_{21}H_{30}O_5$	362.2093	363.2093	5.85
ESI^-				
Metabolite	**Formula**	**Mass**	**$[M+H]^-$**	**RT (min)**
Lactate	$C_3H_6O_3$	90.0317	89.0317	0.65
Creatinine	$C_4H_7N_3O$	131.0695	112.0589	0.74
Fumarate	$C_4H_2O_4^{-2}$	113.9953	112.9953	0.90
Succinate	$C_4H_6O_4$	118.0266	117.0266	0.98
Hypoxanthine	$C_5H_4N_4O$	136.0385	135.0385	0.84
Uric acid	$C_5H_4N_4O_3$	168.0283	167.0283	0.78
Hippuric acid	$C_9H_9NO_3$	179.0582	178.0582	3.90
L-Tyrosine	$C_9H_{11}NO_3$	181.0739	180.0739	3.90
Citric acid	$C_6H_8O_7$	192.0270	191.0270	0.75
L-Tryptophan	$C_{11}H_{12}N_2O_2$	204.0899	203.0899	3.32
Cortisone	$C_{21}H_{28}O_5$	360.1937	359.1937	5.88
Cortisol	$C+H_{30}O_5$	362.2093	361.2093	5.85

8.3.4 Data analysis

8.3.4.1 Univariate analysis of raw data

Details on the univariate analysis of raw data retention time, mass accuracy and variance are presented in Chapter 3 (Section 3.11.5).

8.3.4.2 Data pre-processing

Details of data pre-processing parameters are presented in Chapter 3 (Section 3.11.6) and utilised the XCMS package for R software (Smith *et al.* 2006) which allowed the chromatographic dataset to be reduced into a single matrix for further analysis.

8.3.4.3 Multivariate analysis

Details of multivariate data analyses, specifically PCA and OPLS-DA performed within SIMCA-P (Version 12, Umetrics, Sweden) are presented in Chapter 3 (Section 3.11.7). For each exercise group, OPLS-DA was performed in a pairwise fashion comparing Pre and Post to assess the changes of the urinary metabolome in response to the 6-week exercise training. Additionally, variability in response based on relative $\dot{V}O_{2max}$, relative MMP, and relative TT power data collected within Chapter 6 were also assessed. Comparisons were made between responders vs non-responders, non-responders vs negative responders, and responders vs negative-responders. In addition, non-responder and negative-responder groups were combined into a single group and compared to the responder group based on relative $\dot{V}O_{2max}$, relative MMP, and relative TT power.

8.4 Results

8.4.1 Analysis of raw data

Following all data pre-processing steps, a range of 134 – 146 metabolic features were detected across all ionisation modes (Table 8.3). Comparisons of all cQC BPI chromatograms indicated that the UPLC-MS system used had been conditioned sufficiently for all experiments, in both ESI^+ and ESI^-, and cQC reached stability after 10 injections.

Table 8.3 - Metabolic features present in dataset following each stage of filtering during XCMS pre-processing.

	Raw Data	Post MinFrac Filtering	Post CV Filtering
ESI^+	235	186	134
ESI^-	318	239	146

Inspection of QC samples was performed using the putatively identified metabolites to assess the analytical reproducibility of the UPLC-MS systems' mass accuracy (Table 8.4), retention time (Table 8.5), and peak intensity (Table 8.6). L-alanine failed to meet the ≤ 30 ppm mass accuracy threshold in ESI^+, and was excluded from further analysis, while all other metabolites met the threshold and remained stable for all sample runs for both ESI^+ and ESI^-. The maximum retention time drift observed was 1.8 sec in both ESI^+ and ESI^-. Graphical representation of peak intensity changes in the form of chromatogram overlays are displayed in for ESI^+ and ESI^-. All metabolites met the 30 % CV threshold in both ESI^+ and ESI^-.

Table 8.4 - Mass accuracy for samples in ESI^+ and ESI^- UPLC-MS conditions.

			ESI^+			**ESI^-**		
Metabolite	**[M+H]**	**[M-H]**	**Standard Compound**	**Ppm Error**	**Theoretical Mass Diff.**	**Standard Compound**	**Ppm Error**	**Theoretical Mass Diff.**
Lactate	-	89.0317	-	-	-	89.0	0	0.0317
L-Alanine	90.0555	-	90.04	**-2207.51**	0.0155	-	-	-
Creatinine	114.0667	112.0589	114.1	0	-0.0333	112.1	0	-0.0411
Fumarate	-	112.9953	-	-	-	113.0	0	-0.0047
Succinate	-	117.0266	-	-	-	117.0	0	0.0266
Creatine	132.0773	-	132.1	0	-0.0227	-	-	-
L-Isoleucine	132.1025	-	132.1	0	0.0025	-	-	-
Hypoxanthine	137.0463	135.0385	137.0	0	0.0463	135.0	0	0.0385
Uric acid	169.0362	167.0283	169.0	0	0.0362	167.0	0	0.0283
Hippuric acid	180.0661	178.0582	180.1	0	-0.0339	178.1	0	-0.0418
L-Tyrosine	182.0817	180.0739	182.1	0	-0.0183	180.1	0	-0.0261
Citric acid	193.0348	191.0270	193.0	0	0.0348	191.0	0	0.0270
L-Tryptophan	205.0977	203.0899	205.1	0	-0.0023	203.1	0	-0.0101
Cortisone	361.2015	359.1937	361.2	0	0.0015	359.2	0	-0.0063
Cortisol	363.2172	361.2093	363.2	0	0.0172	361.2	0	0.0093

Table 8.5 – Retention time deviation for QC samples in ESI^+ and ESI^- UPLC-MS conditions.

	ESI^+		ESI^-	
Metabolite	**Average (min)**	**Drift (sec)**	**Average (min)**	**Drift (sec)**
Lactate	-	-	0.65	0.02
L-Alanine	0.44	0.60	-	-
Creatinine	0.42	0.00	0.74	0.01
Fumarate	-	-	0.90	0.01
Succinate	-	-	0.97	0.01
Creatine	0.43	0.00	-	-
L-Isoleucine	1.05	1.20	-	-
Hypoxanthine	0.83	1.20	0.83	0.01
Uric acid	0.77	0.60	0.77	0.02
Hippuric acid	3.88	1.20	3.89	0.02
L-Tyrosine	2.84	1.80	3.89	0.02
Cirtic acid	0.73	0.60	0.73	0.01
L-Tryptophan	3.31	1.20	3.32	0.03
Cortisone	5.87	0.60	5.85	0.02
Cortisol	5.84	0.60	5.78	0.04

Table 8.6 - Peak intensity, variation, and percentage reduction for QC samples in ESI^- and ESI^+.

	ESI^+ (N=7)			ESI^- (N=7)		
Metabolite	**Average**	**CV**	**% Loss**	**Average**	**CV**	**% Loss**
Lactate	-	-	-	2.65E + 05	14.68	-41 %
L-Alanine	4.98E + 05	29.74	-58 %	-	-	-
Creatinine	5.97E + 07	15.41	-32 %	4.33E + 04	5.15	-15 %
Fumarate	-	-	-	5.69E + 03	8.98	-22 %
Succinate	-	-	-	2.84E + 05	6.58	-15 %
Creatine	6.62E + 06	22.85	-50 %	-	-	-
L-Isoleucine	7.17E + 05	7.03	-20 %	-	-	-
Hypoxanthine	6.74E + 06	3.38	-9 %	9.43E + 04	2.50	-7 %
Uric acid	7.78E + 06	15.85	-43 %	3.04E + 06	2.87	-8 %
Hippuric acid	2.42E + 07	3.29	-8 %	9.33E + 06	4.11	-10 %
L-Tyrosine	1.02E + 06	5.83	-15 %	1.11E + 05	4.27	-10 %
Citric acid	4.52E + 05	9.07	-22 %	4.05E + 06	3.13	-10 %
L-Tryptophan	5.23E + 06	13.52	-36 %	1.58E + 04	13.19	-31 %
Cortisone	3.09E + 04	8.80	-23 %	1.65E + 04	23.57	-48 %
Cortisol	1.82E + 04	17.34	-34 %	8.45E + 03	18.37	-40 %

8.4.2 PCA analysis and assessment of QC stability

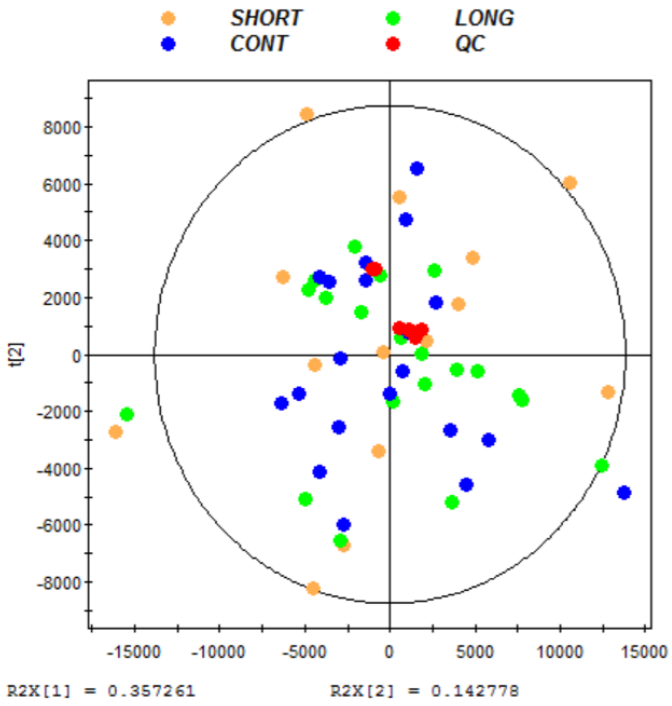
PCA analysis was conducted to observe any trends or patterns present in the datasets related to each training intervention group. The stability of the UPLC-MS system was assessed using repeated QC sample injections interspersed throughout the sample runs. The PCA model statistics are displayed in Table 8.7, outlining the model statistics for Q^2X, the variance (R^2X), and percentage of variance in PC1 and PC2. The PCA scores plots for the first two PCs are shown in Figure 8.2. The PCA plots generated for ESI^+ data display two QC samples that deviate from the central cluster (Figure 8.2A), and upon analysing these samples, the deviation is due to creatinine and uric acid. Excluding the two QC samples and surrounding data from the CON group in ESI^+, the remaining QC is tightly clustered and allows comparison between SHORT and LONG interval groups (Figure 8.2B). From the PCA scores plot the interindividual variability in the experimental samples can be seen to be larger than the analytical variation as displayed by the QC sample cluster. Model statistics for the reduced dataset is also displayed in (Table 8.7). Samples identified as being outliers (Hotelling's $T^2 > 99$ %) were investigated and were due to biological variations in metabolites, not analytical errors. Therefore, all samples were included in further analyses. PCA analysis of pre and post training samples (Figure 8.3A), and participant training group samples (LONG, SHORT, CON [ESI^- only] , Figure 8.3B) did not display any clear clustering. No observable sample clusters were present within the PCA score plots based on either participant training response level; responder, non-responder, or adverse-responder based on relative $\dot{V}O_{2max}$, relative MMP, or relative 20 minute TT power (Figure 8.4).

Table 8.7 – PCA scores details. R^2X = regression coefficient, Q^2 = predictive component of the PCA to assess model validity, PC1/PC2 = principal component 1 (PC1) and principal component 2 (PC2) for each PCA model.

	R^2X	Q^2	PC1	PC2
ESI^+ Full	0.842	0.128	0.357	0.143
ESI^+ Segment	0.853	0.171	0.431	0.161
ESI^-	0.566	0.300	0.447	0.119

ESI$^+$

A



B

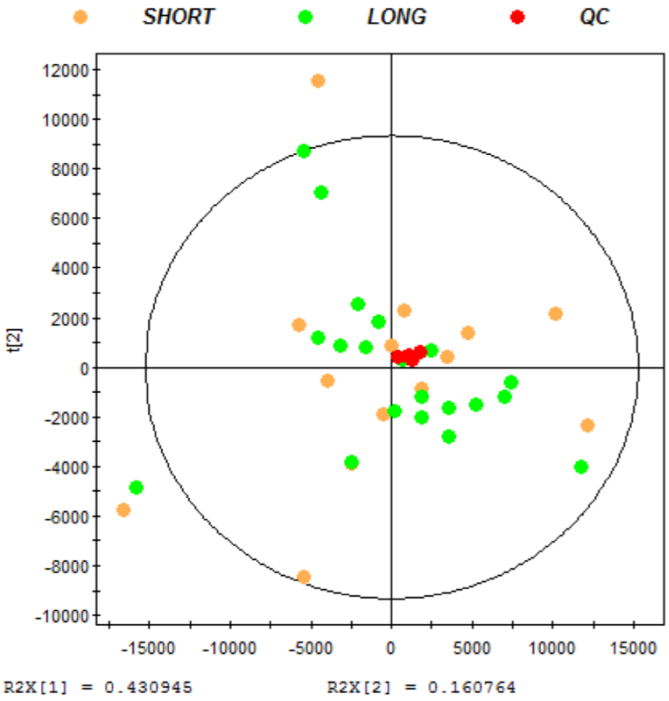


Figure 8.2 - PCA scores plots for ESI$^+$ with A) complete dataset, and B) reduced dataset including only training intervention groups.

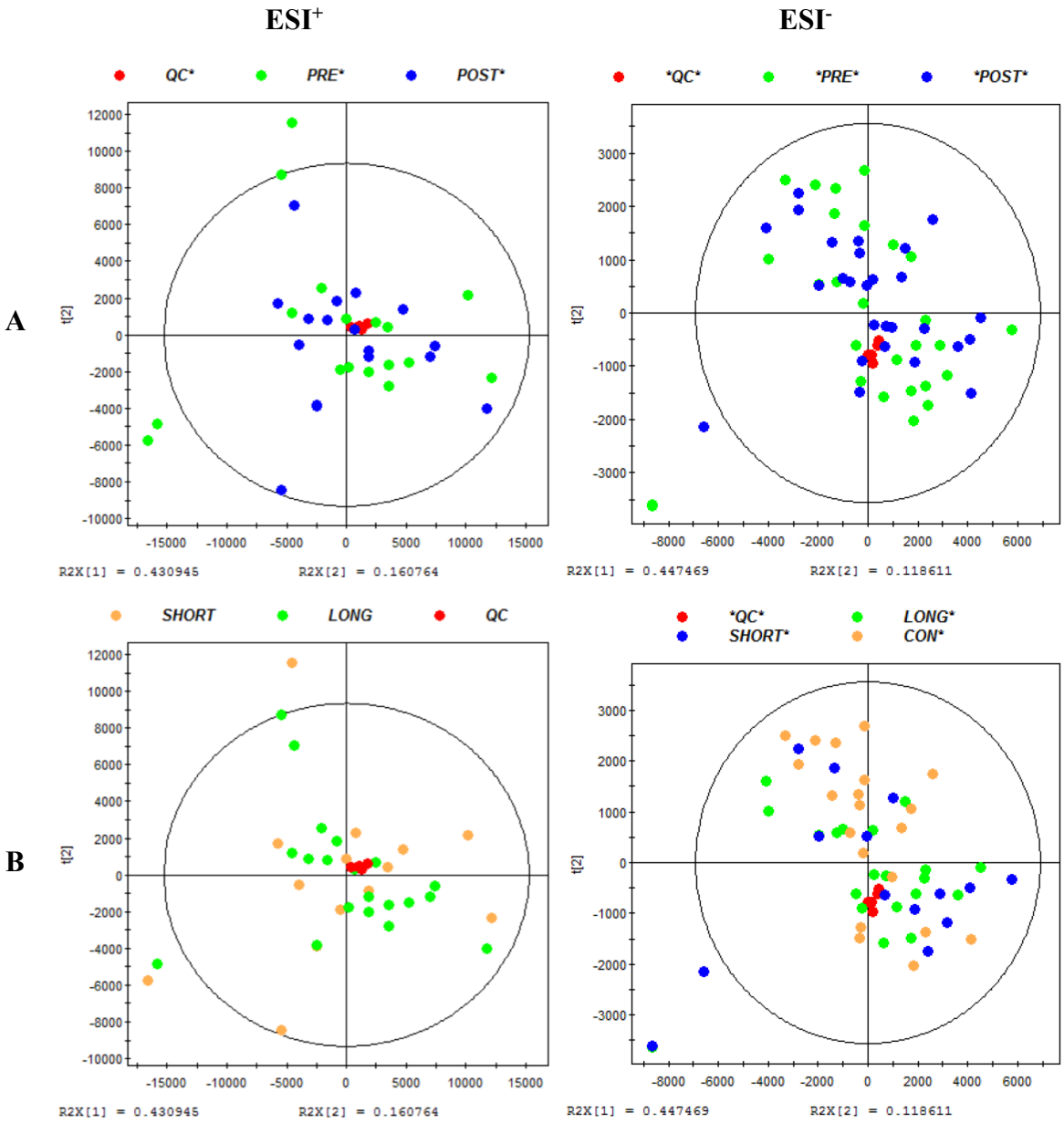


Figure 8.3 - PCA scores plots displaying good analytical reproducibility of the UPLC-MS as shown by QC clustering for in ESI^+ and ESI^-. A) samples identified by time-point (pre vs post-training), B) samples identified by training group (LONG, SHORT, CON).

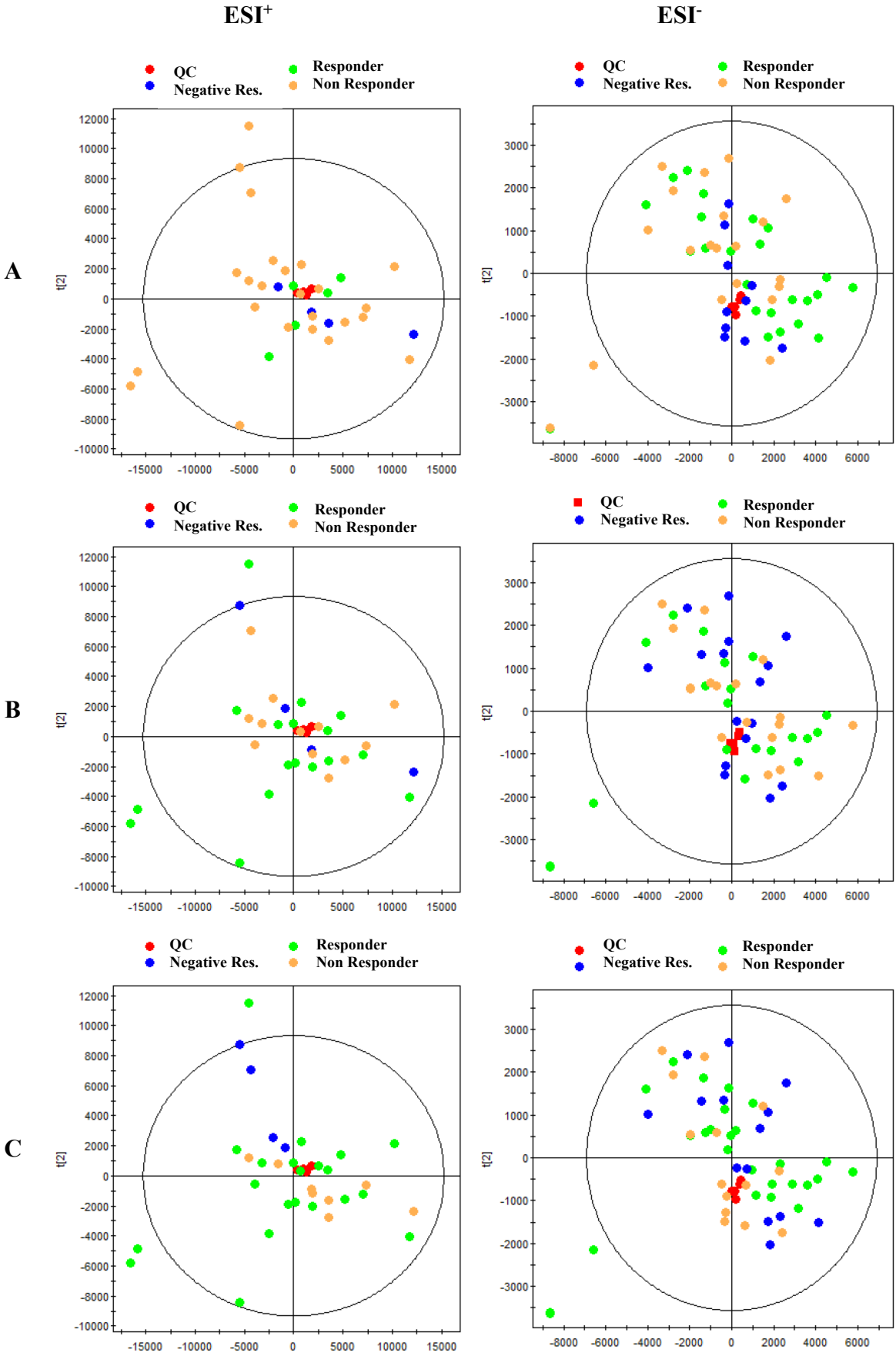


Figure 8.4 - PCA scores plots displaying good analytical reproducibility of the UPLC-MS as shown by QC clustering for in ESI^+ and ESI^-. Individual variability groupings based on; A) $\dot{V}O_{2max}$/kg, B) MMP/kg, C) TT W/Kg. Data points coloured based on; Responder (Green), Non-responder (Orange), or Negative-responder (Blue).

8.4.3 OPLS-DA analysis

8.4.3.1 Urinary metabolome changes following training intervention

To evaluate the chronic effects of each training intervention, OPLS-DA models comparing urine sample collected before and after the 6-week training intervention were performed for each exercise group separately. All initial OPLS-DA models generated resulted in negative Q^2Y values and were subsequently deemed to be invalid ($P > .05$ from permutation testing, $n = 100$), and therefore no further analyses were conducted on these OPLS-DA models.

8.4.3.2 Urinary metabolome differences based on responder status

To evaluate the variability-dependent metabolomic perturbations following the training intervention, OPLS-DA models were generated comparing samples collected from participants that responded to, showed no response, or adversely responded to the training interventions based on three measures. Table 8.8 shows the summary OPLS-DA model statistics with permutation testing results ($n = 100$). In ESI^+ ionisation mode, all OPLS-DA models were excluded based on a negative Q^2 values. In ESI^- ionisation mode, the models which passed permutation testing ($n = 100$) were individual variability based on relative $\dot{V}O_{2max}$ between non-responders and negative-responders ($Q^2 = .165$, $P = .01$), relative MMP between both responders and non-responders ($Q^2 = .164$, $P = .02$) as well as responders and negative-responders ($Q^2 = .212$, $P = .02$). All other models in ESI^- ionisation mode failed permutation testing and were excluded from further analyses. Figure 8.5 displays OPLS-DA scatter plots for relative $\dot{V}O_{2max}$ comparing non-responders to negative-responders (Figure 8.5A), relative MMP comparing responders to non-responders (Figure 8.5B), and relative MMP comparing responders to negative-responders (Figure 8.5C), respectively. Table 8.8 presents model diagnostics, outlining variability in each model due to separation between groups, and due to within-group variability.

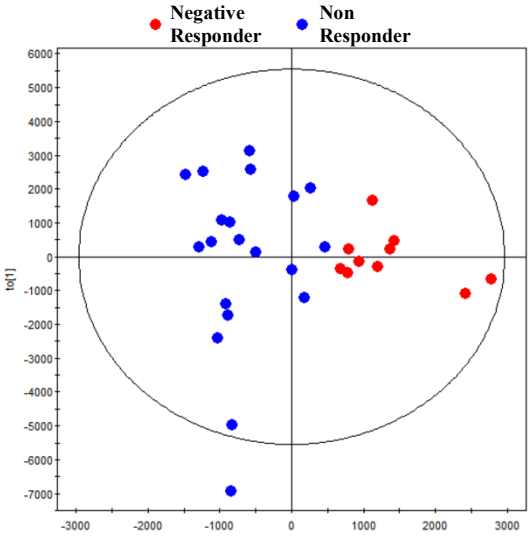
Table 8.8 - OPLS-DA scores details comparing responders, non-responders, and negative-responders to training based on changes in $\dot{V}O_{2max}$, MMP W/kg, and TT W/kg. Model diagnostics presented based on separation of scatter plots between groups, and within-group variability as a % CV. CON ESI^+ and ESI^- OPLS-DA analyses were not performed due to exclusion from the dataset. OPLS-DA analyses which failed to establish valid models have been excluded.

		Response	R^2X	R^2Y	Q^2	A	Between-group separation based on predictive component	Within-group variability based on orthogonal component	P-Value
ESI-	**$\dot{V}O_{2max}$/kg**	Non vs Neg	.595	.719	.165	2	8%	39%	.01
	MMP/kg	Res vs Non	.629	.610	.164	2	6%	47%	.02
		Res vs Neg	.656	.661	.212	2	5%	49%	.02

R^2X = variation in X explained by the model, R^2Y = variation in Y explained by the model, Q^2 = goodness of prediction, A = number of orthogonal components, P-value = permutation test probability of spurious model generation. “Res” = Responder, “Non” = Non-responder, “Neg” = Negative-responder.

ESI-

A



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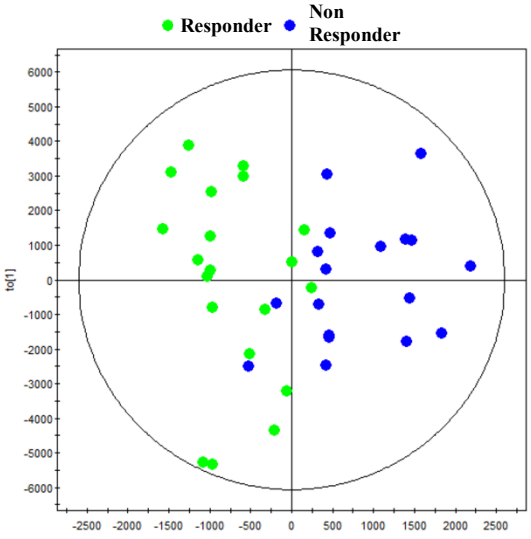


C

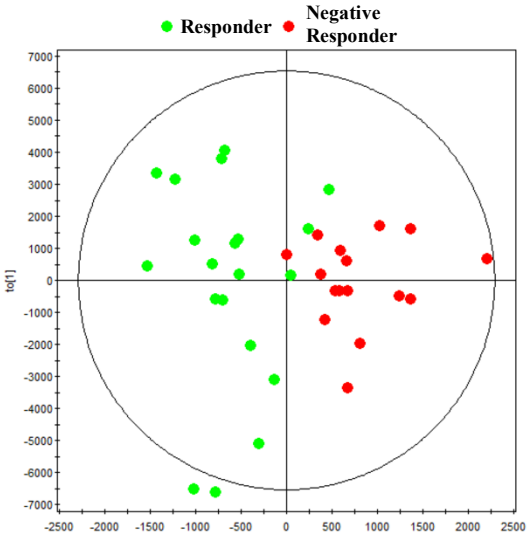


Figure 8.5 – OPLS-DA plots displaying separation of experimental samples collected prior to and following the 6-week training intervention based on A) relative $\dot{V}O_{2max}$ negative-responder vs non-responder, B) relative MMP responder vs non-responder, and C) relative MMP responder vs negative-responder. OPLS-DA analyses which failed to establish valid models have been excluded.

Metabolites contributing to OPLS-DA separation

From S-plot analysis, the OPLS-DA model separations involved a small range of metabolite classes, including dicarboxylic acids, organic acids, and purine derivatives (Table 8.9 and Table 8.10). Succinate excretion was decreased in the responder group versus the non-responder group based on relative MMP in ESI^-. Lactate and hypoxanthine excretion was decreased in the responder group versus the negative-responder group based on relative MMP in ESI^-.

Table 8.9 – Metabolic features responsible for separation of variability groups based on relative $\dot{V}O_{2max}$ as determined by OPLS-DA and S-Plot analysis.

Ionisation mode	**Biological Class**	**Metabolite**	***m/z (M)***	**RT (min)**	Non vs Neg
ESI^-	Unknown	167/159	167.0	2.65	↓
		203.1/221	203.1	3.68	↓
		203.1/237	203.1	3.95	↓
		203.1/243	203.1	4.05	↓

"Non" = non-responder, and "Neg" = negative-responder. ↑ indicates increased urinary excretion of metabolite versus non-responder group. ↓ indicates decreased urinary excretion of metabolite versus non-responder group.

Table 8.10 – Metabolic features responsible for separation of variability groups based on relative MMP as determined by OPLS-DA and S-Plot analysis.

Ionisation mode	Biological Class	Metabolite	*m/z (M)*	RT (min)	Pos vs Non	Pos vs Neg
ESI$^-$	Dicarboxylic acids	Succinate	117.0	0.97	↓	
	Organic acids	Lactate	89.0	0.65		↓
	Purine derivatives	Hypoxanthine	135.0	0.83		↓
	Unknown	130.1/66	130.1	1.10	↓	
		167/56	167.0	0.93		↓
		167/191	167.0	3.18	↓	↓
		180.1/226	180.1	3.77	↑	
		191/110	191.0	1.83	↓	
		191/121	191.0	2.02		↑
		203.1/237	203.1	3.95	↑	↑
		361.2/298	361.2	4.97		↓

"Pos" = responder, "Non" = non-responder, and "Neg" = negative-responder. ↑ indicates increased urinary excretion of metabolite versus responder group. ↓ indicates decreased urinary excretion of metabolite versus responder group.

8.4.3.3 Urinary metabolome differences based on combined responder status within interval training groups

Further evaluation of the variability-dependent metabolomic perturbations following the training intervention within each intervention group was performed. Due to low sample sizes within intervention groups and to further isolate metabolites which can differentiate responders to exercise, the non-responder, and negative-responder groups were combined into a "combined non-responder" group and using OPLS-DA models were compared to the responder groups based on relative $\dot{V}O_{2max}$, relative MMP, and relative TT power. Table 8.11 shows the summary of the comparisons with a valid OPLS-DA model with permutation testing results (n = 100) for SHORT between responders and combined non-responders for relative $\dot{V}O_{2max}$ (Q^2 = .579, P = .03), relative TT power between both responders and combined non-responders (Q^2 = .294, P = .05). All other models in both ESI^+ and ESI^- ionisation modes failed permutation testing and were excluded from further analyses. Figure 8.6 display OPLS-DA scatter plots for relative $\dot{V}O_{2max}$ comparing responders and combined non-responders (Figure 8.6A), and relative TT power comparing responders and combined non-responders (Figure 8.6B), respectively. Table 8.11 also presents model diagnostics, outlining variability in each model due to separation between groups, and due to within-group variability.

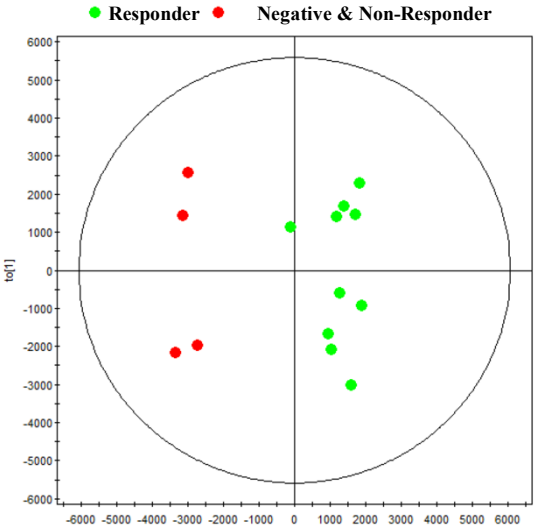
Table 8.11 - OPLS-DA scores details comparing responder group and combined non-responder groups based on changes in $\dot{V}O_{2max}$, MMP W/kg, and TT W/kg for interval intervention groups. Model diagnostics presented based on separation of scatter plots between groups, and within-group variability as a % CV. OPLS-DA analyses which failed to establish valid models have been excluded.

		Response	R^2X	R^2Y	Q^2	A	Between-group separation based on predictive component	Within-group variability based on orthogonal component	P-Value
ESI-	**$\dot{V}O_{2max}$/kg**	SHORT	.759	.942	.579	2	23%	44%	.03
	TT W/kg	SHORT	.758	.901	.294	2	6%	61%	.05

R^2X = variation in X explained by the model, R^2Y = variation in Y explained by the model, Q^2 = goodness of prediction, A = number of orthogonal components, P-value = permutation test probability of spurious model generation.

ESI$^-$

A



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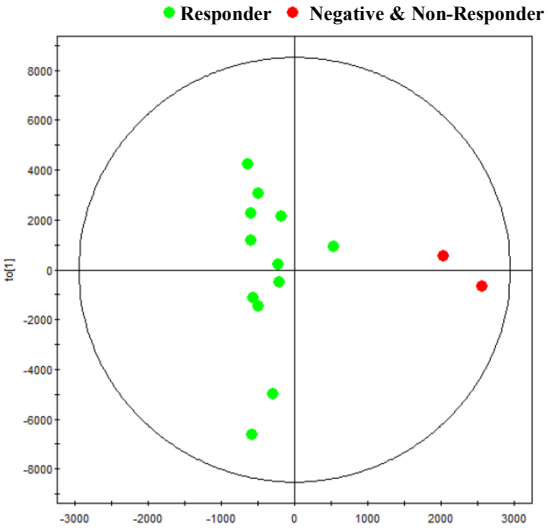

Figure 8.6 – OPLS-DA plots displaying separation of experimental samples collected prior to and following a 6-week training intervention using SHORT intervals based on responder or combined netagive and non-responder status based on A) Relative $\dot{V}O_{2max}$ and B) Relative TT power. OPLS-DA analyses which failed to establish valid models have been excluded.

Metabolites contributing to OPLS-DA separation

From S-plot analysis and based on the classification for significance, the OPLS-DA model separations involved a small range of metabolite classes, including alkaloids, carboxylic acids & derivatives, dicarboxylic acids, and purine derivatives (Table 8.12 and Table 8.13).

Within ESI^+ for SHORT comparing variability groups based on relative $\dot{V}O_{2max}$, increased uric acid, citric acid, succinate, and hippuric acid excretion was observed within responders versus combined non-responders.

Table 8.12 – Metabolic features responsible for separation of variability groups based on relative $\dot{V}O_{2max}$ as determined by OPLS-DA and S-Plot analysis.

Ionisation mode	**Biological Class**	**Metabolite**	***m/z* (M)**	**RT (min)**	**SHORT**
ESI^-	Alkaloids	Uric acid	167.0	0.78	↑
	Carboxylic acids & derivatives	Citric acid	191.9	0.75	↑
	Dicarboxylic acids	Succinate	117.0	0.97	↑
	Purine derivatives	Hippuric acid	178.1	3.90	↑
	Unknown	167/72	167.0	1.20	↑
		167/159	167.0	2.65	↑
		191/32	191.0	0.53	↑
		203.1/213	203.1	3.55	↑
		361.2/298	361.2	4.97	↓

↑ indicates increased urinary excretion of metabolite in responder group versus non-responder group. ↓ indicates decreased urinary excretion of metabolite in responder group versus non-responder group.

Table 8.13 – Metabolic features responsible for separation of variability groups based on relative TT power as determined by OPLS-DA and S-Plot analysis.

Ionisation mode	Biological Class	Metabolite	*m/z (M)*	RT (min)	SHORT
ESI^+	Unknown	145.1/190	145.1	3.17	↑
		167/191	167.0	3.18	↑
		180.1/249	180.1	4.15	↑
		203.1/221	203.1	3.68	↑
		203.1/258	203.1	4.30	↑
		203.1/313	203.1	5.22	↑
		361.2/255	361.2	4.25	↑
		361.2/298	361.2	4.97	↑

↑ indicates increased urinary excretion of metabolite in responder group versus non-responder group. ↓ indicates decreased urinary excretion of metabolite in responder group versus non-responder group.

8.5 Discussion

This study assessed the differences in the urinary metabolome using untargeted UPLC-MS before and after a 6-week training intervention of two different interval training session formats using a maximal effort-based intensity prescription (Chapter 6). The ability to identify metabolites that increase or decrease with exercise training, as well as whether any metabolites can be attributed to exercise responder status, presents itself as a method of optimising exercise training prescription. No difference was observed using OPLS-DA between the samples taken before and after the training intervention when all groups were combined, as well as when each group was analysed individually.

Uric acid is formed as the final compound of purine metabolism (Maiuolo *et al.* 2016; Becker 1993), and exercise results in increased concentrations of Uric acid within the body due to the increased rate of ATP turnover (Sutton *et al.* 1980; Sahlin, Palmskog and Hultman 1978). It has been observed that in the 24 h following bouts of high-intensity exercise increased concentrations of uric acid in human serum (Green and Fraser 1988). Of the total uric acid that is produced within the human body, approximately 30 % is degraded in the biliary and gastrointestinal tract during uricolysis, and the remaining 70 % is excreted through the kidneys (Sorensen and Levinson 1975). Whilst the alterations in uric acid excretion following acute exercise have been extensively examined, the alterations in

uric acid excretion following endurance training have received limited attention. Over the course of a 1-year training cycle, long-distance runners display stable uric acid concentrations, with non-significant elevations in concentrations usually observed during the pre-competition phase (Zieliński *et al.* 2009; Zieliński, Kusy and Rychlewski 2011; Zieliński and Kusy 2012). Interestingly, over a 2-week period within a 6-month military training schedule, uric acid at rest was found to increase (Chevion *et al.* 2003). Conversely, uric acid has been observed to decrease during intense training periods (Yan *et al.* 2009) but was also observed to be higher in more experienced athletes than less experienced athletes (Yan *et al.* 2009). In the present study, uric acid excretion increased in responders versus non-responders based on relative $\dot{V}O_{2max}$ following SHORT training, which supports the previous findings of increased uric acid following training increased improvements in performance. However, the use of uric acid to assess training status has not been supported by previous research due to no observable change following training interventions (Zieliński and Kusy 2012; Lombardi *et al.* 2010).

Whilst uric acid represents the end-product of purine metabolism; the intermediate compound hypoxanthine has been shown to be a strong predictor of performance in highly trained athletes, regardless of sport specialisation (Zieliński, Krasińska and Kusy 2013). Hypoxanthine can not only be used to indicate degradation of adenine nucleotides within muscle and therefore energetic stress during exercise (Zieliński and Kusy 2015a; Zieliński and Kusy 2015b; Sahlin, Tonkonogi and Söderlund 1999) and a marker of exercise intensity (Ka *et al.* 2003), but also it has potential to be utilised as a marker which can be used to differentiate performance levels (Zieliński, Krasińska and Kusy 2013). Acute exercise results in increased urinary excretion of hypoxanthine (Hargreaves *et al.* 1998; Green and Fraser 1988; Hellsten-Westing, Sollevi and Sjödin 1991; Houston and Thomson 1977) and is commonly increased in post-exercise urine samples in several metabolomics investigations (Enea *et al.* 2010; Pechlivanis *et al.* 2010; Muhsen Ali *et al.* 2016; Mukherjee *et al.* 2014; Pechlivanis *et al.* 2015). In the present investigation, decreased hypoxanthine excretion was observed in the responder group versus the negative-responder group based on relative MMP in ESI^-. Elevations in blood hypoxanthine concentrations following exercise were higher during the transition phase versus the competition phase in long- and middle-distance runners, as well as lower pre-exercise hypoxanthine concentration, mainly due to the maintenance of intramuscular IMP by the resynthesis of hypoxanthine (Figure 8.7; Zieliński *et al.* 2009; Zieliński, Kusy and Rychlewski 2011; Zieliński and Kusy 2012; Zieliński, Kusy and Słomińska 2013). Hypoxanthine

concentrations, both at rest and post-exercise, were able to differentiate performance level in both sprint and endurance-trained athletes, highlighting the applicability of this metabolite as a marker for training adaptation and performance status (Zieliński, Krasińska and Kusy 2013). It has also been observed that resting urinary hypoxanthine excretion was decreased following 6 weeks of threshold training, but not polarised training (Neal *et al.* 2013). In contrast to this, increases in resting urinary hypoxanthine excretion after an 18-month training intervention using middle-aged and older men has been observed, potentially highlighting the applicability of hypoxanthine as a training marker to higher trained athletes (Sheedy *et al.* 2014).

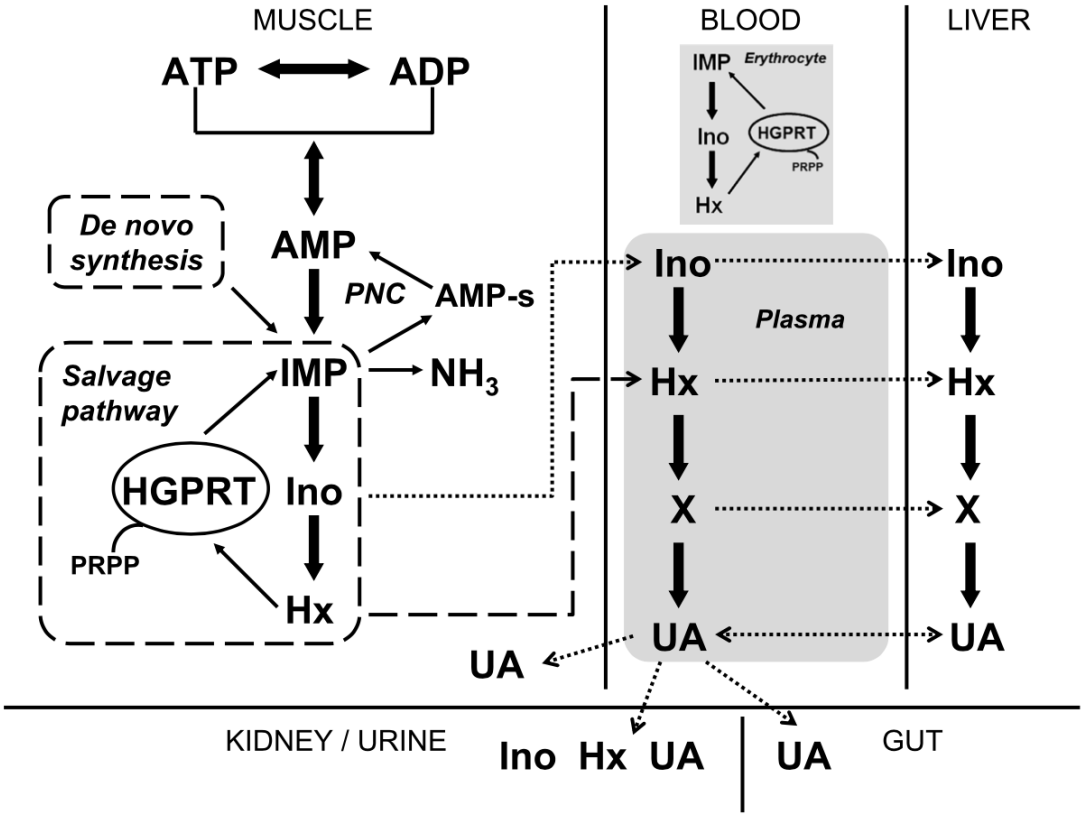

Figure 8.7 – Main metabolic pathways of adenine nucleotide degradation during exercise and recovery period. *Hx, hypoxanthine; AMP-s, AMP synthase; HGPRT, Hx- guanine phosphoribosyltransferase;; Ino, inosine; NH3, ammonia; PNC, purine nucleotide cycle; PRPP, phosphoribosyl pyrophosphate; UA, uric acid; X, xanthine* (Taken from Zieliński and Kusy 2015a).

One of the key intermediates in the Krebs Cycle is citric acid and is essential for oxidative energy production along with its derivative citrate. With the presence of CS, citrate is synthesised from acetyl CoA and oxaloacetate and rate-limited by the presence of ATP (Wiegand and Remington 1986). Within the present chapter, citric acid excretion was

increased in the responder group versus the combined non-responder group based on relative $\dot{V}O_{2max}$. This finding could reflect increased CS activity, as it has been shown that training increases CS activity (Vigelsø, Andersen and Dela 2014), resulting in greater citrate synthesis and increase the excretion of citrate in urine (López *et al.* 2010; Nuñez *et al.* 2012). Succinate has been recently attributed to having an important role in skeletal muscle remodelling following exercise (Reddy *et al.* 2020) through the SUCNR1 pathway. This is reflected by the increased succinate excretion in responders versus non-responders in SHORT based on relative $\dot{V}O_{2max}$. The present investigation also found that succinate was decreased in the responder group versus the non-responder group based on relative MMP. This may indicate the unsuitability for succinate to be a target for metabolic phenotyping based on training-related variability. Previous investigations have identified lower concentrations of excreted lactate following a training intervention (Pechlivanis *et al.* 2013), which indicates increased removal of lactate from the muscle and bloodstream and improvement in exercise performance as a result. Similarly, within the present investigation, lactate excretion was decreased in the whole cohort responder group versus the negative-responder group based on relative MMP. The present investigation indicated an increase in hippuric acid within responders versus combined non-responders based on relative $\dot{V}O_{2max}$. As the exercise-related effects on hippuric acid have not been extensively investigated (Neal *et al.* 2013; Davison *et al.* 2018), this provides an insight into how training-related performance changes may influence hippuric acid metabolism.

Due to the small number of valid OPLS-DA models identifying separations between analysis groups, it is difficult to compare variability within the metabolomes of each group. Of the valid OPLS-DA models, it can be observed that lowest within-group variability can be observed between non-responders and negative responders based on relative $\dot{V}O_{2max}$ (39%), which may highlight the potential small differences in training stimulus that could relate in training non-response or negative-response. Similarly, the OPLS-DA models comparing responders to both non-responders and negative-responders had significantly higher within-group variability at 47% and 49%, respectively. This could indicate that when a training response occurs, it coincides with a wide range of metabolic perturbations. Whether these perturbations are responsible for the training-related performance improvements, or a response to the changes, should be investigated further. However, a limitation within the current investigation is the small sample size within the responder, non-responder, and adverse-responder groups, in addition the unbalanced nature of the sample groups. With a larger sample cohort these limitations could be overcome, allowing

for more confidence to be given to any differences observed between groups from OPLS-DA analysis.

The human metabolome is sensitive to changes in dietary intake of participants (such as caffeine, protein, phytochemical intake etc.) and has been reported within previous nutrition metabolomics investigations (van Velzen *et al.* 2008) and has previously been reviewed (Gibney *et al.* 2005). Within the study design from Chapter 6, from which data was used within the current chapter, dietary intake was moderately controlled but not monitored or extensively controlled. Participants were instructed to avoid caffeine intake and fish in the days preceding testing and to repeat a similar dietary intake to pre-training testing following the training intervention. The presence of metabolites that are responsive to variations in dietary intake of fruits, tea, or coffee could indicate dietary standardisation was not sufficient within the current investigation (Toromanović *et al.* 2008; Clifford *et al.* 2000; Pero 2010). The number of unknown metabolites identified within the present study highlights the limited number of putatively identified metabolites, potentially resulting in biomarkers of exercise variability being overlooked. Further limitations of this investigation relate to the lack of validated OPLS-DA models and small size of sample groups during OPLS-DA analyses. This may have been due to a smaller sample size within this investigation, but previous research has been able to successfully implement NMR (C. Enea *et al.* 2010), LC-MS (Rainer Lehmann *et al.* 2010), and GC-MS (Chorell *et al.* 2012; Nieman *et al.* 2012; Peake *et al.* 2014) methods with sample sizes ranging from 6 to 14 participants. It is also possible that with the urine samples being collected at rest prior to and following an exercise training intervention, there may not have been a metabolic challenge present, such as exercise, to display differences in training adaptations between groups. The metabolomics investigation conducted by (Pechlivanis *et al.* 2013) measured the serum metabolome before and after the first session of the training intervention and compared to before and after the last session of the training intervention. Future investigations should examine the acute metabolomic response to exercise before and after a training intervention, as this may provide further insight into the physiological changes which differ between intervention groups.

8.6 Conclusion

No differences were observed in the resting metabolomic profile prior to and following the training interventions, whether data were grouped with all training groups or separated into the different training prescription groups. The findings of the present investigation indicate differences in metabolomic profiles were observed between responders and non-responders across the whole study cohort, and further metabolites were identified to differentiate between responders and non-responders following LONG and SHORT interventions. While the present investigation identified a limited number of metabolites, the available data indicate that metabolomics can provide a promising approach to explore individual variability in chronic exercise response, especially if more sensitive analytical systems are utilised.

Chapter 9 - General discussion

The aim of this thesis was to assess the efficacy of using effort-based intensity prescriptions as a tool to produce low levels of individual variability in response to training and whether metabolomics could identify potential metabolite markers which could help to indicate training responsiveness prior to training.

The first experimental chapter (Chapter 4) investigated the within-athlete and between-athlete variability in response to different effort-based intensity targets during single self-paced exercise bouts of varying durations. As both intensity and duration of the exercise bouts were increased, within- and between-athlete variability in power output, work done, heart rate, $\dot{V}O_2$, $\dot{V}CO_2$, and $\dot{V}_E$ decreased, but within- and between-athlete variability in muscle oxygenation data increased. This relationship agrees with previous findings by Bagger, Petersen and Pedersen (2003), who observed lower variability in physiological variables as intensity increases, with the lowest variability being recorded under maximal conditions. When exercising at higher absolute exercise intensity, a small change in power output can result in large changes in physiological response and fatigue compared to lower absolute exercise intensities (Burnley and Jones 2018); thus, participants are likely to control their exercise intensity within a closer bandwidth. However, it has also been observed that whilst variability may be low when athletes complete maximal or near-maximal intensities, increasing the duration of the exercise trials may increase variability (Schabort *et al.* 1998). This is in contrast with the findings of Chapter 4, possibly due to differences in methodology. Schabort *et al.* (1998) instructed participants to complete the exercise "*as fast as possible*", whereas in Chapter 4 the participants were instructed to modulate the external workload to achieve the desired RPE anchor. Schabort *et al.* (1998) found lowest within-athlete variability during a 100 km simulated time trial (1.7 %), followed by a 1 km sprint lasting 1 min 16 sec ± 6 sec (1.9 %), and highest variability during a 5 km effort lasting 5 min 31 sec ± 16 sec (2.0 %) It may have been the case that a shift of attentional focus towards internal-associative modes at the higher intensities and durations may have occurred in Chapter 4, as the task requires constant attention and modulation of workload in order to maintain the set RPE anchor (Noble and Robertson 1996). The findings of this study are in-line with previous investigations showing that within-athlete variability is greater when exercising below $\dot{V}O_{2max}$ (ranging from between 5.6 - 55.9 % CV) versus exercising above $\dot{V}O_{2max}$ (ranging from between 1.7 – 17 % CV; Gleser and Vogel 1971; Maughan, Fenn and Leiper 1989; McLellan, Cheung and Jacobs 1995; Jeukendrup *et al.* 1996; Laursen *et al.* 2007; Coggan and Costill 1984; Lindsay *et al.* 1996). However, it is uncertain whether high or low levels of within- and between-athlete

variability in acute exercise translates to differences in chronic training adaptations. Previous research has demonstrated that improving the individualisation of training intensities results in a reduction of training non-response (Wolpern *et al.* 2015). In (Wolpern *et al.* 2015), the prescription relative to the first and second ventilatory threshold resulted in all athletes responding to the training intervention, versus when intensity was prescribed using %HRR (41.7 % responders vs 58.3 % responders). With lower levels of within- and between-athlete variability in power output, work done, heart rate, $\dot{V}O_2$, $\dot{V}CO_2$, and $\dot{V}_E$ as duration decreases and intensity increases when using self-paced targets, there may be a more homogenous and predictable training stimulus being generated. Further investigations may look to explore the differences in within- and between-athlete variability between self-paced exercise intensities and other individualised intensity prescription methods to evaluate which method generates the least variability in physiological stimulus from training. Once established, lower variability on both a within- and between-athlete basis would allow practitioners to prescribe training in a manner that targets a specific physiological response and is consistent between athletes. The practical applications of Chapter 4 would relate to the implementation of self-paced exercise bouts, or whole sessions, within an athletes' training programming. The findings also provide a background understanding of the levels of variability a coach or practitioner may look to expect following efforts of differing durations or intensities.

The findings of lower within- and between-athlete variability as intensity increases and duration decreases, suggest that using maximal effort-based intensity prescriptions during interval sessions may result in less variability in the acute physiological response to each training session. The second experimental chapter (Chapter 5) analysed the within-athlete and between-athlete variability in response to three different interval training session formats (SHORT, LONG, and CONT), during which an effort-based intensity prescription was used to achieve maximal exertion during each session. Interestingly, different levels of variability in physiological response were observed between LONG, SHORT and CONT exercise bouts. Within-athlete variability of session power output was greatest during SHORT, whereas between-athlete variability was greatest in CONT, and total variability was greatest in SHORT. Overall, on a single-session basis, the use of LONG formats provides the least individual variability in acute response compared to SHORT and CONT. This is in contrast with the finding that higher intensities during individual exercise bouts resulted in lower variability, but likely this is due to the prolonged undulating nature of the SHORT interval training format. In comparison to other investigations in the literature, the

findings of Chapter 5 display lower levels of between-athlete variability in power output and $\dot{V}O_2$ during SHORT (Nicolò *et al.* 2014). The between-athlete variability observed in BLa^{-1} in response to LONG within Chapter 5 is observed to be consistent with variability levels previously reported for longer duration efforts. Seiler and Sylta (2017) displayed increased consistency as interval duration decreases; 16 min (CV: 34 %), 8 min (CV: 26 %), and 4 min intervals (CV: 21 %), and therefore the between-athlete CV of 26.1 % from the 5 min intervals, and 34.2 % from the 40 min continuous effort. This indicates that between-athlete variability likely reduces gradually as effort duration shortens, however, there is a time duration of interval training format at which this reduction in between-athlete variability is reversed, exemplified by the 31.6 % between-athlete variability in BLa^{-1} during SHORT interval training sessions. The reason for this may relate to the combination of many factors, such as the similarities and differences in performance levels between the athletes within the study cohort relating to the power-duration relationship. The similarities in performance level of athletes may result in a common duration of effort which is most similar across all participants, reducing training-induced variability in acute exercise performance. Such an investigation into not only the between-athlete variability during interval training sessions but also the within-athlete variability has not been conducted and provides a valuable tool for understanding the training process and adaptive responses to training (Chrzanowski-Smith *et al.* 2020). With the differences in within-athlete and between-athlete variability observed between the LONG and SHORT session performance and physiological response, it remains to be established whether high or low variability in acute training stimulus results in greater or lower chronic training response or higher or lower degrees of individual variability in training response. The findings of Chapter 5 may be implemented by coaches or practitioners to identify training sessions which athletes could repeat at regular intervals during training cycles. The expected within-athlete variability levels for each training session could be used to indicate when an athlete has exceeded the critical difference range, and therefore the change is likely to be reflective of true performance changes.

The third experimental chapter (Chapter 6) investigated the effects of two maximal effort-based training interventions (SHORT and LONG) on physiological adaptations and performance and a control group (CON). Over a six-week intervention period, only the SHORT group increased MMP and both absolute and relative TT power, compared to no change in LONG or CON. However, a low magnitude of training response can be observed when comparing the findings of Chapter 6 with studies which report ~ 0.5 $L.min^{-1}$

improvements in $\dot{V}O_{2max}$ following HIT training, potentially due to the trained nature of the participants within Chapter 6 (Bacon *et al.* 2013). The reasoning for this may also be due to a mixture of relatively low training volumes and absolute work intensity within Chapter 6. Training durations within Chapter 6 were ~ 8 h per week in SHORT, ~ 5.6 h per week in LONG, and ~ 6.6 h per week in CONT. The work bout power output during LONG interval sessions within Chapter 6 was lower than those performed in Chapter 5 (Ch 5; 310 ± 45 W vs Ch 6; 274 ± 52 W) and much lower than commonly reported 5-min interval training power output in the literature (334 ± 39 W; Weston *et al.* 1997 and 333 ± 27 W; Lindsay *et al.* 1996)). The recent findings of Rønnestad *et al.* (2020) demonstrated slightly higher work bout power output during 5-min interval sessions in comparison to the sessions completed within Chapter 6 (ranging from 295 W to 315 W). Moreover, the recovery period used within Rønnestad *et al.* (2020) was 2.5 min with 4 repeats being performed, indicating a higher training load was achieved compared to Chapter 6. It is also of note that the work bout power output during SHORT in both Chapter 5 (382 ± 55 W) and Chapter 6 (391 ± 72 W) is lower than has been previously reported in the literature, with 464 ± 51 W being reported during 30:30 format intervals (Nicolò, Bazzucchi, Haxhi, *et al.* 2014). However, the recent publication from Rønnestad *et al.* (2020) reported work bout power outputs to range from 381 W to 390 W in a 1-week intensive training intervention which resulted in a 5.7 % improvement in $\dot{V}O_{2max}$, demonstrating that these work bout power outputs are high enough to deliver a sufficient training stimulus. Despite the differences in work bout power between Chapter 5 and Nicolò, Bazzucchi, Haxhi, *et al.* (2014), similar levels of BLa^{-1} (9.5 ± 3.0 $mmol.L^{-1}$ vs 8.5 ± 2.4 $mmol.L^{-1}$, respectively), HR (164 ± 10 bpm vs 169 ± 5 bpm, respectively), and $\dot{V}O_2$ (3310 ± 470 $ml.min^{-1}$ vs 3147 ± 234 $ml.min^{-1}$, respectively) were observed, showing that training stimulus may have been in-line with previously reported investigations. Despite the small magnitude of training-related performance improvements, SHORT intervals resulted in higher rates of training response in both $\dot{V}O_{2max}$ and absolute TT power (71 %, and 86 %, respectively) compared to LONG (40 %, and 60 %, respectively) or CON (27 % and 27 %, respectively). A high degree of between-athlete variability in training response was still observed across all groups, ranging from as small as 10 % (Absolute $\dot{V}O_{2max}$ in LONG) to 86 % response (Absolute and relative TT power in SHORT). This demonstrates that using short maximal effort-based intensity prescriptions may be optimal for reducing the level of participant non-response, but further research is required to establish other factors relating to individual training response. The findings of Chapter 6 support previous literature on the efficacy of the use of SHORT intervals within training programming (Rønnestad *et al.* 2015; Rønnestad *et al.* 2020; Rønnestad *et al.* 2021) and highlight the importance of

ensuring training stimuli during LONG interval sessions is high enough due to the long work bout durations. However, despite the SHORT format being the most effective intervention of those investigated within this thesis, the occurrence of non-response or adverse-response was still present within the intervention. Future research should compare interval training interventions using short undulating intervals using effort-based intensity prescriptions and standardised prescription methods of varying intensities to further examine whether effort-based intensity prescriptions are more effective at lowering levels of non-response to training. Future research should also further investigate whether there is a difference in chronic training response magnitude and variability following training prescribed using sessions which result in either high levels of individual variability versus low levels of individual variability. Chapter 6 also indicates that despite efforts to reduce between-athlete variability in training improvements using effort-based intensity prescriptions, individual variability remains in training response when individual measures are investigated in isolation, but improvement can interestingly be observed across $\dot{V}O_{2max}$, MMP, and TT performances for each individual.

The incorporation of metabolomics into this thesis allowed the comparison of the global metabolic response to acute training performance and physiological response and the alterations in chronic training changes in the metabolome. The finding of the importance of urinary hypoxanthine, uric acid, lactate, and citric acid in identifying variable participants could be used to explain the higher levels of physiological stimulus in response to these acute training sessions. Of the metabolites that were identified to be associated with variable acute training performance and response, some were identified as metabolites of interest following chronic training interventions. Both hypoxanthine and uric acid reflect exercise-induced alterations in ATP, AMP, and IMP utilisation during exercise, and can be used to establish metabolic differences to different exercise intensities or formats (Sutton *et al.* 1980; Stathis, Carey and Snow 2005; Stathis *et al.* 1999; Gerber *et al.* 2014; Zieliński and Kusy 2012; Zieliński and Kusy 2015a; Zieliński and Kusy 2015b; Stathis *et al.* 2006; Hellsten-Westing *et al.* 1993; Zieliński, Kusy and Rychlewski 2011; Zieliński *et al.* 2009; Sahlin, Tonkonogi and Söderlund 1999; Hellsten-Westing, Sollevi and Sjödin 1991; Pechlivanis *et al.* 2015; Kaya *et al.* 2006; Lewis *et al.* 2010). Excretion of hypoxanthine was increased in all acute exercise sessions; decreased hypoxanthine excretion was associated with variable performance during LONG interval sessions, whereas increased hypoxanthine excretion was associated with variable performance during SHORT interval sessions. On a chronic basis, hypoxanthine was decreased in positive training responders

based on MMP improvements. This highlights a potential carry-over from acute to the chronic metabolomic response, but also indicates that there may be an intensity-based difference in hypoxanthine response to exercise. This agrees with an investigation monitoring long-distance runners, which identified that factors including the exercise intensity, rate of reconversion of hypoxanthine to IMP by hypoxanthine-guanine phosphoribosyl-transferase (HGPRT), and excretion rate of hypoxanthine itself (Zieliński *et al.* 2009). Overall, it was found that chronic training resulted in reduced excretion of hypoxanthine (Zieliński, Kusy and Rychlewski 2011; Zieliński *et al.* 2009). Acute decreases in uric acid were observed following both LONG and SHORT training, but on a chronic basis, increased uric acid excretion was found to be associated with positive training response versus non-responders based on relative $\dot{V}O_{2max}$ following the SHORT training intervention. This is in contrast to the previous finding of elevated uric acid during intense training periods (Yan et al. 2009), but it has also been shown that uric acid is elevated in more experienced athletes compared to less experienced athletes (Yan et al. 2009), and other observations of increased uric acid excretion following acute exercise (Gerber *et al.* 2014; Kanďár *et al.* 2014; Stathis *et al.* 2006) and over chronic training interventions (Chevion *et al.* 2003; Zieliński *et al.* 2009; Zieliński, Kusy and Rychlewski 2011).

The finding of increased urinary lactate following acute training sessions was not unexpected, being one of the most abundantly detected metabolites within sports metabolomics investigations and an end product of glycolysis (Goodwin *et al.* 2007; Berton *et al.* 2017; Daskalaki, Easton and Watson 2015; Lewis *et al.* 2010a; Yan *et al.* 2009; Pechlivanis *et al.* 2010; Mukherjee *et al.* 2014; Santone *et al.* 2014; Kujala *et al.* 2013; Netzer *et al.* 2011; Enea *et al.* 2010; Sheedy *et al.* 2014; Nieman *et al.* 2012). The increased lactate following the maximal self-paced acute training sessions reflects previous findings of increased lactate excretion after exhaustive exercise This increase in lactate excretion is commonly seen following maximal exercise (Hood *et al.* 1988; Cairns 2006; Allen, Lamb and Westerblad 2008). However, the finding that increased urinary lactate excretion was associated with individual variability in session performance in both SHORT and CONT, but not the LONG training format, was a novel finding of this thesis. This pattern of variability is also highlighted within the BLa^{-1} samples collected within the sessions themselves, with the within- and between-athlete variability being similar in both CONT (18.6 % and 34.3 %, respectively) and SHORT (16.8 % and 31.3 %, respectively), whilst being lower in LONG (14.1 % and 25.9 %, respectively). Interestingly, when

compared to a previous investigation comparing acute response to continuous and intermittent self-paced interval sessions, the between-athlete variability in acute BLa^{-1} response in the current thesis was lower during CONT and higher during SHORT (43.1 % and 28.2 %, respectively; Nicolò, Bazzucchi, Haxhi, *et al.* 2014). Another investigation which compared effort-matched short intervals (3 sets of; 13 x 30/15 sec) and long intervals (4 x 5 min) reported extremely low between-athlete variability in BLa^{-1} response 16 % and 15 % (Almquist *et al.* 2020). These differences may be due to differences in the performance level of the athletes included in the studies, as Almquist *et al.* (2020) included athletes of level 4 – 5, and the present thesis included athletes of level 3 – 4 (de Pauw *et al.* 2013). This suggests that maximal effort-based training may be most effective for use with higher level athletes at reducing between-athlete variability. On a chronic training basis, reduced excretion of lactate was able to differentiate between training responders and negative-responders based on relative MMP across all intervention groups. This is similar to previous interventions in trained participants (Pechlivanis et al. 2013), and it has been observed that trained endurance athletes are able to efficiently re-utilise lactate for both gluconeogenesis and oxidative metabolism (Emhoff *et al.* 2013).

In addition to lactate, another metabolite which could provide an insight into energy demand and carbohydrate metabolism is citric acid, or the intermediate citrate (Krebs and Johnson 1980; Krebs, Salvin and Johnson 1938; Peake *et al.* 2014; Chorell *et al.* 2009). On an acute-session basis, decreased citric acid excretion was observed immediately following LONG and SHORT sessions, likely reflecting the high levels of metabolic acidosis during the sessions (Unwin, Capasso and Shirley 2004; Simpson 1983). Interestingly, decreases in citric acid excretion was observed between the start of the training session and one hour following the training session, but only in LONG and CONT session formats. Whilst this may reflect changes in CS activity, it is surprising as previous investigations have found increased excretion of the citric acid derivative citrate following acute running exercise (Pechlivanis et al. 2013). Following acute sessions of CONT, increased citric acid excretion was observed within variable versus consistent athletes, likely highlighting the fluctuations in CS activity when athletes complete continuous exercise (Leek *et al.* 2001). Increased citric acid excretion was observed in training responders versus non-responders to chronic training using SHORT based on relative $\dot{V}O_{2max}$, which is supported by previous observations of increased CS activity has been observed following training (Vigelsø, Andersen and Dela 2014), and urinary excretion of citric acid and citrate (López *et al.*

2010; Nuñez *et al.* 2012). However, following chronic training, differences in CS activity have been reported to range from between 0 % and 100 % (Green *et al.* 1992; Holloszy *et al.* 1970.; Starritt, Angus and Hargreaves 1999), highlighting that the use of citric acid as a biomarker may be challenging due to the many influences on the Krebs Cycle, CS activity, and citric acid excretion (Wiegand and Remington 1986). Increases in excretion of the dicarboxylic acid succinate was found to be associated with training responders versus non-responders based on $\dot{V}O_{2max}$ following chronic SHORT training. However, contrary to this, reduced succinate excretion was associated with training responders versus non-responders based on MMP across all training groups. Whilst succinate has been attributed to having an important role in skeletal muscle remodelling following exercise (Reddy *et al.* 2020) through the SUCNR1 pathway, it is more commonly associated with its role within the TCA cycle for energy generation. With increased succinate excretion associated with training response following SHORT, this could reflect the higher energy turnovers encountered during chronic training using this format. However, this would not explain the observation of reduced succinate excretion in training responders versus non-responders based on MMP across all training groups, requiring further investigation into the pathways of excretion of succinate and associated metabolic consequences.

Amino acids were observed to respond to acute exercise in the one hour following training, but no amino acids were observed to respond to chronic exercise interventions. The findings within this thesis of increased urinary excretion of tyrosine in the one hour following acute LONG training sessions are in apparent contradiction of decreased tryptophan excretion following sub-maximal exercise of varying lengths (Thysell *et al.* 2012) and maximal sprint-based HIIT (Pechlivanis *et al.* 2010). The mechanisms for this may be related to high demand for NAD^+ following high-intensity exercise for short periods (Sahlin *et al.* 1976; Coelho *et al.* 2016). The excretion of another amino acid, creatine, was observed to be increased in the hour following acute LONG training sessions, which may be due to excess creatine within the trained participants in this thesis (Forbes, Slade and Meyer 2008), with creatine and associated metabolites being regarded as indicators of muscle damage (Jang *et al.* 2018; Baird *et al.* 2012). In contrast to this finding, reduced creatine excretion has been reported from trained and untrained individuals following maximal sprint exercise, highlighting that in some exercise formats, creatine excretion may be reduced due to the resynthesis into phosphocreatine for energy (Bezrati-Benayed *et al.* 2014). A reduction in histidine in the hour following acute LONG training sessions is in contrast to previous research displaying an increase following

maximal exercise (Pechlivanis *et al.* 2013). However, this may be due to the body utilising histidine as an antioxidant following endurance exercise in the present investigation (Son, Satsu and Shimizu 2005), in contrast to the sprint exercise of Pechlivanis *et al.* (2013). Increased excretion of the amino acid tyrosine was able to differentiate between variable and consistent performance in acute LONG format training sessions. This is again in contrast with the previous findings of increased tyrosine excretion following maximal sprint training sessions (Pechlivanis *et al.* 2015). However, there may be a necessary threshold of exercise duration for tyrosine excretion to occur, with previous findings of increased tyrosine excretion following endurance exercise lasting between 15 and 765 min (Haralambie and Berg 1976), and the finding that no differences in tyrosine excretion was observed following the SHORT format exercise in the present thesis.

9.1 Thesis limitations

Whilst the athletes recruited to take part within the experiments reported in this thesis were all competitive cyclists, their associated performance level (Level 3 – 4; de Pauw *et al.* 2013) may highlight that effort-based training could be more effective in populations of a higher performance level. A previous investigation utilising maximal effort-based intensity prescriptions displayed lower between-athlete variability (Almquist *et al.* 2020), highlighting an extremely homogenous group of high-level athletes. Future investigations should look to screen participants based on performance level to maintain a small range of performances within experimental groups. In addition, future research may investigate the differences in within- and between-athlete variability dependent on performance level may highlight whether the use of effort-based intensity prescriptions is more suited to use within athletes above a certain performance level. The inclusion of a comparison method of intensity prescription would have allowed this thesis to establish whether effort-based intensity prescriptions result in greater or lower within- and between-athlete variability to other common prescription methods. It has previously been shown that when training is prescribed based on graded exercise test data versus self-paced exercise test data, no differences in training responses were observed between groups (Hogg *et al.* 2018). However, other investigations have shown that some methods of training individualisation can be more effective than traditional standardised methods at improving exercise performance (Weatherwax *et al.* 2019). This highlights that more research is required to identify the most optimal standardised method for prescribing exercise which results in low levels of within- and between-athlete variability. The metabolomics investigations within

this current thesis were limited by the relatively low number of detected compounds, and by the large number of unknown compounds that were not able to be identified. The Waters ACQUITY QDa mass analyser used within this thesis would likely be better suited for targeted analyses within exercise metabolomics, rather than untargeted metabolomics investigations due to the low number of detected compounds. The QDa utilises a single quadrupole mass analyser design, which does increase the affordability of the system (Bu *et al.* 2016), there are limitations in the accuracy and sensitivity of the QDa system versus others such as TOF analysers with multiple quadrupole setup and ion mobility analysis capability (Marshall and Hendrickson 2008). Future untargeted investigations into the metabolomic responses to exercise should seek to utilise a system which results in greater number of detected compounds, and greater identification of unknown metabolites may be improved by the inclusion of more standard compounds. The observation that L-alanine failed the mass error threshold in both Chapters 7 and 8 indicates that there may have been an issue with the standard compound produced to identify this metabolite. More extensive comparisons of standard compound mixtures against analytical urine samples prior to experimental runs may ensure this is identified and rectified. The findings within Chapter 8 suggest that there are only marginal differences in the resting metabolome following a six-week training intervention, and it is possible that differences would have been observed if the metabolomic response to acute exercise was assessed prior to and following the training intervention. Future investigations may look to incorporate a study design similar to (Pechlivanis *et al.* 2013), where samples for metabolomic analysis were collected before and after the first session of the training intervention and compared to before and after the last session of the training intervention. This would provide further insight into the physiological changes which differ between intervention groups, further to any changes in the resting metabolome before and after a training intervention.

9.2 Future directions of research

The findings of this thesis highlight the importance of exploring the use of effort-based training intensity prescriptions further. Future investigations should compare the within- and between-athlete variability of effort-based prescriptions against other methods such as $\%\dot{V}O_{2max}$, %LT, %HRR, and intensities prescribed relative to VT_1/VT_2 or LT_1/LT_2. This would establish whether effort-based intensity prescriptions allow athletes to effectively individualise their training to a greater extent than traditional standardised methods.

This thesis also highlights how metabolomics techniques could be integrated into exercise training research, providing a wealth of information regarding metabolites which are upregulated or downregulated, or baseline metabolites which are associated with positive training response. Future research may also look to perform repeated training interventions on individuals who are non-responsive to one form of training, to further examine whether training modality changes can improve the occurrence of training response.

9.3 Conclusions

The results of this thesis provide a comprehensive overview of the individual variability that occurs when exercise training intensity is prescribed using an effort-based approach. The first study investigated self-paced exercise bouts of different durations and intensity targets, and it has been established that effort-based intensity prescriptions at higher RPEs and shorter durations result in lower levels of individual variability. Following this, the use of maximal effort-based intensity prescriptions was applied to either long or short interval or continuous training sessions. It was found that long intervals resulted in the lowest levels of variability in both how the session was performed in addition to the physiological response to exercise, compared to the short interval and continuous sessions. Following metabolomic analysis of urine samples collected before, immediately after, and 1 h following each training session, it was found that metabolites relating to energy turnover, purine metabolism, and amino acid metabolism were associated with individuals that were consistent or variable in session performance. With the levels of within- and between-athlete variability in acute session performance established, the chronic use of short and long interval formats using maximal effort-based intensity prescriptions were explored. It was found that chronic training using short interval formats and maximal effort-based intensity prescriptions resulted in greater training response versus the long interval formats and a control group. Despite the effectiveness of short interval format at increasing group-mean performances, the between-athlete variability in chronic training response was also found to be highest when utilising this session format. A small number of metabolites relating to energy turnover were able to differentiate between training responders and non-responders, as well as being associated with increased MMP or $\dot{V}O_{2max}$ across all training groups.

Chapter 10 - References

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